THE NEUROPSYCHIATRIC PHENOTYPE IN DARIER'S DISEASE

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Abstract

Darier's Disease (DD) is a rare autosomal dominantly inherited skin disorder in which co-occurrence of neuropsychiatric abnormalities has been frequently reported by dermatologists. The disease is caused by mutations in a single gene, *ATP2A2*, which maps to 12q23-q24.1 and is expressed in the skin and brain. This gene encodes SERCA2 (sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2), a calcium pump involved in intracellular calcium transport.

This study aimed to conduct the first systematic investigation of the neuropsychiatric phenotype in DD, investigate possible genotype-phenotype correlations, and compare the neuropsychiatric features in DD individuals and their first-degree unaffected relatives.

One hundred unrelated individuals with DD and 24 of their unaffected relatives were assessed using a battery of standardised neuropsychiatric measures. The relationship between the mutations detected in the *ATP2A2* gene and the presence/severity of neuropsychiatric phenotypes was examined.

DD individuals reported high rates of mood disorders, specifically major depression (30%), suicide attempts (13%) and suicidal thoughts (31%), and these were significantly more common in DD when compared with normative population data. Further, individuals with DD reported higher scores on measures of neuropsychiatric dysfunction than their unaffected relatives. These associations cannot be explained by psychosocial factors. Mutations found among individuals with similar neuropsychiatric phenotypes clustered in certain locations within the SERCA2b protein. Together, these findings support the hypothesis that mutations in *ATP2A2* confer susceptibility to neuropsychiatric dysfunction, in particular mood disorders, in individuals with DD.

These findings highlight the need for assessment and recognition of psychiatric symptoms in DD. The findings may also have implications for identification of other genetic factors involved in conferring susceptibility to neuropsychiatric features in individuals without DD. Further research is needed into other neuropsychiatric phenotypes in DD and into the specific functional effects of mutations in *ATP2A2* and the relationship of these to the presence of certain neuropsychiatric phenotypes.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 OVERVIEW OF THESIS

Darier's Disease (DD) is a rare, genetic skin disorder characterised by a rash mainly affecting seborrheic areas. It is caused by mutations in a single gene, *ATP2A2,* which encodes a protein involved in intracellular calcium signalling. A range of neuropsychiatric features, including depression, bipolar disorder, epilepsy and learning difficulties, have been reported to co-occur in individuals and families with DD. The psychological consequences of having a chronic skin disorder are likely to contribute to, but cannot fully explain, this association. There is evidence to suggest that mutations in *ATP2A2* could have pleiotropic effects in the skin and brain suggesting neuropsychiatric features may be part of the DD phenotype. To date there has been no systematic investigation of the neuropsychiatric phenotype in DD using standardised assessment measures.

The reported association between DD and neuropsychiatric symptoms has obvious implications for management and treatment of individuals with DD. Furthermore, investigations into the extent and causes of the relationship between neuropsychiatric features and DD may identify both genetic and non-genetic factors involved in conferring susceptibility to neuropsychiatric disorders in individuals without DD.

This thesis investigates the neuropsychiatric phenotype in DD. The three main aims are:

1. To conduct a systematic investigation of the neuropsychiatric characteristics in a sample of 100 unrelated individuals with DD using a battery of standardised neuropsychiatric measures.
2. To investigate possible genotype-phenotype correlations between the type and/or locations of pathogenic mutations detected in the *ATP2A2* gene and neuropsychiatric features observed in this sample.

3. To compare the presence of neuropsychiatric features in this sample of individuals and their first-degree relatives, unaffected by DD.

A brief overview of the thesis is given below:

Chapter 2 describes the clinical and genetic features of DD. This is followed by a review of the literature reporting the presence of neuropsychiatric features among individuals with DD. The possible explanations for the co-occurrence of neuropsychiatric features in DD are then discussed. Finally, the main aims of the thesis are outlined in further detail.

Chapters 3 to 5 are concerned with describing and discussing the investigations carried out to examine the clinical features and prevalence of neuropsychiatric features in 100 unrelated individuals with DD (Aim 1).

Chapter 6 provides a more detailed introduction to the genetics of DD, describing the single gene, *ATP2A2,* in which mutations causing DD have been found to occur and describing the protein, SERCA2, which is encoded by *ATP2A2.*

Chapters 7 to 9 describe the investigations carried out into the relationship between the type and/or location of DD causing mutations detected within *ATP2A2* and the neuropsychiatric phenotypes observed (Aim 2).

Chapters 10 to 12 describe and discuss the investigations carried out to compare the presence of neuropsychiatric features in a subset of individuals with DD and their unaffected relatives (Aim 3).

Chapter 13 summarises the key findings of the investigations, discusses the limitations and implications of these findings, and makes suggestions for further research.

2 INTRODUCTION TO DD AND REVIEW OF THE NEUROPSYCHIATRIC FEATURES REPORTED IN DD

2.1 Introduction to DD

2.1.1 DD Clinical Features

Darier's Disease (DD), also known as Darier-White Disease / keratosis follicularis (Online Mendelian Inheritance in Man (OMIM) 124200), is a rare autosomal dominantly inherited skin disorder. Estimates of prevalence range from 1 in 100 000 (Svendsen & Albrectsen, 1959) to 1 in 55 000 (Wilkinson *et al.*, 1977) to 1 in 30 000 (Tavadia *et al.*, 2002). Based on these estimates, there are between 600 and 2000 individuals in the UK with DD. The disorder is characterised by a rash of hyperkeratotic/warty papules, which can coalesce to form large plaques, predominantly present in seborrheic areas (central truck, neck, scalp, hair margins and flexures). Additional features include distinctive nail abnormalities including fragile nails with red and white lines and V-shaped notches at the free end. Pits and/or keratotic papules, which can be present on the palms or soles, are also a characteristic feature of the disease. Histological features include loss of cell-cell adhesion between cells in the epidermis (acantholysis) and abnormal, premature keratinisation of epithelial cells (dyskeratosis) with abnormal keratinocytes (called corps ronds). Age of onset is generally between 11 and 20 years of age and the disorder is equally prevalent in males and females and across all ethnic groups. The course of the disorder is characterised by remissions and relapses, which can be triggered by heat, sweating, infections, friction and sun exposure (Burge & Wilkinson, 1992). The severity of DD varies greatly between individuals including those in the same family, ranging from individuals having a few scattered papules or mild nail abnormalities to individuals with severe DD having widespread itchy, malodorous plaques. In addition to the classical form of DD described above, a small number of individuals have different clinical forms including a haemorrhagic form of the disorder, characterised by

red/black lesions on the palms and soles, and a vegetating form affecting the flexures. DD is currently clinically diagnosed by a skin biopsy and is commonly and most effectively treated with topical or oral retinoids (Cooper & Burge, 2003). Examples of classic DD affecting the back and neck are shown in Figure 2.1 and Figure 2.2 respectively. The characteristic nail fragility, ridging and splitting observed in the majority of individuals is illustrated in Figure 2.3. The haemorrhagic lesions observed on the palms of individuals with the haemorrhagic form of the disorder are illustrated in Figure 2.4.

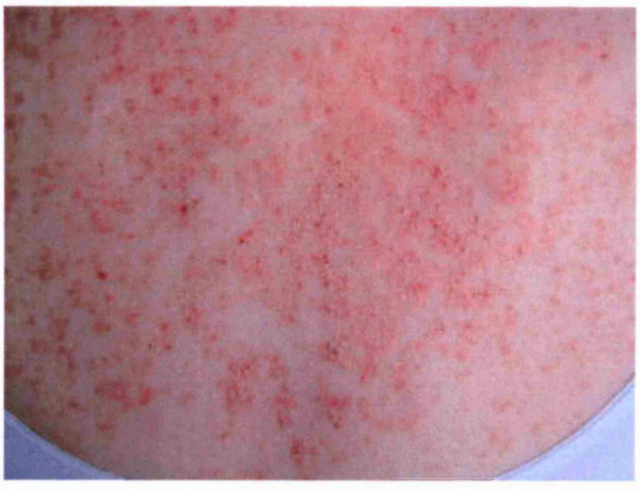

Figure 2.1 DD Affecting the Back

Photograph taken by the author of the current thesis after obtaining consent

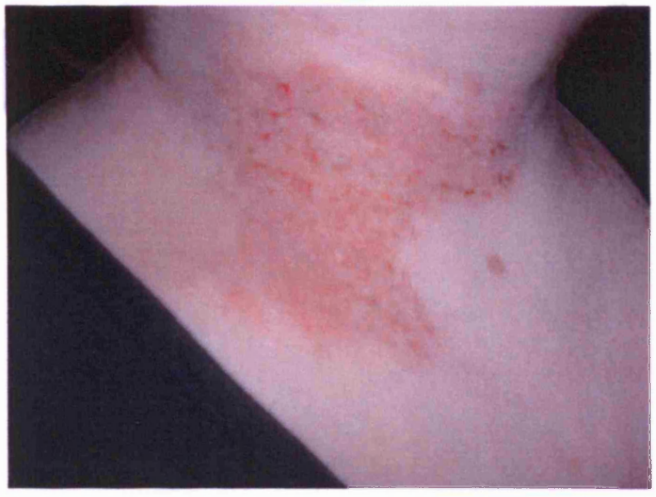

Figure 2.2 DD Affecting the Neck

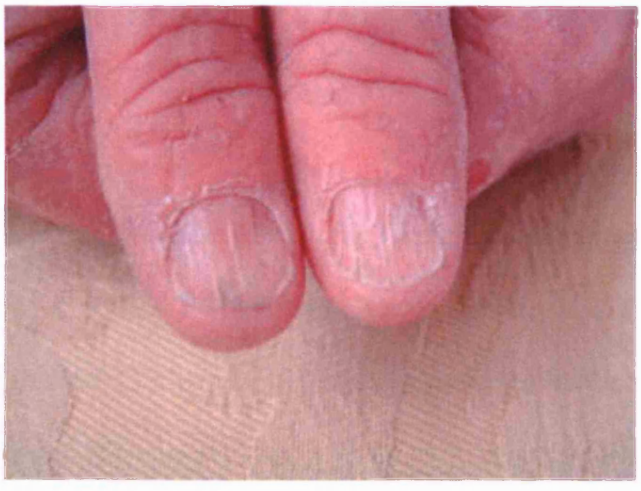

Figure 2.3 Nail Fragility, Ridging and Splitting in DD

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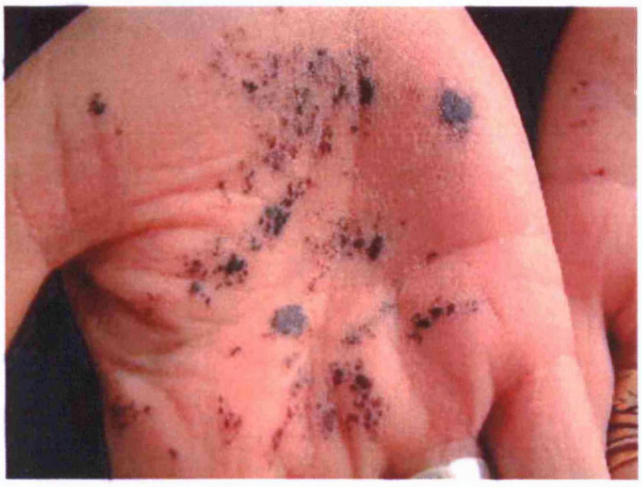


Figure 2.4 Haemorrhagic Lesions on Palms

Photograph taken by the author of the current thesis after obtaining consent

2.1.2 Genetics of DD

DD is autosomal dominantly inherited with high penetrance although phenotypic expression is variable (Munro, 1992). The disease rarely, if ever, skips a generation (Cooper & Burge, 2003) although some family members may have very mild features and be unaware that they have the condition. Most individuals with DD will report a family history and/or will be found to have relatives with mild disease (Munro, 1992) although spontaneous *de novo* mutations can arise. Linkage analysis mapped DD to chromosome 12q23-q24.1 (Bashir *et al.*, 1993; Craddock *et al.*, 1993) and the disease was subsequently shown to be caused by mutations in a single gene *ATP2A2* (Sakuntabhai *et al.*, 1999b). *ATP2A2* encodes the sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2), a calcium pump involved in intracellular calcium signalling (MacLennan *et al.*, 1997). The *ATP2A2* gene spans 21 exons and has three splice variants (a, b and c) encoding three isoforms of the SERCA2 protein namely SERCA2a, SERCA2b and SERCA2c (Gelebart *et al.*, 2003; Lytton & MacLennan,

1988; Lytton *et al.*, 1992; Verboomen *et al.*, 1994). These three isoforms differ in their end amino acid sequences (carboxyl termini) and show different tissue expression patterns, summarised in Table 2.1. Both the SERCA2a and SERCA2b isoforms have been found to be present in the epidermis although the SERCA2b isoform predominates (Ruiz-Perez *et al.*, 1999; Tavadia *et al.*, 2004).

Table 2.1 Three Isoforms of SERCA2

Isoform	Length (amino acids)	Expression
SERCA2a	997	Heart and slow-twitch skeletal muscle
SERCA2b	1042	Smooth muscle and all non-muscle tissue including the epidermis. Ubiquitously expressed -'Housekeeping isoform'
SERCA2c	999	Epithelial, mesenchymal & hematopoietic cell lines and monocytes

All three isoforms are identical up to amino acid 993.

2.1.3 The Sarco/Endoplasmic Reticulum Ca^{2+} ATPase Isoform 2 (SERCA2)-Function & Structure

The sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2) is a calcium pump located in the endoplasmic reticulum (ER) membrane. SERCA2 belongs to a family of SERCA pumps, which includes two other members namely SERCA1 and SERCA3 that are encoded by 2 different genes (Lytton *et al.*, 1992). The pump actively transports calcium ions (Ca^{2+}) against a concentration gradient from the cell cytosol into the lumen of the ER by using ATP hydrolysis. (MacLennan *et al.*, 1997), see Figure 2.5. The pump is therefore responsible for maintaining low cytostolic and high ER Ca^{2+} concentrations. The ER constitutes the main intracellular Ca^{2+} store and plays a central role in Ca^{2+} homeostasis.

The predicted secondary structure of the SERCA2 protein is based on functional studies and the published X-ray crystal structures of the SERCA1 pump (Toyoshima & Mizutani, 2004; Toyoshima *et al.*, 2000; Xu *et al.*, 2002). The structure of the SERCA2 protein and its functional domains are described in further detail in Chapter 6.

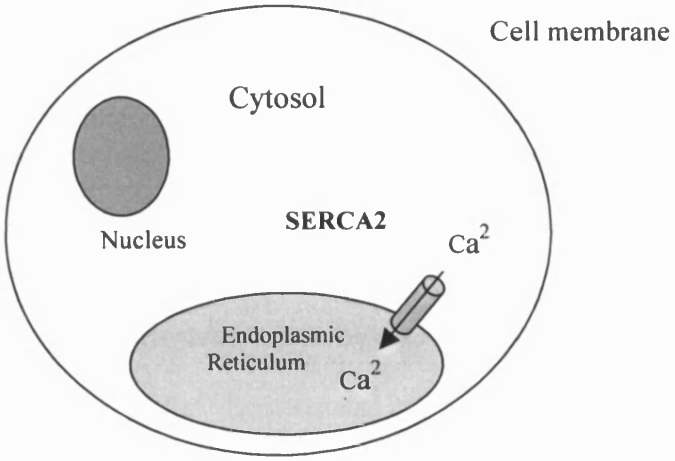


Figure 2.5 Basic Function of SERCA2

2.1.4 Dual Role of SERCA2

The SERCA2 protein plays a role both in intracellular Ca^{2+} signalling and in the synthesis and post-translational modification of proteins within the ER. These are described briefly below.

2.1.4.1 Intracellular Ca^{2+} Signalling

In response to external stimuli, Ca^{2+} ions are released from the ER. This release is counterbalanced by the SERCA2 pump actively transporting Ca^{2+} ions back into the ER. Repeated cycles of Ca^{2+} release and reuptake generate Ca^{2+} oscillations in the cytosol. These complex fluctuations in the intracellular concentration of Ca^{2+} are responsible for controlling many cellular functions including cell differentiation and proliferation and gene transcription (Berridge *et al.*, 2000).

2.1.4.2 *Protein Synthesis and Post-translational Modification of Proteins in the ER*

By maintaining high Ca^{2+} concentrations in the ER lumen, SERCA2 plays an essential role in protein synthesis and post-translational modifications of proteins. These processes, which take place in the ER and include protein folding, assembly and trafficking, require a Ca^{2+} rich environment as several of the ER resident chaperone proteins are Ca^{2+} dependent. Disruption of Ca^{2+} homeostasis in the ER leads to impaired protein synthesis, protein mis-folding and abnormal trafficking of proteins. The accumulation of unfolded proteins in the ER causes ER stress and the activation of the unfolded protein response (UPR) (Zhang & Kaufman, 2004). The UPR involves increasing the protein folding capacity of the ER and has been found to include up-regulation of SERCA2b expression (Caspersen *et al.*, 2000). In severe circumstances prolonged ER stress can lead to cell apoptosis (Schroder & Kaufman, 2005).

2.1.5 Mutations in ATP2A2 Cause DD

Mutations in *ATP2A2* have been shown to cause DD (Sakuntabhai *et al.*, 1999b). To date, 143 *ATP2A2* mutations have been reported in 175 unrelated individuals with DD in the literature (Chao *et al.*, 2002; Dhitavat *et al.*, 2003b; Dode *et al.*, 2003; Foggia *et al.*, 2006; Godic *et al.*, 2004; Ikeda *et al.*, 2003; Jacobsen *et al.*, 1999; Jones *et al.*, 2002; Onozuka *et al.*, 2004; Racz *et al.*, 2005; Racz *et al.*, 2004; Ren *et al.*, 2006; Ringpfeil *et al.*, 2001; Ruiz-Perez *et al.*, 1999; Sakuntabhai *et al.*, 1999a; Sakuntabhai *et al.*, 2000; Sakuntabhai *et al.*, 1999b; Takahashi *et al.*, 2001; Wada *et al.*, 2003; Wang *et al.*, 2006; Yang *et al.*, 2004; Yang *et al.*, 2001). These mutations, which have been found to be distributed across the gene, are described in further detail in Chapter 6.

There have been no reports in the literature of individuals with DD being homozygous for mutations in *ATP2A2* and mice studies have found that mice homozygous for null mutations

in the gene are not produced (Zhao *et al.*, 2001). Therefore as individuals with DD are heterozygous for mutations in *ATP2A2,* one normal allele continues to encode a normal SERCA2b protein. Recent functional studies looking at the effects of specific mutations in *ATP2A2* on the functioning of the SERCA2b protein have found the majority lead to a 'mutant' SERCA2b with very limited or loss of Ca^{2+} transport activity due to a lack of protein expression and/or function (Ahn *et al.*, 2003; Dode *et al.*, 2003; Miyauchi *et al.*, 2006; Sato *et al.*, 2004). This suggests that the expression of wild type pump from the single normal allele is not sufficient to provide normal function (haploinsufficiency) resulting in an increase in cytoplasmic Ca^{2+} and a decrease in ER lumenal Ca^{2+}.

The exact cause of DD remains uncertain but accumulating evidence suggests that reduced Ca^{2+} concentrations in the lumen of the ER may impair the processing and trafficking of newly synthesised proteins that are needed for normal adhesion between skin cells. This is supported by the finding that trafficking of desmoplakin, a plasma membrane protein involved in cell adhesion, is impaired in DD keratinocytes (Dhitavat *et al.*, 2003a). It has also been proposed that a further consequence of the impaired processing of proteins in DD keratinocytes could be the accumulation of misfolded proteins in the ER, leading to ER stress and the unfolded protein response (UPR). In such circumstances, DD epidermal cells would have a limited capacity to increase the level of functional SERCA2 pumps and be unable to control the UPR which, if it remained persistent, would lead to apoptosis of epidermal cells (Hovnanian, 2004). The round dyskeratotic keratinocytes (corps ronds) which are highly specific to DD have been suggested to be apoptotic keratinocytes (Foggia & Hovnanian, 2004). A recent study provided evidence for the possible role of ER stress in the pathogenesis of DD by finding when ER stress was induced in keratinocytes from an individual with DD, the cells showed an increased expression of three proteins involved in the UPR compared to normal keratinocytes from a control individual (Onozuka *et al.*, 2006). These findings suggest

that the pathological pathway from mutations in *ATP2A2* to the clinical features of DD is complex and probably involves abnormal trafficking of proteins involved in cell to cell adhesion, the UPR and the induction of apoptosis (Hovnanian, 2004).

SERCA2 is widely expressed however, the reasons why mutations in *ATP2A2* produce a disease that appears to be largely limited to the skin are still unclear. One reason could be that the relative importance of SERCA2 may vary in different tissues. SERCA2 pumps may be more critical in the epidermis than in other tissues and as a result epidermal cells may be more susceptible to a reduction in SERCA2 activity. Another explanation is that certain tissues could compensate for the loss of SERCA2 activity by the up-regulation of other members of the SERCA pump family such as SERCA3. Cells in the epidermis have been found to lack SERCA3, which may indicate a decreased ability of these cells to compensate for loss of SERCA2 function (Tavadia *et al.*, 2004). It is also possible that factors that trigger DD such as friction, sun exposure and temperature affect the skin most predominantly as it is exposed to the external environment whereas internal organs are protected.

2.2 Review of Neuropsychiatric Features in DD

The following sections provide review of the literature reporting neuropsychiatric features in individuals with DD including mood/affective disorders (including bipolar affective disorder and major depressive disorder) and psychosis, learning difficulties, epilepsy and other neurological features. Evidence for the co-occurrence of neuropsychiatric features in DD has come from a small number of case series of unrelated individuals with DD in addition to family studies and individual case reports. These studies are summarised in Table 2.2, Table 2.3 and Table 2.4 and are discussed in the following sections.

Table 2.2 Summary of Reports of Neuropsychiatric Features in DD- Cases Series

Reference	Sample	Neuropsychiatric Features
Svendsen & Albrectsen (1959)	51 cases (included 12 members of one family and 15 sporadic cases). Identified through dermatology departments across Denmark.	22 (43%) individuals described as being either 'mentally subnormal', 'mentally deranged or psychopaths' or 'destitutes'. One individual had been admitted to a psychiatric hospital with psychosis and another had committed suicide.
Medansky & Woloshin (1961)	5 cases (1 family and 2 sporadic cases) identified from a review of dermatology records at a Chicago hospital, US.	A mother and two children with neuropsychiatric abnormalities including depression, 'unrealistic thinking', 'schizophrenic delusions' and 'mild cerebral deterioration' Female described as a 'high grade mental defective with intermittent symptoms of depression' Male 'of low average intelligence, anxious and depressed'.
Denicoff *et al.* (1990)	11 cases of DD and control group of 11 individuals with skin disorders of equal severity.	Prevalence of psychiatric illness did not differ between the groups. History of suicidal thoughts was higher in the DD group (64%) than the controls (27%). Most individuals with DD reported that their suicidal ideation was not necessarily due to their DD.
Burge & Wilkinson (1992)	163 cases from 107 families recruited via dermatologists in the UK.	7 patients from 6 families had epilepsy, one of these individuals also had BPAD. 8 individuals, 2 from the same family, had learning difficulties. 2 females had been treated for depression.
Munro (1992)	75 adult cases (including 13 families with two or more cases) recruited via dermatologists in north-east England.	2 cases of learning difficulties including a female with severe learning difficulties and epilepsy of unknown cause. Individuals from 2 families had been treated for fits. One individual and her mother had required admissions to psychiatric hospitals. Neuropsychiatric features were noted to be inconsistent within families.
Ringpfeil *et al.* (2001)	50 cases from 24 families in Germany and the US.	A high incidence of depression, suicide, and suicide attempts was reported as well as individual cases of schizophrenia, seizure disorder, learning difficulties, hospitalisation for psychiatric diagnoses not otherwise specified and violent behaviour. Several suicides were reported in one family and substance abuse was a problem for a number of individuals in another family.
Racz *et al.* (2004)	Eight unrelated cases identified through a dermatology department in Hungary.	One female with learning difficulties One female with mild depression and a history of temporary losses of consciousness, she also had endocrinological symptoms. This case was described in further detail in (Racz *et al.*, 2006).
Goh *et al.* (2005)	24 unrelated cases of DD in Singapore identified through the National Skin Centre.	Neuropsychiatric disorders recorded in three patients: epilepsy, major depressive disorder and severe antisocial personality disorder.

Table 2.2 Continued

Reference	Sample	Neuropsychiatric Features
Miljkovic *et al.* (2005)	28 cases of DD (9 families and 13 sporadic cases) identified through major dermatological departments in Slovenia.	3 cases of learning difficulties, 2 patients reported to have periodic epileptic seizures and one case of affective psychosis. An impairment of memory and concentration was observed in the majority of patients.
Zeglaoui *et al.*(2005)	12 cases (from 1 family and 7 sporadic cases) identified through a dermatology department in Tunisia.	One case of learning difficulties.

Table 2.3 Summary of Reports of Neuropsychiatric Features in DD- Family Studies

Reference	Neuropsychiatric Features
Getzler & Flint (1966)	*20 family members, 3 generations*; eight out of the 12 members with DD had multiple neuropsychiatric problems including alcoholism, below average intelligence, 'schizophrenic episodes' and 'depressive psychosis'. Two out of the six unaffected family members also had neuropsychiatric problems.
Craddock *et al.* (1994b), Craddock *et al.* (1994a)	*17 family members, 3 generations;* all six family members with DD had an affective illness including bipolar disorder and major depression. All unaffected relatives did not have a history of mood disorder.
Sidenberg *et al.* (1994)	*17 family members, 2 generations:* Multiple family members with DD and schizophrenia although not always found together.
Venencie *et al.* (1996)	*4 family members, 2 generations;* both family members with DD had a slowly progressive encephalopathy. Their unaffected family members had normal clinical examinations.
Cordeiro *et al.* (2000)	*7 family members, 2 generations;* all three family members with DD had a diagnosis of depression including a male with associated mood congruent psychotic symptoms. All unaffected relatives had no history of affective illness.
Jones *et al.* (2002)	*17 family members, 4 generations*; all of the six family members with DD in addition to 4 unaffected relatives had a lifetime diagnosis of affective disorder including bipolar disorder and major depression.

Table 2.4 Summary of Reports of Neuropsychiatric Features in DD- Case Reports

Reference	Neuropsychiatric Features
Pendlebury (1964)	Female with epilepsy. Attacks of hysteria noted whilst receiving inpatient hospital treatment for DD.
Peck *et al.* (1976)	Male with a unique variant of DD, disabled by large painful cutaneous horns on all extremities. Chronically depressed with suicidal ideation.
Clark *et al.* (1986)	Female experienced her first manic episode following childbirth and a further episode 6 months later. Lithium treatment exacerbated DD.
Milton *et al.* (1990)	Female diagnosed with BPAD had also made multiple suicide attempts. Lithium treatment exacerbated DD.
Rubin (1995)	Young male diagnosed with BPAD. Lithium treatment induced symptoms of DD.
Hellwig *et al.* (1996)	Female with schizophreniform psychosis, auditory hallucinations, depression, suicidal ideation. Temporal relationship between DD and psychotic symptoms.
Jones *et al.* (1996)	Female diagnosed with BPAD. Lithium treatment exacerbated DD.
Koutroumanidis *et al.* (1998)	Male with tonic-clonic epileptic seizures.
al-Homrany *et al.* (1997)	Male with learning difficulties, epilepsy, chronic renal failure, cataract and corneal opacities.
Ehrt & Brieger (2000)	Female with diagnosed with BPAD. Lithium treatment exacerbated DD.
Lange (2000)	Male diagnosed as having a psychotic disorder. Temporal relationship observed between DD and psychotic symptoms.
Mei *et al.* (2000)	Male with Vesiculo-Bullous DD and diagnosis of BPAD.
Wang *et al.* (2002)	Male diagnosed with BPAD.
Dhitavat *et al.* (2003b)	Male with learning difficulties and depression.
Dortzbach *et al.* (2003)	Male developed DD at the age of 74 , which appeared in conjunction with other serious problems including metastatic carcinoma and an unusual progressive fibroproliferative process. Subsequently became severely depressed and committed suicide.
Chopra *et al.* (2004)	Female developed DD at the age of 46 following radiotherapy treatment for carcinoma of the cervix. This was associated with marked mood variations characterised by extreme phases of elation and depression.
Godic *et al.* (2004)	Male with a severe case of DD also "complained about learning difficulties and being moody".
Yang *et al.* (2004)	Female with an unspecified mood disorder.

Table 2.4 Continued	
Reference	**Neuropsychiatric Features**
Racz *et al.* (2005)	Female with childhood temporal epilepsy, brother with DD had been hospitalised for aggressive behaviour.
Racz *et al.* (2005)	Male with dysthymic disorder.
DD; Darier's Disease, BPAD; Bipolar Affective Disorder	

2.2.1 Mood Disorders and Psychosis

The presence of psychiatric symptoms, in particular mood disorders and suicidal ideation, in DD has frequently been reported in the literature although the prevalence of psychiatric illness in the case series studies of unrelated individuals with DD varies greatly.

Case Series - In an examination of 51 individuals with DD, identified through dermatology departments across Denmark, 22 (43%) individuals were described as being either 'mentally subnormal', 'mentally deranged or psychopaths' or 'destitutes'. More specifically one patient had been diagnosed with 'psychosis' and another had committed suicide (Svendsen & Albrectsen, 1959). Medansky & Woloshin (1961) administered a battery of psychological measures to five individuals with DD identified from a review of dermatology records at a Chicago hospital in the US. A mother, her son and daughter were described being 'extremely suspicious', 'a chronic paranoid schizophrenic' and having 'unrealistic thinking, compatible with that of an underlying schizophrenic process' respectively. A further unrelated female and male were described as having 'intermittent symptoms of depression' and being ' anxious and depressed' respectively. More recently, larger studies of individuals with DD have reported small numbers of psychiatric illness. Out of 163 individuals with DD in the UK studied by two dermatologists, Burge & Wilkinson (1992), two individuals had been treated for depression and another had a diagnosis of bipolar affective disorder (BPAD). Similarly, of 75 individuals with DD living in the north-east of England interviewed by a dermatologist, one individual and an additional affected family

member had been admitted to a psychiatric hospital (Munro, 1992). In a study of 24 unrelated individuals with DD in Singapore, one individual had major depressive disorder and another is reported as having an antisocial personality disorder (Goh *et al.*, 2005). Racz *et al.* (2004) studied eight unrelated cases of DD recruited through a dermatology department in Hungary with one female being reported as having mild depression. One case of affective psychosis was found in an examination of 28 cases of DD in Slovenia (Miljkovic *et al.*, 2005). In contrast, Ringpfeil *et al.* (2001) assessed 50 individuals with DD from 24 German and US families and noted a high incidence of depression, suicide, and suicide attempts. Individual cases of schizophrenia, hospitalisation for psychiatric diagnoses not otherwise specified and hospitalisation for violent behaviour were also reported. Several suicides were reported in one family and substance abuse was a problem for a number of individuals in another family.

Denicoff *et al.* (1990) compared the psychiatric histories of 11 patients with severe DD and a control group of 11 individuals with other skin disorders of equal severity. Prevalence of psychiatric illness did not differ between the groups. Four individuals with DD had a psychiatric diagnosis including one individual with BPAD, two with depression and one individual with an adjustment disorder. In the control group two individuals had a history of major depression and another had a history of an adjustment disorder with depressed mood. History of suicidal ideation was higher in the DD group (64%) than the controls (27%), which was thought to be clinically significant. Although the study did not examine the relationship between DD severity and the presence of suicidal ideation, most individuals did not relate their suicidal ideation to their DD. However, the authors noted that DD might indirectly lead to suicidal ideation by causing problems such as job and relationship difficulties.

Family Studies – Several families have been reported in which there is co-occurrence of mood spectrum disorders and DD. Getzler & Flint (1966) studied three generations of a large family with multiple family members with DD. Information was obtained regarding 20 family

members, and the authors reported eight out of the 12 family members with DD had multiple neuropsychiatric features including alcoholism, 'schizophrenic episodes' and 'depressive psychosis'. However, two of the six unaffected family members studied, including the mother of the second generation, had also been admitted to 'an institution'. Sidenberg *et al.* (1994) identified a large Canadian family with DD during an ongoing linkage study of schizophrenia. Of the 17 family members that were studied, multiple members were found to have DD and schizophrenia although these were not always found together. The authors did not report the proportion of individuals with and without DD who also had a diagnosis of schizophrenia. Cordeiro *et al.* (2000) report on a family in which three family members with DD had a diagnosis of depression, which in one case was associated with mood congruent auditory hallucinations. Four unaffected relatives did not have any history of affective illness.

Craddock *et al.* (1994b), from our research group, administered a standardised psychiatric interview to available adult members of three generations of a family with DD in the UK. All five family members with DD also had a lifetime diagnosis of major affective illness according to two sets of psychiatric diagnostic criteria, including one family member with a diagnosis of BPAD (see Figure 2.6a). All unaffected relatives did not have a history of mood disorder. At follow-up an unaffected family member displayed very mild features of DD, which they had never been aware of (Craddock *et al.*, 1994a), the individual later developed a severe mood disorder (Green *et al.*, 2005). The authors note that it is unlikely that the co-occurrence seen in this family was due to chance or a psychological reaction to having DD since individuals did not relate their mood symptoms to their skin disorder. This is particularly highlighted by the individual found to have mild features of DD at follow-up who subsequently developed a mood disorder despite being unaware that they had DD. In addition, there was no temporal relationship between worsening of skin symptoms and the symptoms of affective illness. Jones *et al.* (2002), also from our research group, used a modified

standardised psychiatric interview to assess 17 members from four generations of one family with DD. Ten family members, including six individuals with DD, had a lifetime diagnosis of affective disorder according to standard diagnostic criteria, including BPAD spectrum illness and major depressive disorder, shown in Figure 2.6b.

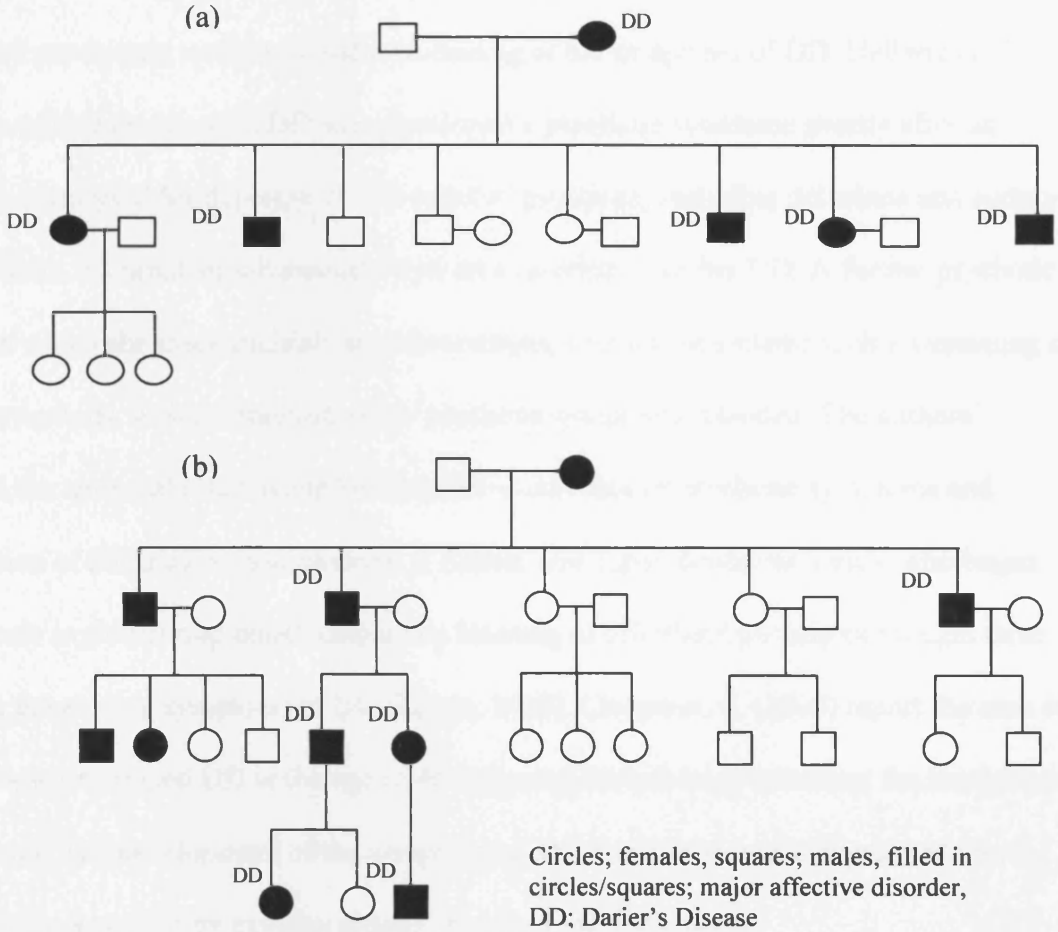


Figure 2.6 Two Families Displaying Co-occurrence of Major Affective Disorder and DD Investigated by our Research Group. Figure a; Craddock *et al.* (1994a), Figure b; Jones *et al.*, (2002)

Case Reports - In the literature there are reports of individuals with DD also having mood disorders including depression (Dhitavat *et al.*, 2003b; Dortzbach *et al.*, 2003; Peck *et al.*, 1976; Yang *et al.*, 2004), dysthymic disorder (Racz *et al.*, 2005) and BPAD (Clark *et al.*, 1986; Ehrt & Brieger, 2000; Jones *et al.*, 1996; Mei *et al.*, 2000; Milton *et al.*, 1990; Rubin, 1995; Wang *et al.*, 2002). Treatment with lithium for BPAD has been reported to exacerbate (Burge & Wilkinson, 1992; Clark *et al.*, 1986; Ehrt & Brieger, 2000; Milton *et al.*, 1990) and induce (Rubin, 1995) the symptoms of DD. In the case of one male reported to be

chronically depressed with suicidal ideation (Peck *et al.*, 1976) and another male who committed suicide (Dortzbach *et al.*, 2003), their psychiatric symptoms are reported as being a psychological reaction to the severity of the symptoms of DD.

Further case reports have suggested a possible temporal relationship between the presence of psychiatric symptoms and a worsening of the symptoms of DD. Hellwig *et al.* (1996) describe a female with DD who developed a psychotic syndrome shortly after an admission to hospital for depression. The onset of psychosis, including delusions and auditory hallucinations, occurred simultaneously with an exacerbation of her DD. A further psychotic relapse, in which she made multiple suicide attempts, was also associated with a worsening of her DD symptoms, which improved as her psychotic symptoms subsided. The authors suggested the temporal relationship between the occurrence of psychotic symptoms and exacerbation of DD may be non-random. A further case report describes a male who began 'talking only in riddles and puns', displaying blunting of affect and poverty of thought three days after developing symptoms of DD (Lange, 2000). Chopra *et al.* (2004) report the case of a female who developed DD at the age of 46 following radiotherapy treatment for carcinoma of the cervix. The development of the symptoms of DD was associated with marked mood variations characterised by extreme phases of elation and depression.

2.2.2 Learning Difficulties

Case Series - Learning difficulties (LD) were frequently noted among individuals with DD in early studies of unrelated individuals with DD in the literature although the criteria used were not specified (Medansky & Woloshin, 1961; Svendsen & Albrectsen, 1959). Svendsen & Albrectsen (1959) suggested that individuals with DD differed from 'normal' in terms of their mental development and described 22 (43%) of the 51 individuals examined as being either 'mentally subnormal', 'mentally deranged or psychopaths' or 'destitutes'. Three unrelated individuals out of the five assessed by Medansky & Woloshin (1961) were

described as being 'a borderline mental defective', 'a high grade mental defective' and 'of low average intelligence' respectively. In contrast, during a thorough examination of 163 patients from 107 unrelated families with DD, only eight patients (two from the same family) were found to have learning difficulties (Burge & Wilkinson, 1992). In another recent examination of 75 cases of DD there were only two reports of learning difficulties (Munro, 1992). Of the 50 individuals from 24 families with DD examined by Ringpfeil *et al* (2001), one individual with mild DD had learning difficulties. Higher frequencies of LD in individuals with DD have been found in more recent studies, one individual was found to have LD out of 12 patients with DD in Tunisia (Zeglaoui *et al.*, 2005) and one case was reported in a study of eight cases of DD in Hungary (Racz *et al.*, 2004). In an examination of 28 individuals from 22 families with DD in Slovenia by Miljkovic *et al.* (2005) three patients were reported to have learning difficulties and the majority of individuals were found to have an impairment of memory or concentration although it is not reported how these features were measured. Evaluation of educational level in this sample revealed 61% had only finished a primary or vocational school.

Family Studies- Getzler & Flint (1966) reported below average intelligence in several of the individuals with DD in the family they investigated. Venencie *et al.* (1996) describe two siblings with DD and a slowly progressive encephalopathy including moderate learning difficulties.

Case Reports- Further reports of individuals with DD also having learning difficulties are reported in the literature (al-Homrany *et al.*, 1997; Dhitavat *et al.*, 2003b; Godic *et al.*, 2004). In a few cases individuals with DD and LD have been found to have additional neurological abnormalities and psychiatric symptoms. The three individuals described by Medansky & Woloshin (1961) as being a "borderline mental defective", "a high grade mental defective" and "of low average intelligence" all had additional psychiatric symptoms including

"unrealistic thinking compatible with that of an underlying schizophrenic process", anxiety and depression. The sample collected by Munro (1992) included a woman with learning difficulties in addition to epilepsy of an unknown cause. One individual with LD and DD interviewed by Burge & Wilkinson (1992) also had spinocerebellar dysfunction, pes cavus and scoliosis and another had congenital hydronephrosis, partial spina bifida and hemitransitional vertebrae. In the large family reported by Getzler & Flint (1966), two family members described as having below average intelligence were also described as being "emotionally labile" and having "schizophrenic episodes" respectively. The two siblings described by Venencie *et al.* (1996) are described as having LD in addition to a slowly progressive encephalopathy. In addition to LD and DD, the patient described by al-Homrany *et al.* (1997) had multiple additional problems including chronic renal failure, epilepsy, cataract and corneal opacities. Two further males in the literature reported to have DD and LD are also reported to have depression (Dhitavat *et al.*, 2003b) and complaints of "being moody" (Godic *et al.*, 2004). Together these studies suggest that severe LD is present in a minority of individuals and/or families with DD and in some cases may be associated with additional complications. Whether mild LD or problems in particular areas of learning, such as memory or concentration affects a larger proportion of individuals with DD, as suggested by Miljkovic *et al.* (2005), remains unclear.

2.2.3 Epilepsy and Other Neurological Features

Case Series- In the data collected from 163 patients with DD by Burge & Wilkinson (1992), seven individuals from six families were found to have epilepsy. The authors highlighted that the prevalence of epilepsy in the sample (42.9 / 1000) was higher than that for the general population (7 / 1000) (Wade & Hewer, 1987). The other case series studies of unrelated individuals with DD found cases of epilepsy in their samples in varying small frequencies. Of 75 individuals interviewed by Munro (1992), epilepsy of an unknown cause

was found in one patient with severe learning difficulties and individuals from two further families had been treated for fits. Individuals in one of the 24 families examined by Ringpfeil *et al.* (2001) were found to have a range of neuropsychiatric problems including depression, schizophrenia and a case of seizure disorder. One case of epilepsy was found in 24 unrelated cases of DD in Singapore (Goh *et al.*, 2005). Two of the 28 individuals examined by Miljkovic *et al.* (2005) were reported to have periodic epileptic seizures.

Case Reports- Further case reports in the literature have described individuals with DD also having epilepsy (al-Homrany *et al.*, 1997; Koutroumanidis *et al.*, 1998; Pendlebury, 1964; Racz *et al.*, 2005). Pendlebury (1964) described a female with DD and grand mal epilepsy who is also described as having attacks of hysteria whilst receiving hospital treatment for her DD. One member of a Hungarian family with DD was found to have temporal childhood epilepsy and her brother with DD had been hospitalised for aggressive behaviour (Racz *et al.*, 2005). As previously reported, the individual with DD and epilepsy described by al-Homrany *et al.* (1997) also suffered from learning difficulties, chronic renal failure, cataract and corneal opacities.

Studies of unrelated individuals with DD suggest the prevalence of epilepsy may be higher in DD than in the general population, but is not a common feature of the disease. In a small number of cases, individuals with DD and epilepsy may have additional neuropsychiatric features.

2.2.4 Summary of Review of Neuropsychiatric Features in DD

This review of the literature indicates that a range of neuropsychiatric features including psychiatric illness, epilepsy and learning difficulties are consistently reported in case series studies of unrelated individuals with DD. However, the reported prevalence rates of neuropsychiatric features in these studies vary greatly. This variation is likely to be a result of methodological differences in the measurement and/or reporting of such features in these

studies. Studies reporting additional neuropsychiatric features in a relatively small proportion of individuals have tended to report more severe features requiring treatment (Burge & Wilkinson, 1992; Munro, 1992). In contrast, case series studies reporting a much higher prevalence have tended to include reports of milder features such as mild learning difficulties (Miljkovic *et al.*, 2005) and depression (Ringpfeil *et al.*, 2001). Studies reporting neuropsychiatric features in families with multiple family members with DD have consistently reported a greater prevalence of neuropsychiatric features in family members with DD than family members without DD, including families with complete co-segregation of DD and neuropsychiatric features (Cordeiro *et al.*, 2000; Craddock *et al.*, 1994a; Venencie *et al.*, 1996). The small number of case reports in the literature highlight the range of neuropsychiatric features found to co-occur in individuals with DD. Of particular interest are the reports of individuals where there has been a temporal relationship between the presence of severe psychiatric symptoms and a worsening of the symptoms of DD. The next section discusses the possible explanations for the co-occurrence of neuropsychiatric features in DD.

2.3 Explanations for the presence of Neuropsychiatric Features in DD

There are several possible explanations for reported co-occurrence of neuropsychiatric abnormalities in DD including a chance association, psychosocial factors, genetic linkage between DD and a susceptibility gene for neuropsychiatric illness and pleiotropic effects of mutations in *ATP2A2* in the skin and brain. These are discussed below.

2.3.1 Chance Relationship

The first possibility is a chance co-occurrence between DD and neuropsychiatric features. The most common neuropsychiatric features that have been reported to co-occur with DD are mood disorders and mild learning difficulties, which are also relatively common

in the general population. It is therefore possible that the many reports of a co-occurrence of DD and neuropsychiatric features are due to chance and/or ascertainment bias. However, there are a large number of reports of neuropsychiatric features among individuals with DD in the literature and the reports of families where multiple family members with DD have additional neuropsychiatric symptoms provide evidence against a chance relationship.

2.3.2 Psychosocial Effects of Skin Disease

The psychological consequences of having a chronic skin disorder are likely to contribute to the reported association between DD and neuropsychiatric features, in particular symptoms of depression. Onset of the symptoms of DD during adolescence could also lead to greater psychosocial impairments than might be expected if the symptoms started later in adult life. The perceived impact the disorder has on various aspects of everyday functioning, rather than the severity of DD, may be related to the presence of depressive symptoms. This is supported by Harris *et al.* (1996) who found a poor correlation between patient self-rated scores on the Dermatology Life Quality Index (DLQI) and clinicians' ratings of DD severity. This indicates that for some individuals a mild form of the disease may cause severe impairment. In addition, the onset of a skin disorder at a young age may affect performance at school and could contribute to the reports of poor school performance in individuals with DD.

Denicoff *et al.* (1990) reported that of the 64% individuals with DD in their study who had a history of suicidal thoughts, most did not relate their suicidal ideation to their DD. Similarly the family members with mood disorders and DD interviewed by Craddock *et al.* (1994b) did not relate their mood symptoms to their skin disorder and one family member with very mild features of DD which they were never aware of, later developed a severe mood disorder (Green *et al.*, 2005). Also, anecdotally, dermatologists report that DD patients in general differ in terms of their behaviour from their other patients with similar dermatological morbidity. These findings suggest that for a proportion of individuals with DD the additional

presence of mood disorders and suicidal ideation may be due to factors other than a psychological reaction to having a chronic skin disorder.

2.3.3 Genetic Linkage

Genetic linkage between DD and a susceptibility gene for affective illness has been proposed as an explanation for the reported association between DD and affective illness. Recent genome-wide scans have provided evidence for the presence of a susceptibility locus for affective disorders on chromosome 12 in the region of the *ATP2A2* gene including bipolar disorder (Shink *et al.*, 2005) and major depressive disorder (Abkevich *et al.*, 2003; McGuffin *et al.*, 2005). Genetic linkage is a possibility in families where DD co-segregates with affective illness however, it cannot explain the majority of the reported associations between DD, affective disorders and other neuropsychiatric phenotypes. This is because most DD mutations are specific to families and therefore the majority would not be in linkage disequilibrium (very closely linked) with a variant that predisposes to susceptibility to mood disorders.

2.3.4 Pleiotropic Effects of Mutations in *ATP2A2* in the Skin and Brain

A further explanation is that mutations in the *ATP2A2* gene itself, in addition to causing DD, confer susceptibility to neuropsychiatric features (pleiotropy). This is supported by the fact that SERCA2b has been found to be highly expressed in the brain (Baba-Aissa *et al.*, 1998). The role of intracellular Ca^{2+} signalling in a range of neuronal functions including neuronal excitability, neurotransmitter release, gene expression neuronal growth and synaptic plasticity (Berridge, 2002; Berridge *et al.*, 1998; Verkhratsky, 2005) also supports this suggestion.

Furthermore, there is evidence suggesting the role of ER function in the pathophysiology and treatment of affective disorders. A recent study has found that ER stress responses were

impaired in B lymphocyte cell lines from a small sample of individuals with bipolar disorder compared to controls (So *et al.*, 2007). In addition, in primary cultured rat cerebral cortical cells, two mood stabilizing drugs, lithium and valproate have been shown to regulate the expression of proteins involved in the ER stress response (Shao *et al.*, 2006). As previously reported, the SERCA2 protein is located in the ER membrane and has been implicated to be involved in ER stress pathways (Caspersen *et al.*, 2000). The finding that ER stress pathways have also been suggested to be disrupted in bipolar affective disorder and are a potential target of pharmacological agents used to treat mood disorders, further supports the suggestion that mutations in *ATP2A2* may be involved in conferring susceptibility to neuropsychiatric features, in particular mood disorders, in individuals with DD.

Interestingly, another disorder reported to have associated neuropsychiatric features, Wolfram Syndrome, has been found to be caused by mutations in a single gene encoding a protein located in the ER membrane (WFS1) (Strom *et al.*, 1998; Takeda *et al.*, 2001). This protein, expressed in various tissues including the brain and pancreas, has recently been indicated to play a role in regulating cellular Ca^{2+} homeostasis, in part by modulating the filling state of the ER Ca^{2+} store (Takei *et al.*, 2006). WFS1 deficiency has also been shown to increase ER stress in pancreatic β-cells (Yamada *et al.*, 2006). Wolfram Syndrome is a rare autosomal-recessive disorder characterised by juvenile onset diabetes mellitus and optic atrophy. Individuals with Wolfram Syndrome have been described as having a diverse range of neuropsychiatric abnormalities including severe psychiatric symptoms (Swift *et al.*, 1990), learning difficulties and seizures (Kinsley *et al.*, 1995). In one study of 68 individuals with the disorder, 60% of individuals were found to have severe psychiatric symptoms including severe depression, psychosis, organic brain syndrome or impulsive aggression with 25% of individuals requiring an admission to a psychiatric hospital and/or making a suicide attempt (Swift *et al.*, 1990). Reports of a high prevalence of neuropsychiatric features among

individuals with a disorder caused by mutations in a gene encoding a protein located in the ER membrane with a very similar function to SERCA2, also provides support for the hypothesis that mutations in the *ATP2A2* gene itself, in addition to causing DD, confer susceptibility to neuropsychiatric features.

The pleiotropy hypothesis suggests that mutations in *ATP2A2* could confer susceptibility to a wide range of different neuropsychiatric features including major depressive disorder, bipolar affective disorder, psychotic episodes, schizophrenia, epilepsy and learning difficulties that might be expected to have different aetiologies. However, there is evidence that a number of these neuropsychiatric phenotypes have overlapping pathogenic mechanisms. For example, first-degree relatives of individuals with bipolar affective disorder appear to have an increased risk of developing both bipolar affective disorder and major depressive disorder suggesting the two disorders have shared susceptibility factors (McGuffin & Katz, 1989). Similarly, family studies have suggested the presence of overlapping susceptibility factors in affective disorders and schizophrenia including the finding of high rates of major depressive disorder in the relatives of individuals with schizophrenia (Maier *et al.*, 1993). The prevalence of schizophrenia (Turner, 1989) and mood disorders (Hurley, 2006) have also been reported as increased in individuals with learning difficulties A review of studies assessing the type and prevalence of psychiatric disorders in individuals with epilepsy found that reports of the lifetime prevalence of affective disorders in individuals with epilepsy ranged from 24% to 72% (Gaitatzis *et al.*, 2004). In addition, family history of depression is frequently observed among individuals with both epilepsy and depression (Kanner & Balabanov, 2002).

Further support for mutations in *ATP2A2* having pleiotropic effects in both the skin and brain would come from a population-level association between neuropsychiatric features and DD. As discussed above, such an association has not been consistently or robustly found in

the case series studies of unrelated individuals with DD. However, these studies have had methodological problems and small sample sizes. Previous case series of DD have been undertaken by dermatologists untrained in neuropsychiatric assessment and have been mainly based on recording individuals' general medical history and treatment. Systematic investigations using standardised measures of neuropsychiatric features in large samples of individuals with DD are required to confirm and determine the extent of the relationship between DD and neuropsychiatric features.

Genotype-phenotype correlations between the types and/or locations of mutations within the *ATP2A2* gene and the presence of neuropsychiatric phenotypes would also provide support for the pleiotropic hypothesis and one study has reported such a correlation (Jacobsen *et al.*, 1999). This study, and others investigating genotype-phenotype correlations in DD are described in further detail in Chapter 6.

The aims of this thesis are to address these two questions by conducting a systematic investigation of the neuropsychiatric phenotypes in a large sample of individuals with DD, and to look for correlations between the neuropsychiatric phenotypes observed and *ATP2A2* mutations within this sample. The three main aims of this thesis are:

1. To conduct a systematic investigation of the neuropsychiatric characteristics in a sample of 100 unrelated individuals with DD using a battery of standardised neuropsychiatric measures. *This will include investigations of possible relationships between the clinical features of DD, such as severity and age of onset, and neuropsychiatric features observed.* (Chapters 3 to 5).
2. To investigate possible genotype-phenotype correlations between the type and/or locations of pathogenic mutations detected in the *ATP2A2* gene and neuropsychiatric features observed in a large sample of unrelated individuals with DD. *This will include investigations of*

possible relationships between the mutations and type and severity of neuropsychiatric phenotypes. (Chapters 6-9).

3. To compare the presence of neuropsychiatric features in a sample of individuals with DD and their first-degree relatives, unaffected by DD. *This method will allow controlling for additional genetic and non-genetic factors that could influence the presence of neuropsychiatric features in individuals with DD.* (Chapters 10-12).

The following chapter (Chapter 3) describes the recruitment and clinical and neuropsychiatric assessment of 100 unrelated index participants with DD.

3 DEMOGRAPHICS, CLINICAL AND NEUROPSYCHIATRIC FEATURES IN 100 INDIVIDUALS WITH DD: METHODS

This chapter describes the recruitment and clinical and neuropsychiatric assessment of 100 unrelated index participants with DD. First, the systematic and non-systematic approaches to recruiting study participants are described. This is followed by a description of the clinical and neuropsychiatric assessment of participants, which included an assessment of demographic characteristics, clinical features of DD and impact on quality of life, lifetime neuropsychiatric features, IQ and family history of neuropsychiatric features. Finally a summary is provided of the analyses carried out to investigate possible relationships between the clinical features of DD and the neuropsychiatric features observed. The study was approved by the Multi-Centre Research Ethics Committee for Wales (MREC) Reference: 03/9/08. Additional information relating to this chapter can be found in section A of the Appendix starting at pg. 276, and will be referenced throughout the chapter.

3.1 Recruitment of Participants

The recruitment of participants took place between January 2004 and May 2006. In order to assess the neuropsychiatric features in a representative sample of individuals with DD, participants were recruited on the basis of having DD mainly through dermatologists and a UK support group for DD. Both systematic and non-systematic approaches were used to recruit participants, described below.

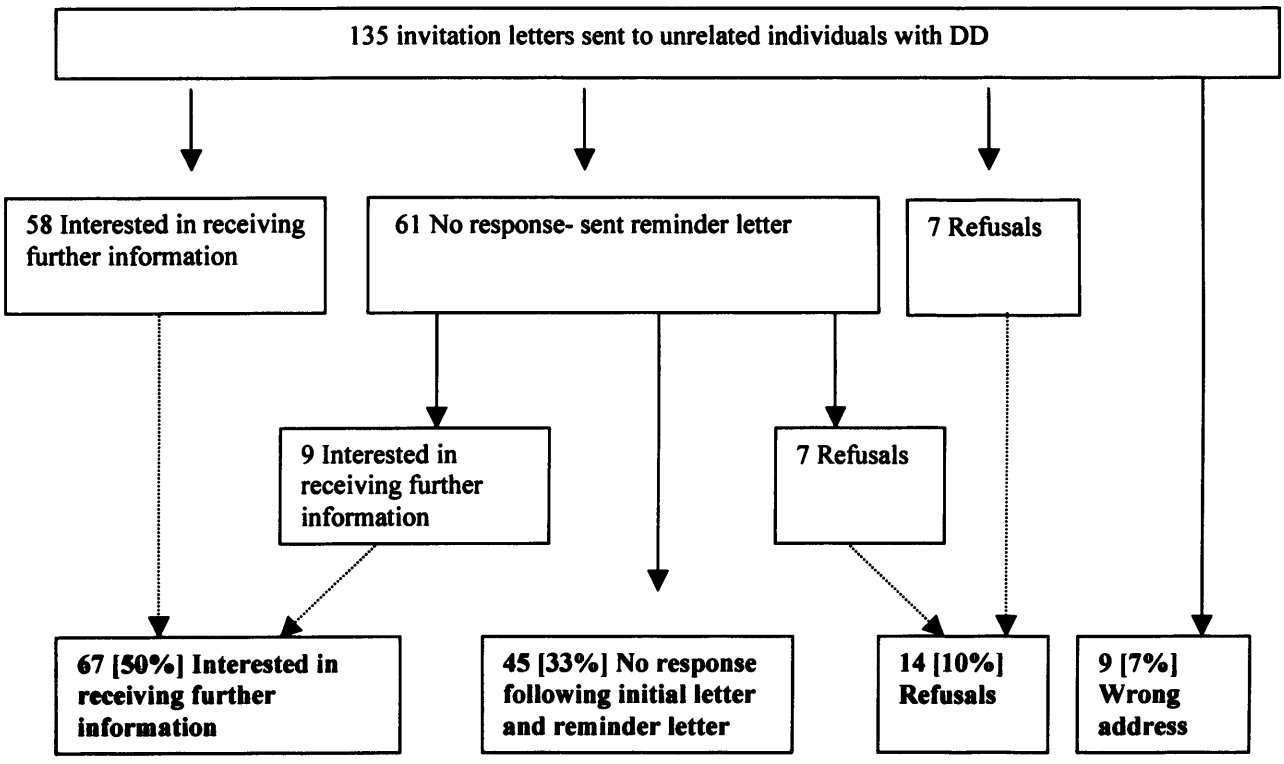
3.1.1 Systematic Approaches to Recruiting Participants

The main systematic recruitment of participants was achieved with the help of a Consultant Dermatologist, Dr Susan Burge (Dermatology Department, Churchill Hospital, Oxford). Dr Burge has extensively researched DD including conducting a study investigating the clinical features of 163 individuals with DD in the UK (Burge & Wilkinson, 1992). Dr

Burge is in contact clinically with a large number of individuals with DD in the UK and wrote to those individuals that she was in recent clinical contact with asking if they would be willing to participate in the current study. Invitation letters were sent to 135 individuals between the ages of 18 and 75 years (51 males (38%) and 84 females (62%)). The letter provided brief details about the research and asked if individuals may be willing to take part (shown in Appendix A.i. pg. 276). Individuals who were interested in receiving further information about the study were asked to complete an attached contact form, to be returned in the stamped addressed envelope enclosed with the letter. Individuals were given the opportunity to refuse by ticking a box on the form indicating that they did not wish to take part in the research.

The numbers of individuals responding to the invitation letter are summarised in Figure 3.1. After one month a reminder letter was sent to individuals who had not responded to the initial invitation letter. In total, 67 individuals (50%) replied indicating that they would be interested in receiving further information about taking part in the study. 14 individuals (10%) refused. The address details were incorrect for 9 individuals (7%). The remaining 45 individuals (33%) did not respond to either the initial or reminder invitation letter.

Figure 3.1 Systematic Recruitment of Index Participants with DD via Dr. Burge



Individuals were also systematically recruited with the help of Dr Sherine Tavadia and Professor Colin Munro (Department of Dermatology, Southern General Hospital, Glasgow). Dr Tavadia and Professor Munro have also carried out extensive research into DD including a survey of the prevalence of the disorder in the west of Scotland (Tavadia *et al.*, 2002). They sent 20 invitation letters, identical to the letter sent by Dr Burge, to individuals with DD with whom they were currently in contact. Three individuals (15%) replied indicating that they would be interested in receiving further information about taking part in the study.

3.1.2 Non-Systematic Approaches to Recruiting Participants

The non-systematic approaches to recruiting individuals included approaching members of the UK Darier's Support Group, contacting dermatologists in the Midlands Area, a study webpage and the referral of a small number of individuals from medical disciplines other than dermatology. The main non-systematic recruitment of index participants was achieved by contacting members of the DD Support Group, which is currently run by Mrs Jenny Davies. Individuals usually contact the group after being given the details by their dermatologist, seeing a poster in a dermatology waiting room or finding the details on the internet. The current role of the support group is to provide individuals with further information about DD in the form of information leaflets and answering queries over the telephone and email. Mrs Davies sent out 60 letters to members of the support group with information about the research. Individuals who were interested in receiving further information about the study were asked to complete an attached contact form, which could be returned in the stamped addressed envelope enclosed with the letter. A copy of the letter is shown in Appendix A.i. pg. 277). Letters were not sent to members of the support group who had already expressed an interest in the research after receiving an invitation letter from Dr Susan Burge.

A further non-systematic approach to recruiting participants was achieved by writing to dermatologists in the Midlands area asking whether they had any patients with DD who they would be willing to write to and provide information about the research. Willing dermatologists were emailed the standard template invitation letter (Appendix A.i. pg 276). Eight individuals receiving letters from their dermatologist replied indicating they would be interested in receiving further information about taking part in the study.

Three individuals expressed an interest in the research after reading about the research on the internet. The information contained on the website is shown in Appendix A.i. pg. 278. A further three individuals were recruited for the study from three other disciplines namely medical genetics, neurology/learning disability service and psychiatry. It should be noted that the individual who was recruited from psychiatry was a family member of an individual who received an invitation letter from Dr Burge. Therefore this family/individual could have potentially been recruited systematically on the basis of having DD rather than being treated for a psychiatric condition.

A summary of the numbers of individuals expressing an interest in the research according to method of recruitment is displayed in Table 3.1.

Table 3.1 Frequencies of Individuals Expressing an Interest in the Research according to Method of Recruitment

Method Of Recruitment	Frequency of individuals	%
Dr Susan Burge, Oxford	67	58
Support Group	32	26
Dermatologists in Midlands Area	8	7
Internet	3	3
Professor Colin Munro and Dr Sherine Tavadia, Glasgow	3	3
Referral from other medical disciplines	3	3
Total	**116**	**100**

Individuals expressing an interest in the research were sent a letter from myself along with a detailed participant information sheet with further details about the study and what

taking part would involve (shown in Appendix A.i. pg. 279). The letter also informed individuals that they would be contacted by telephone around one week after they had received the letter to answer any questions they may have and to discuss whether they would be interested in taking part in the research. During the telephone conservation individuals were briefly screened to check that they had been diagnosed with DD by a dermatologist and were over 18. Individuals were asked if they had any further questions about the research before being asked if they would be willing to participate. Once individuals had said they would be willing to participate, a convenient time and place for an interview to take place was arranged. In all cases participants were visited in their own homes.

A summary of the frequencies of individuals agreeing to participate in the research and the reasons for individuals not participating are summarised in Table 3.2. Of the 116 individuals initially expressing an interest in the research 102 individuals agreed to participate and were visited in their homes. A further two individuals were willing to participate in the research but unfortunately due to their location (North Scotland) it was not possible for them to participate. Five individuals who initially expressed an interest in the study could not be contacted by telephone to discuss participating in the study. One individual became too physically unwell, not related to their DD, to be able to participate in the study and six individuals decided that they would not like to take part.

Table 3.2 Frequencies of Individuals Agreeing to Participate in the Research and Reasons for Individuals not Participating

	Frequency
Participated in study	102
Unable to participate due to location (N. Scotland)	2
Unable to contact via telephone to discuss participating in the research	5
Became physically too unwell	1
Decided not to participate	6
Total	**116**

102 unrelated individuals with a diagnosis of DD participated in the research. Two of these individuals had to be excluded from the study. One male participant who had previously been told he might have DD actually had a diagnosis of another skin disorder, Hailey-Hailey disease, and did not have DD. This diagnosis was confirmed by his dermatologist. A further male participant was excluded due to the limited amount of information that was obtained during his interview. The remaining 100 individuals were included in the study.

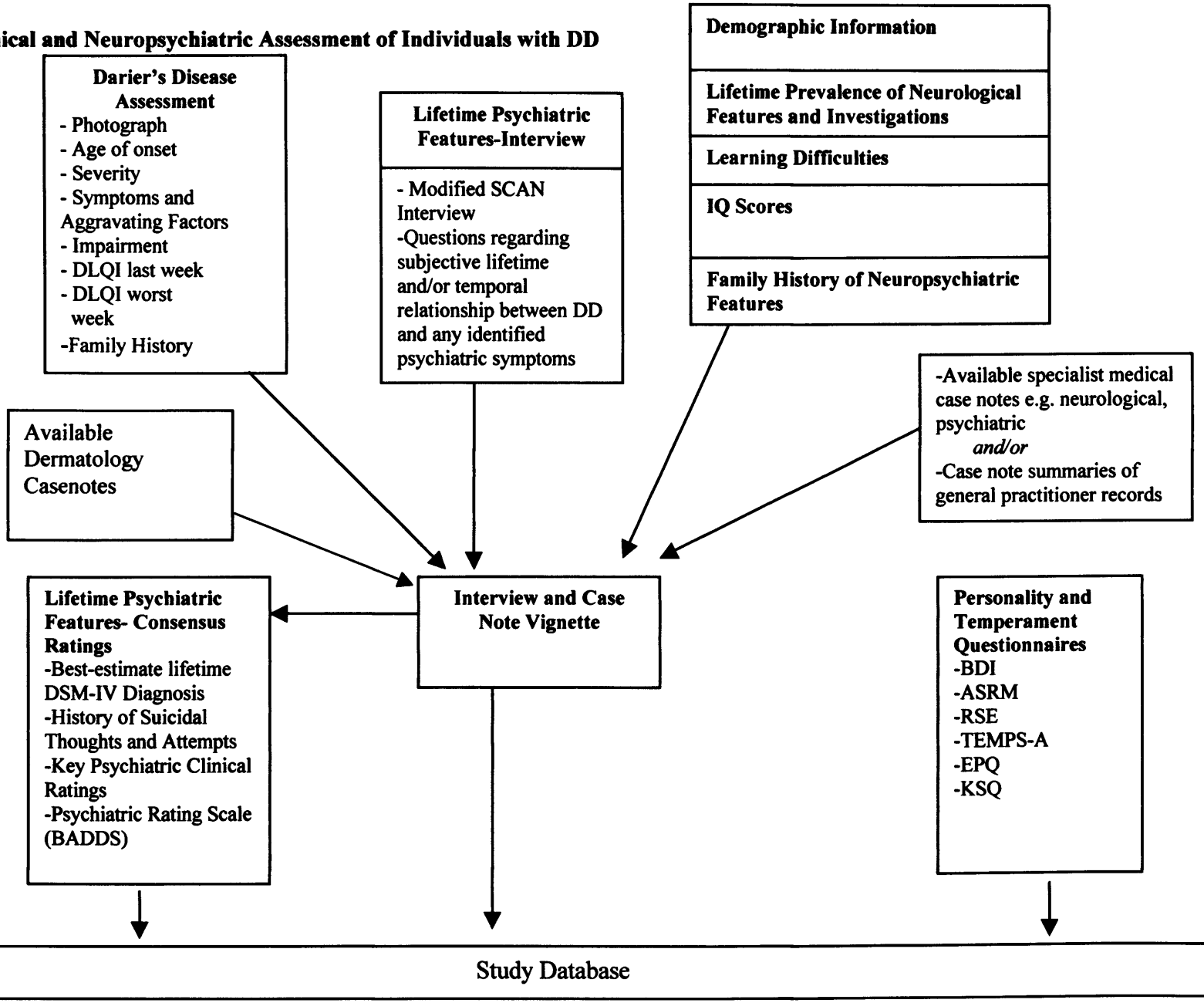
3.2 Clinical & Neuropsychiatric Assessment of Participants

The following section describes the clinical and neuropsychiatric assessment of the 100 index participants with DD. I interviewed all individuals in their homes in one session lasting approximately 2-2 ½ hours. All participants gave written informed consent (see Appendix A.i. pg. 281 for copy of the consent form). A summary of the clinical and neuropsychiatric assessment carried out with 100 unrelated individuals with DD is displayed in Figure 3.2 pg. 36. All data were entered into a relational Microsoft Windows Access Database that I designed.

3.2.1 Demographic Characteristics of the Sample

Information was collected on a selection of demographic variables including current age, gender, marital status, current social circumstances, main lifetime occupation and highest level of educational qualifications.

Figure 3.2 Clinical and Neuropsychiatric Assessment of Individuals with DD



DLQI; Dermatology Life Quality Index, SCAN; Schedules for Clinical Assessment in Neuropsychiatry, DSM-IV; Diagnostic and Statistical Manual of Mental Disorders-fourth edition, BADDS; Bipolar Affective Disorder Dimension Scale, BDI; Beck Depression Inventory, ASRM; Altman Self-Rating Scale for Mania, RSE; Rosenberg Self-Esteem Scale, TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-autoquestionnaire version, KSQ; Kings Schizotypy Questionnaire.

3.2.2 Assessment of Clinical Features of DD and Impact on Quality of Life

A series of structured questions were asked to obtain data on the clinical features of DD including age of onset and duration, severity, symptoms, aggravating factors and the impairment caused by having DD and the impact on quality of life. Photographs of areas of skin currently affected by DD were taken once consent to take a photograph had been obtained. A copy of the DD clinical assessment is displayed in Appendix A.ii pg. 282.

3.2.2.1 Age of Onset and Duration of DD

Individuals were asked to recall the age when their symptoms of DD first appeared. The number of years individuals had had DD for was subsequently calculated.

3.2.2.2 Lifetime Severity of DD

Individuals were classified as having mild, moderate or severe DD according to the descriptions given in Table 3.3. This classification of severity has been used in previous published studies of DD (Sakuntabhai *et al.*, 1999a).

Table 3.3 Classification of Severity of DD

Severity	**Description**
Mild	Keratotic papules scattered sparsely over the trunk or flexures or disease limited to one or two areas (e.g. hands)
Moderate	More extensive papular lesions or localised verrucous plaques
Severe	Coalescent verrucous plaques involving most of the trunk or grossly hypertrophic flexured disease

3.2.2.3 Symptoms and Aggravating Factors of DD

Individuals were asked if they had ever experienced pain, itching or malodour due to their DD, rated as either yes, no or unknown. Individuals were also asked whether factors including stress, sunlight and heat or sweating made the symptoms of their DD worse, rated as yes, no, unknown or not applicable.

3.2.2.4 Impairment caused by DD

To assess the impairment caused by having DD, individuals were asked whether they had ever required inpatient treatment for their DD and if so how many times. Individuals were asked how much time they had taken off work due to the symptoms of their DD and if they had ever had to stop work due to their DD. Individuals were also asked to rate on a scale of mild, moderate, severe, not applicable or unsure, how much impact having DD had had on their school life, work/career, relationships and social activities.

3.2.2.5 Dermatology Life Quality Index (DLQI)

Individuals were asked to complete the Dermatology Life Quality Index (DLQI), a brief validated questionnaire designed to assess the impact of skin conditions on quality of life (Finlay & Khan, 1994). This measure was included in the assessment battery to allow investigations of possible relationships between quality of life, the severity of DD and, the presence of neuropsychiatric symptoms.

The DLQI consists of 10 questions relating to the impact of having a skin disorder on six domains of quality of life *'Symptoms and feelings', 'Daily activities', 'Leisure', 'Work and school', 'Personal relationships' and 'Treatment'*. Each of the 10 items relates to the last week and has four possible responses *"not at all", "a little", " a lot" and "very much"* scored as 0, 1, 2 3 respectively. Total scores can range from 0 (no impairment of life quality) to 30 (maximum impairment of life quality). The questionnaire has been used in over 137

studies with 36 different skin conditions (Lewis & Finlay, 2004) and has previously been completed by 137 individuals with DD (Harris *et al.*, 1996). For the purposes of the current study the questionnaire was adapted to create a second version of the DLQI where the items related to the worst week ever. Both versions of the DLQI were used in the present study and are displayed in Appendix A.iii pg. 284.

3.2.2.6 Family History of DD

Individuals were asked if they had any known family history of DD rated as either none, possible, definite or unknown.

3.2.3 Assessment of Lifetime Psychiatric Features- Interview

3.2.3.1 Schedules for Clinical Assessment in Neuropsychiatry (SCAN) Interview

All individuals were asked a series of brief screening questions to assess whether they had a history of experiencing or receiving treatment for psychiatric illness including anxiety and panic attacks, depression, mania, psychosis, obsessive-compulsive symptoms, eating disorders and history of alcohol or substance abuse/dependence (displayed in Appendix A.iv pg. 286). If individuals responded affirmatively to one or more screening questions, the presence of these features was examined in further detail using the relevant sections of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing *et al.*, 1990). The SCAN is a semi-structured interview designed to elicit the presence of symptoms and behaviours associated with major psychiatric disorders. In the present study the standard SCAN sections were modified to reduce the length of the interview. This was achieved by selecting the questions assessing the presence of clinical features required for a lifetime diagnosis of a psychiatric disorder according to standard diagnostic criteria. Careful questioning was needed in the depression section of the interview due to the potential

difficulties in distinguishing between symptoms of depression and symptoms which were a direct result of having a skin disorder (e.g. itchiness during the exacerbation of the symptoms of DD could potentially affect an individual's sleep pattern). Information obtained regarding the presence of psychiatric symptoms during the interview was supplemented by inspecting individuals' psychiatric notes and/or relevant GP notes.

3.2.3.2 Subjective Relationship between DD and Lifetime Psychiatric Features

Individuals reporting a history of psychiatric illness were asked whether they felt there had been a lifetime and/or temporal relationship between the symptoms of their DD and the occurrence of episodes of psychiatric illness.

3.2.4 Assessment of Lifetime Psychiatric Features- Consensus Ratings

Information regarding the presence of lifetime psychiatric symptoms collected at interview and from inspecting available medical case notes was combined to produce a clinical vignette for each individual. The vignettes were used to make a number of psychiatric lifetime ratings on the features outlined in the following sections (see Appendix A.v. pg. 287 for a copy of the rating sheet). In all cases, Dr Lisa Jones, Professor Nick Craddock and I made the psychiatric lifetime ratings independently and consensus was reached by discussing all ratings.

3.2.4.1 *Best-Estimate Lifetime Psychiatric Diagnoses*

Best-estimate lifetime psychiatric diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000). In cases where individuals were diagnosed as having more than one lifetime psychiatric disorder, the disorder judged to have caused the most impairment was recorded as the main lifetime psychiatric disorder.

3.2.4.2 *History of Suicidal Thoughts and Suicide Attempts*

Lifetime history of suicidal thoughts and attempts was rated on a 5-point scale ranging from Absent (0) to Multiple suicide attempts likely to result in death (5), displayed in Table 3.4.

Table 3.4 Rating of Lifetime History of Suicidal Thoughts and Suicide Attempts

	Rating
Absent	0
Tedium Vitae (*feelings that life is not worth living that lacks the active, intrusive quality of suicidal ideation)*	1
Suicidal Ideation	2
Suicide attempt unlikely to result in death	3
Suicide attempt likely to result in death	4
Multiple suicide attempts likely to result in death	5

3.2.4.3 *Key Psychiatric Clinical Ratings and Psychiatric Rating Scale*

Other key psychiatric clinical variables were rated where relevant including:

- Age of onset of any psychiatric illness and age of onset of specific psychiatric illnesses causing clinically significant impairment (including self-sought treatment, being referred for treatment and disruption to work, family or social life)
- Number of episodes of affective illness
- Longest duration of affective illness

3.2.5 The Bipolar Affective Disorder Dimension Scale (BADDS)

The Bipolar Affective Disorder Dimension Scale (BADDS) is a measure designed to allow lifetime ratings to be made on four domains of psychopathology relevant to individuals with bipolar spectrum illness namely Mania (M), Depression (D), Psychosis (P) and Incongruence (I) (Craddock *et al.*, 2004). The main advantage of the scale is that it allows richer information to be obtained regarding an individual's psychopathology than can be obtained using standard diagnostic systems. In the present study the Mania (M) and Depression (D) dimensions of BADDS were rated in index individuals with DD. Based on all available information, including information obtained from the semi-structured SCAN interview and case notes, each dimension is rated on a scale of 0 (no evidence of manic/depressive symptoms) to 100 (evidence of 10 or more incapacitating episodes of mania/depression). A copy of the BADDS ratings guidelines is provided in Appendix A.vi pg. 289.

3.2.5.1 Reliability of Lifetime Psychiatric Ratings

Reliability of the lifetime psychiatric ratings made by myself, Dr Jones and Professor Craddock was assessed using Cohen's Kappa (categorical variables) and intra-class correlation coefficient (continuous variables) and are displayed in Table 3.5 and Table 3.6 respectively. Inter-rater reliability was high with mean kappa statistics of 0.86 and 0.93 for DSM-IV diagnoses and suicidal ideation respectively and mean intra-class correlation coefficients for other key psychiatric clinical variables ranging from 0.82-1.

Table 3.5 Inter-Rater Reliability of Lifetime DSM-IV Diagnoses and History of Suicidal Thoughts and Suicide Attempts

	Mean Kappa	95% CI
Lifetime DSM-IV Diagnoses	0.86	0.85-0.86
History of Suicidal Thoughts and Attempts	0.93	0.8-1.00

Table 3.6 Inter-Rater Reliability of Key Psychiatric Clinical Variables

	Mean Intra-class Correlation	95% CI
Age of Onset of any Psychiatric Disorder	0.86	0.54-1
Age of Onset of Depression	0.82	0.39-1
Age of Onset of any Anxiety Disorder	1	-
Age of onset of Mania	1	-
Number of Episodes of Mania	0.99	0.98-1
Number of Episodes of Depression	0.99	0.98-1
Longest Duration of Mania	0.99	098-1
Longest Duration of Depression	0.91	0.54-1
BADDS Mania	0.99	0.96-1
BADDS Depression	0.92	0.84-1

BADDS; Bipolar Affective Disorder Dimension Scale.

3.2.6 Other Neuropsychiatric Features

3.2.6.1 Lifetime History of Neurological Features and Investigations

Lifetime history of neurological features and neurological investigations was assessed using a brief checklist (Appendix A.vii pg. 295) to obtain information on the presence of neurological symptoms and disorders, including headaches, episodes of loss of consciousness, epilepsy, memory difficulties and infections of the brain. Individuals responding affirmatively to any of the items in the checklist were asked for further information including dates the symptoms occurred, whether any treatment was received and the degree of impairment caused by the symptoms. Information obtained regarding the presence of neurological disorders and/or symptoms during the interview was supplemented by inspecting individuals' available hospital notes and/or relevant GP notes.

3.2.6.2 Learning Difficulties

Lifetime history of learning difficulties was assessed by the following:

- Asking individuals whether they had ever had a diagnosis of dyslexia and/or whether they received extra help at school for problems with reading and/or writing and/or spelling and/or sums/numbers. It was likely that responses to these questions would

depend on the participants' age, with older participants being less likely to have been given a diagnosis of dyslexia and/or received extra help whilst at school.

- Due to the likelihood that the frequencies of individuals diagnosed with dyslexia would vary according to age groups, a brief questionnaire was also used to screen for dyslexia in the sample, The Adult Dyslexia Checklist (Vinegrad, 1994). The dyslexia checklist contains 20 (yes/no) items e.g. "Is your spelling poor?", "Do you mix up bus numbers like 95 and 59?" (Appendix A.viii. pg. 297). Scores are calculated by adding up the number of "YES" answers, with 9 or more YES responses reported as being a powerful indicator of difficulty. The dyslexia checklist was not initially part of the clinical assessment therefore the questionnaire was posted to individuals at a later date along with a stamped addressed envelope in which the questionnaire could be returned.

3.2.7 IQ Scores

IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (The Psychological Corporation, 1999). The WASI is a battery of four subsets of the full WAIS (Wechsler Adult Intelligence Scale) namely, Similarities, Compensation, Block Design and Matrix Reasoning. It is possible to administer either a four- or two-subset form of the measure. Due to time constraints, the two-subset version of the WASI, which includes the Comprehension and Matrix Reasoning subsets, was administered in the present study. An advantage of this measure is that it provides an estimate of general intellectual ability and can be administered in approximately 15 minutes. A disadvantage is that it only provides a full scale IQ score and not separate verbal and performance IQ scores, which can be obtained when using the four-subset version.

3.2.8 Family History of Neuropsychiatric Features

Information was collected regarding known family history of probable neuropsychiatric illness in first-degree relatives (parents, siblings, children) with and without DD. Psychiatric illnesses were recorded if they could be included in one of the five following categories of psychiatric illness:

- Major Depression for which individuals had received treatment and/or the symptoms of which had caused significant impairment including time off work and causing family problems.
- Other psychiatric illnesses -anxiety disorders, alcohol misuse/dependence, substance misuse/dependence, obsessive compulsive disorder, eating disorders for which individuals had received treatment and/or the symptoms of which had caused significant impairment.
- Bipolar Disorder
- Schizophrenia
- Other Severe Psychiatric Disorders- psychiatric illnesses where the diagnosis was uncertain but the symptoms were severe enough to require treatment by psychiatric services and/or inpatient psychiatric treatment.

3.2.9 Personality, Temperament and Current Mood State Questionnaires

At the end of the clinical and neuropsychiatric assessment, individuals were asked if they would be willing to have a pack of personality, temperament and current mood state questionnaires to be completed in their own time and returned in a stamped addressed envelope. The questionnaires included in the pack are listed in Table 3.7 below. Further descriptions of these questionnaires are given in Chapter 10, which describes the measures

and design used to compare the presence of neuropsychiatric features in a smaller subset of individuals with DD and their unaffected relatives.

Table 3.7 Personality, Temperament and Current Mood State Questionnaires

	Reference
Beck Depression Questionnaire (BDI)	(Beck & Steer, 1987)
Altman Self-Rating Scale for Mania Scale (ASRM)	(Altman *et al.*, 1997)
Rosenberg Self-Esteem Scale (RSE)	(Rosenberg, 1965)
Eysenck Personality Questionnaire (EPQ)	(Eysenck & Eysenck, 1975)
Temperament Evaluation of Memphis, Pisa, Paris and San Diego-autoquestionnaire version (TEMPS-A)	(Akiskal *et al.*, 2005)
Kings Schizotypy Questionnaire (KSQ)	(Jones *et al.*, 2000; Williams, 1993)

A small number of individuals (N=9) did not wish to complete the questionnaire pack. The pack was not given to a further three individuals as they reported having a reading disability and would have found it difficult to complete the questionnaires. A questionnaire pack was left with the remaining 88 individuals. A reminder letter with a further copy of the questionnaire pack and another stamped addressed envelope was sent to individuals if the questionnaires had not been returned within 2 weeks. Seventy-three individuals returned the questionnaire pack, figures are summarised in Table 3.8.

Table 3.8 Returned Questionnaire Packs

	Frequency
Returned questionnaire pack	73
Returned- incomplete	1
Not returned	14
Declined- not given	9
Not suitable- not given	3
Total	**100**

3.3 Analysis of Demographics, Clinical and Neuropsychiatric Features in 100 Individuals with DD

Analysis of the demographic characteristics, clinical features of DD and neuropsychiatric features was conducted in the following ways:

1. Describing the demographic characteristics, clinical features of DD, neuropsychiatric features, IQ scores and family history of neuropsychiatric features in 100 unrelated individuals with DD. Including:
    - Examining the relationship between the perceived impact of DD on quality of life and DD severity.
    - Describing the prevalence of neuropsychiatric features to allow comparisons to be made to the prevalence of these features in other populations reported in the literature to assess whether there is evidence for a population-level association between DD and neuropsychiatric features.
    - Comparing the prevalence of DD among first-degree relatives with a history of psychiatric illness and epilepsy and identifying any families displaying co-segregation of DD and neuropsychiatric features.
2. Investigating the presence of multiple neuropsychiatric features in individuals with DD, which may provide support for the hypothesis that mutations in *ATP2A2* have pleiotropic effects.
3. Investigating possible relationships between DD clinical features, psychiatric features, learning difficulties and IQ scores. Including:
    - Investigating possible subjective and objective relationships between the clinical features of DD (including severity and age of onset) and lifetime history of psychiatric symptoms. Such relationships (particularly subjective relationships) may provide support for the contribution of psychosocial factors in the presence of

psychiatric features in individuals with DD but objective relationships may also provide support for pleiotropy (certain mutations in *ATP2A2* may lead to the presence of both severe DD and severe neuropsychiatric features including psychiatric features).

- Investigating possible relationships between the perceived impact of having DD on everyday functioning and lifetime history of psychiatric symptoms which again may provide support for the contribution of psychosocial factors in the presence of psychiatric features in individuals with DD.
- Investigating relationships between age of onset and severity of DD and IQ scores and learning difficulties. Such relationships may support the role of psychosocial factors (severe symptoms of DD from an early age may affect school attendance and subsequently school performance) but may also provide support for pleiotropy (certain mutations in *ATP2A2* may lead to the presence of both severe DD and severe neuropsychiatric features including learning difficulties).

3.4 Statistical Analysis

Data were extracted from the study Access database into the statistical package SPSS version 12.0.1 (SPSS Inc., 2003). Normality of the data was assessed using the Kolmogorov-Smirnov Test. The majority of the data were not normally distributed so non-parametric statistical tests were used, outlined below. Statistical tests were considered significant at the $p<0.05$ level, (two tailed).

Continuous data:

- Differences between two groups were assessed using Mann-Whitney U tests (in one case where data were found to be normally distributed, an independent samples *t*-test was used).

- Differences between 3+ groups were assessed using Kruskal-Wallis Analysis of Variance. If a significant difference was found, *post hoc* comparisons were performed on each pair of groups using Mann-Whitney U tests.
- Correlations between two continuous variables were assessed using Spearman's rho.

Categorical data

- Relationships between two categorical variables were assessed using 2x2 and 2x3 chi-square tests. In cases where 20% or more of the cells in a chi-square table had an expected count of less than five, Fisher's exact tests (2x2 tables) and exact significance tests for Pearson's chi-square (2x3 tables and greater) were used. For 2x3 chi-square tables, if a significant relationship was found, *post hoc* comparisons were performed on each pair using 2x2 chi-square tests.

Due to the modest size of the sample and limited power, corrections were not routinely made for multiple testing. Therefore, any significant findings were treated with caution and an emphasis was placed on looking for trends in the relationships between the clinical features of DD and the neuropsychiatric features observed.

The following chapter (Chapter 4) describes the demographic, clinical and neuropsychiatric features observed among 100 unrelated individuals with DD.

4 DEMOGRAPHICS, CLINICAL AND NEUROPSYCHIATRIC FEATURES IN 100 INDIVIDUALS WITH DD: RESULTS

This chapter describes the demographic, clinical and neuropsychiatric features of 100 unrelated individuals with DD. First the demographic characteristics of the sample are described followed by the clinical features of DD and impact on quality of life. This is followed by a description of the lifetime prevalence of neuropsychiatric features in the sample. The final part of the chapter reports investigations of possible relationships between the clinical features of DD and the presence and severity of lifetime neuropsychiatric features observed in the sample. Additional information relating to this chapter can be found in section B of the Appendix starting at pg. 298, and will be referenced throughout the chapter.

4.1 Demographic Characteristics

The demographic characteristics of the sample are summarised in Table 4.1 and Table 4.2. The mean age of the sample was 48.2 years and participants were predominantly female (71%). The majority of the sample were UK Caucasian (92%). Most individuals (63%) were living in their own home with a spouse and/or children. Fifty-one percent of individuals were working either full or part time and 23% of individuals were retired. Sixteen percent of individuals were currently not working and receiving benefits. The most frequent category of main lifetime occupation was service, shop and market worker (30%).

The frequencies and percentages of the highest level of educational qualifications individuals achieved by age group are summarised in Table 4.2. A large proportion of the sample had no qualifications (41%). Similar proportions of individuals had obtained other qualifications, which included CSEs and GCSE grades D-G (13%), GCSE grades A-C or equivalent (17%) and A Levels or equivalent (12%). Fourteen percent of individuals had obtained higher educational qualifications including teaching and nursing qualifications and

degrees. A higher proportion of individuals in the 55-64 and 65-74 age groups had no qualifications, 69% and 50% respectively, compared to individuals in the 18-34 and 35-44 age groups, 14.3 % and 18.2% respectively.

Table 4.1 Demographic Characteristics of 100 Individuals with DD

Demographics	Descriptives and Percentages
Age (years)	
Mean (95% CI)	48.2 (45.8-50.7)
Standard Deviation	12.4
Range	20-71
	%
Female	71
Ethnic Origin	
UK Caucasian	92
Other Caucasian	6
Asian	2
Marital Status	
Has married/lived as married	88
Has never married/lived as married	12
Current Social Circumstances	
Lives in own home with spouse and/or children	63
Lives alone	14
Lives in home of parents or children	13
Lives with partner at least one year but not married	7
Other	3
Current Employment Status	
Employed full time	34
Employed part time	17
Not working-receiving benefits	16
Full-time student	2
Homemaker	8
Retired	23
Main Lifetime Occupation	
Legislator/senior officials, managers and professionals	18
Technicians & associate professionals (& civil servants)	9
Clerks	18
Service workers & shop & market workers	30
Craft & related trade workers	7
Plant & machinery operators and assemblers	9
Elementary occupations	7
Never worked	1
Full-time student	1

Table 4.2 Highest Level of Educational Qualifications by Age Groups

	Age at Interview										
	18-34 N=14		35-44 N=22		45-54 N=28		55-64 N=26		65-74 N=10		All N=100
	N	%	N	%	N	%	N	%	N	%	%
No qualifications	2	14.3	4	18.2	12	42.9	18	69.2	5	50.0	41
Other qualifications*	4	28.6	5	22.7	2	7.1	2	7.7	0	0.0	13
GCSE A-C or equivalent	3	21.4	4	18.2	7	25.0	3	11.5	0	0.0	17
A Level or equivalent	2	14.3	3	13.6	4	14.3	0	0	3	30	12
Teaching, HND, Nursing	1	7.1	3	13.6	1	3.6	3	11.5	2	20	10
Degree	1	7.1	1	4.5	2	7.1	0	0	0	0.0	4
Unknown	1	7.1	2	9.1	0	0	0	0	0	0.0	3

* Other qualifications include CSEs and GCSE grades D-G.

4.2 DD Clinical Features and Impact on Quality of Life

A clinical diagnosis of DD was confirmed in 95 cases either by obtaining copies of individuals' dermatology records or by the fact that they were recruited for the study through a dermatologist on the basis of having a diagnosis of DD. The remaining five individuals reported that they had been given a diagnosis of DD by a dermatologist and four could recall their diagnosis had been confirmed with a biopsy. Two individuals had a diagnosis of unilateral/segmental DD affecting one side of the body only.

4.2.1 Age of Onset and Duration of DD

Age of onset of DD was known for ninety-six individuals. The descriptives and distributions are summarised in Table 4.3 and Figure 4.1 respectively. The median age of onset of DD in the sample was 14 years. The youngest age of onset was a female who had been born with DD and the oldest was a female who had no symptoms until the age of 48. The peak age of onset of DD was between the ages of 11-20 (49% of individuals). The

median duration of having DD in the sample was 31 years and ranged from 3 to 60 years (Table 4.3).

Table 4.3 Age of Onset and Duration of DD (Years)- Descriptives

	N	Median	Range	Inter quartile range
Age of onset of DD	96	14	0-48	10
Duration of DD	96	31	3-60	21

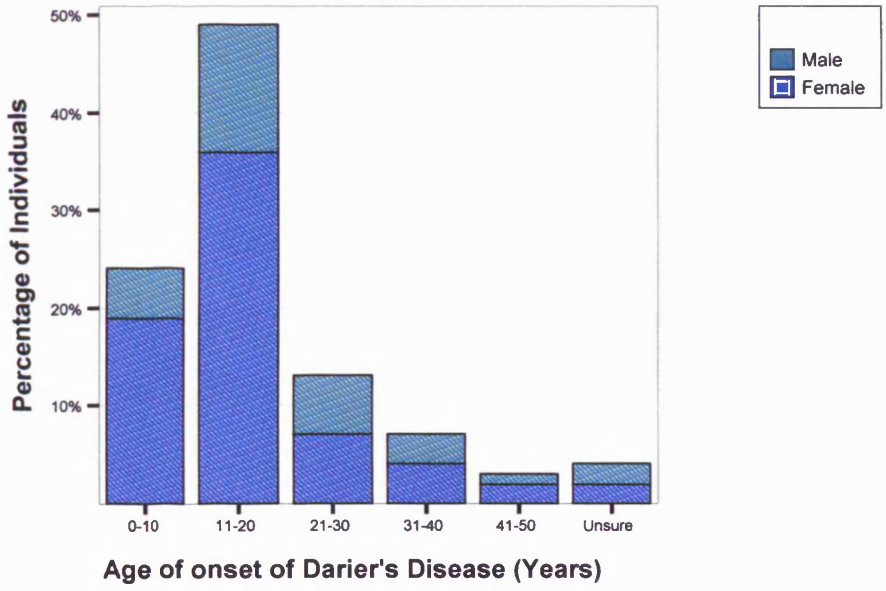


Figure 4.1 Age of Onset of DD- Distributions

4.2.2 Severity of DD

Table 4.4 displays the severity of the cases of DD using the classification criteria described in Chapter 3 Table 3.3 pg.37. The majority of individuals (67) were classified as having moderate DD. Twenty-two individuals were classified as having mild DD and 11 were classified as having severe DD. The distribution of mild, moderate and severe DD was similar in males and females.

Table 4.4 Severity of DD –Frequencies and Percentages

	Total N=100	Male N=29		Female N=71	
	N	N	%	N	%
Mild	22	7	24	15	21
Moderate	67	19	66	48	68
Severe	11	3	10	8	11

4.2.3 Symptoms and Aggravating Factors of DD

Ninety-two individuals reported itching as a symptom of their DD, 54 individuals reported that their skin could be painful and 60 reported they had noticed body malodour as a result of their DD. The frequencies of symptoms reported in DD are summarised in Figure 4.2 pg. 56. Twenty-two individuals reported that their skin could at times be completely clear whereas the majority of individuals reported their skin had never been completely clear since the onset of the condition.

Eighty-two individuals reported that heat or sweating made their skin worse while 52 stated sunlight caused exacerbations. Sixty-eight individuals felt that their skin was worse under times of stress. The frequencies of common aggravating factors reported in DD are summarised in Figure 4.3 pg. 56. One individual reported a severe flare of their DD after using a sun bed. Three individuals' dermatology notes indicate that they experienced an outbreak of DD following surgery. Two individuals who had taken the mood stabiliser lithium in the past felt this had possibly made their skin worse. This was supported by evidence from their medical notes that their skin seemed to improve when lithium was stopped. It is also noted in one of these individual's case notes that their skin flared whilst they were taking carbamazepine, another mood stabiliser. Another individual's dermatology case notes reported that their DD flared the same time their antidepressant, mirtazapine, was increased.

4.2.4 Impairment and Impact on Quality of Life

The numbers of individuals who had been admitted to hospital for treatment of their DD are summarised in Table 4.5 pg. 56. The majority of individuals had not required inpatient treatment (67 individuals). Twenty-three individuals had been treated in hospital between one and four times and 10 individuals had required an admission to hospital on five or more occasions. Nine individuals reported having to stop work due to the severity of their DD and a further 15 individuals had taken a total of months off work due their skin condition (shown in Table 4.6).

The subjective impairment individuals felt having DD had had on their school life, work/career, relationships and social activities are summarised in Figure 4.4 pg. 57. Twenty-eight individuals reported that they felt having DD had an impact on their school life (either mildly, moderately or severely). Areas of school life affected were the comments of other children and bullying, sports and swimming lessons and having to take time off due to the severity of their skin condition. Thirty individuals reported having DD had caused relationship problems, 35 individuals reported problems at work and in their career and 53 individuals reported social activities being impaired by having DD.

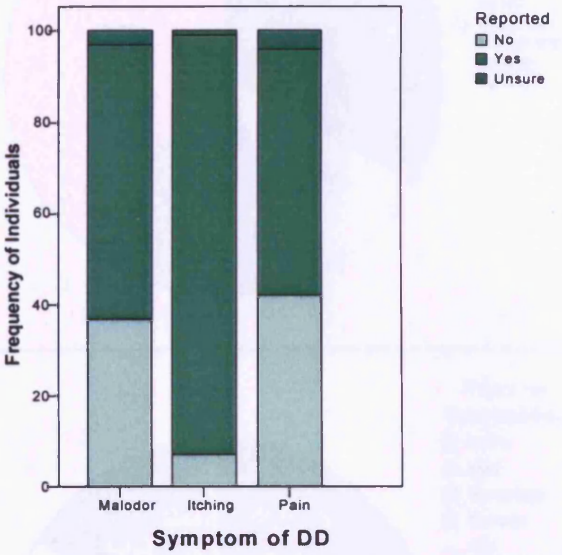


Figure 4.2

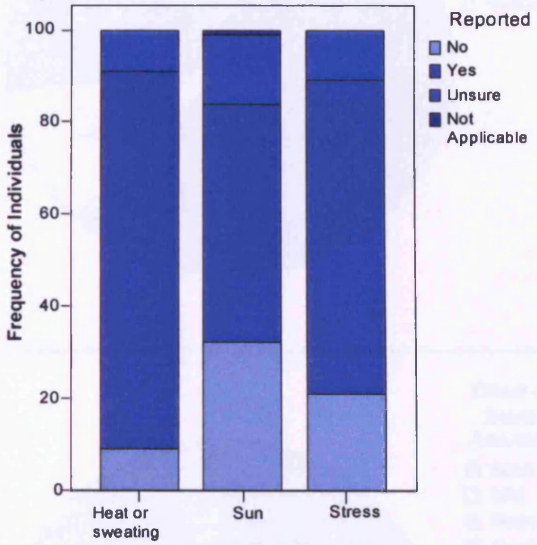


Figure 4.3

Figure 4.2 Frequencies of Individuals Reporting Malodour, Itching and Pain as a Symptom of DD

Figure 4.3 Frequencies of Individuals Reporting Heat, Sun and Stress as an Aggravating Factor of DD

Table 4.5 Number of Admissions to Hospital for Treatment of DD

Number of Admissions	Frequency
0	67
1-4	23
5+	10
Total	100

Table 4.6 Total Amount of Time Taken Off Work due to the Symptoms of DD

Time Taken Off Work	Frequency
None	51
Days	11
Weeks	11
Months	13
Greater than one year	2
Stopped work due to DD	9
Not applicable	3
Total	100

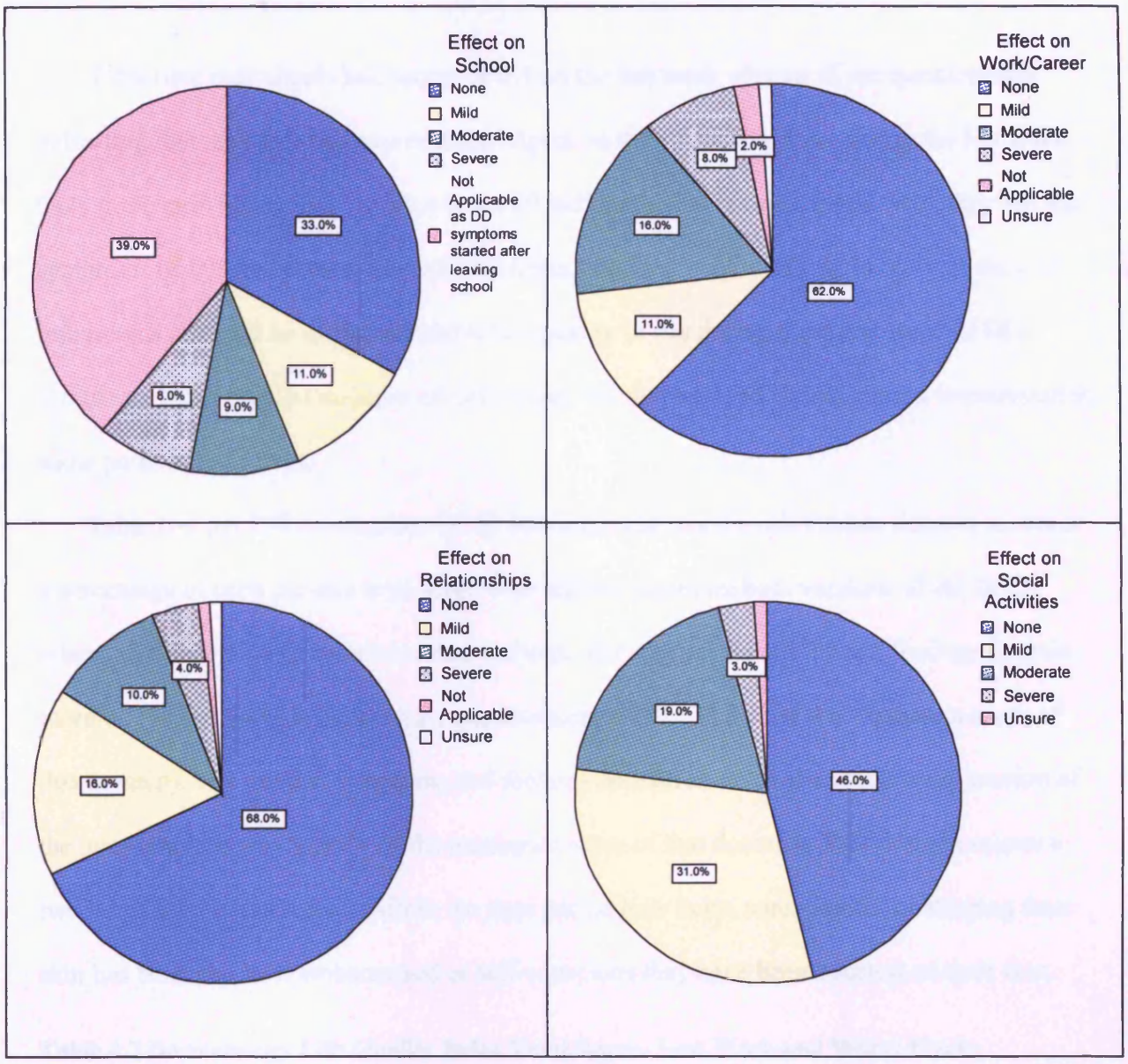


Figure 4.4 Subjective Impairment of DD on School Life, Work/Career, Relationships and Social Activities

4.2.4.1 Dermatology Life Quality Index

Ninety-nine individuals completed two versions of the Dermatology Life Quality Index (DLQI) relating to the last week and the worst week ever respectively. The measure has been previously described in Chapter 3 section 3.2.2.5 pg. 38. The descriptives and distributions of DLQI total scores are summarised in Table 4.7 and Table 4.8. The median total score on the last week and worst week version of the questionnaire was 4 (13% of the maximum score of 30) and 15 (50% of the maximum score of 30) respectively.

Fifty-nine individuals had scores of 0-5 on the last week version of the questionnaire indicating that their DD had caused little impact on their everyday lives during the last week. Only three individuals had a score of over 20 indicating that during the last week they felt the symptoms of DD had caused a significant impact on their quality of life. In contrast only 12 individuals reported no or limited impact on quality of life during the worst week of DD symptoms indicating the majority of individuals felt that having DD had caused impairment at some point in their lives.

Table B-1 pg. 298 summarises DLQI last week and worst week median domain scores as a percentage of each domain total score. The highest scores on both versions of the DLQI related to the domain of symptoms and feelings. The median symptoms and feelings domain score on the last week version of the questionnaire was 2 (33.3 % of the maximum score of that domain). The median symptoms and feelings domain score on the worst week version of the questionnaire was 5 (83% of the maximum score of that domain). This domain relates to two items asking individuals within the time period how itchy, sore, painful or stinging their skin has been and how embarrassed or self-conscious they have been because of their skin.

Table 4.7 Dermatology Life Quality Index Total Score– Last Week and Worst Week-Descriptives

	N	Median	Range	Inter quartile range
Last Week	99	4	0-28	8
Worst Week	99	15	0-30	12

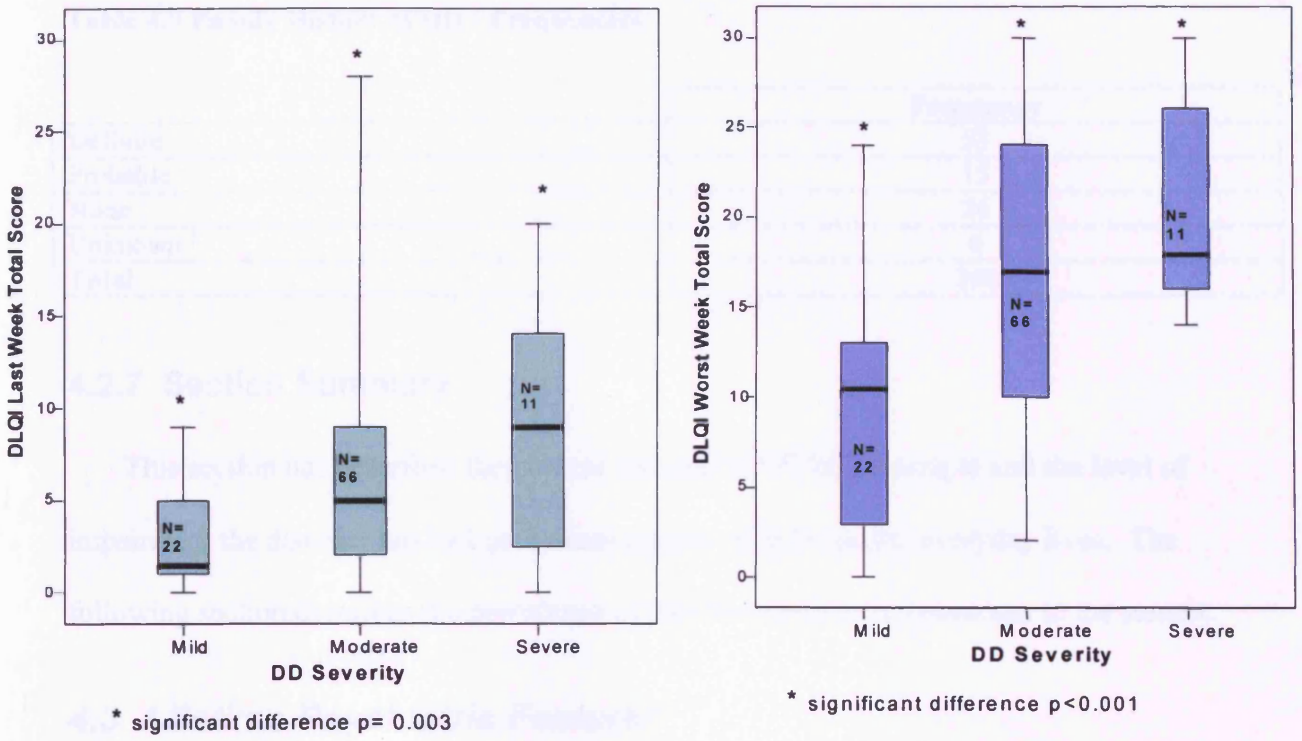
Table 4.8 Distribution of Dermatology Life Quality Index Total Scores – Last Week and Worst Week

	Number of Individuals	
DLQI Score	**Last Week**	**Worst Week**
0-5	59	12
6-10	23	17
11-15	9	21
16-20	5	20
>20	3	29

4.2.5 Relationship between Impact on Quality of Life and DD Severity

The relationship between DLQI last and worst week total scores and DD severity is illustrated in Figure 4.5. Kruskal-Wallis H test indicated that there was a significant difference between the total median DLQI last week scores of individuals with mild, moderate and severe DD ($H = 11.496$, $df=2$, $p=0.003$). Mann-Whitney U tests indicated that individuals with moderate and severe DD scored significantly higher on the DLQI last week version than individuals with mild DD (5 vs. 1.5; $U=387$, $n=88$, $p=0.001$) and (9 vs. 1.5; $U=65.5$, $n=33$, $p=0.032$) respectively. There was no significant difference between the DLQI last week scores of individuals with moderate and severe DD (5 vs. 9; $U=308$, $n=77$, $p=0.422$).

There was also a significant difference between the total median DLQI worst week scores of individuals with mild, moderate and severe DD ($H=19.051$, $df=2$, $p<0.001$). Mann-Whitney U tests indicated that individuals with moderate and severe DD scored significantly higher on the DLQI worst week version than individuals with mild DD (17 vs. 10.5; $U=336.5$, $n=88$, $p<0.001$) and (18 vs. 10.5; $U=17$, $n=33$, $p<0.001$) respectively. There was no significant difference between the DLQI worst week scores of individuals with moderate and severe DD (17 vs. 18; $U=279.5$, $n=77$, $p=0.224$).



Numbers in the boxes reflect numbers of individuals in each group.
Box plots represent the median, interquartile range and minimum and maximum DLQI scores. DLQI; Dermatology Life Quality Index.

Figure 4.5 Relationship between Last Week and Worst Week Dermatology Life Quality Index Scores and DD Severity

4.2.6 Family History of DD

The numbers of individuals with a definite, possible or no known family history of DD are summarised in Table 4.9. Fifty-three individuals reported a definite family history of DD. A further 15 felt that another family member probably had DD either because they had observed that they had a similar skin condition to themselves that had not been diagnosed or another family member had mentioned that they had a similar looking skin condition. Twenty-six individuals reported no family history of DD despite knowing or having information on all other immediate family members. Six individuals were unsure if they had a

family history of DD, four of these individuals had been adopted and had no information on either of their parents and two individuals had no information about their fathers.

Table 4.9 Family History of DD - Frequencies

	Frequency
Definite	53
Probable	15
None	26
Unknown	6
Total	**100**

4.2.7 Section Summary

This section has described the clinical features of DD in the sample and the level of impairment the disorder has had on various aspects of individuals' everyday lives. The following section describes the prevalence of lifetime psychiatric symptoms in the sample.

4.3 Lifetime Psychiatric Features

4.3.1 Main Best-Estimate Lifetime DSM-IV Diagnoses

The frequencies and percentages of the main best-estimate lifetime DSM-IV psychiatric diagnoses of individuals in the sample are summarised in Table 4.10 and Table 4.11. Fifty-five individuals had a lifetime DSM-IV diagnosis. In the case of a further eight individuals (classified as unknown) there was a suggestion of a history of psychiatric illness but not enough symptoms could be elicited to make a definite lifetime DSM-IV diagnosis. A significantly higher percentage of females than males had a history of psychiatric illness (65% vs. 31%; $\chi^2 = 9.478$, df= 1, p=0.002).

Thirty individuals in the sample had a history of Major Depressive Disorder, which was the most common category of psychiatric illness. One individual with a diagnosis of Major Depressive Disorder developed hypomanic symptoms for one month after being treated with antidepressant medication, the symptoms stopped after her GP discontinued the

medication. Two individuals had a diagnosis of Dysthymic Disorder and twelve individuals had a lifetime diagnosis of Depression Not Otherwise Specified (NOS). This category is used for individuals with a history of depressive features that do not reach the criteria for other depressive disorders. Four individuals had a lifetime diagnosis of Bipolar I Disorder and a further individual was diagnosed as having Cyclothymic Disorder. Three individuals had history of Panic Disorder and a further three individuals had a diagnosis of Anxiety NOS.

Table 4.10 Summary of Main Best-Estimate Lifetime DSM-IV Diagnoses

	Total N=100	**Male N=29**		**Female N=71**	
	%	N	%	N	%
Any DSM-IV Disorder	55	9	31.0	46	64.8
Unknown	8	4	13.8	4	5.6
No DSM-IV Disorder	37	16	55.2	21	29.6

Table 4.11 Main Best-Estimate Lifetime DSM-IV Diagnoses

	Total N=100
DSM-IV Diagnosis	N
Major Depressive Disorder	30
Recurrent Episodes	*24*
Single Episode	*6*
Dysthymic Disorder	2
Depression NOS	12
Bipolar I Disorder	4
Cyclothymic Disorder	1
Panic Disorder	3
With Agoraphobia	*1*
Without Agoraphobia	*2*
Anxiety Disorder NOS	3
Unknown	8
Unaffected	37

NOS; Not otherwise specified

4.3.2 Co-morbid DSM-IV Diagnoses

Table 4.12 summarises the co-morbid DSM-IV diagnoses of eleven individuals. Ten individuals whose main diagnosis was a depressive disorder also had a history of an anxiety disorder and one individual with a main diagnosis of an anxiety disorder also had a history of a depressive disorder. Table 4.13 and Table 4.14 summarise the percentages of all best

estimate lifetime DSM-IV diagnoses in the sample and includes the individuals with two co-morbid diagnoses twice. In total 50 individuals had a lifetime diagnosis of a mood disorder and 16 individuals were diagnosed as having a history of an anxiety disorder.

Table 4.12 Co-morbid DSM-IV Diagnoses

Main DSM-IV Diagnosis	Second DSM-IV Diagnosis	Number of Cases
Major Depressive Disorder (R)	Panic Disorder without agoraphobia	3
Major Depressive Disorder (R)	Panic Disorder with agoraphobia	1
Major Depressive Disorder (R)	Generalised anxiety disorder	1
Major Depressive Disorder (R)	Anxiety Disorder NOS	1
Major Depressive Disorder (S)	Anxiety Disorder NOS	1
Dysthymic Disorder	Panic Disorder without agoraphobia	1
Depression NOS	Panic Disorder with agoraphobia	1
Panic Disorder with agoraphobia	Depression NOS	1
Panic Disorder without agoraphobia	Anxiety Disorder NOS	1

R; recurrent episode, S; single episode, NOS; not otherwise specified.

Table 4.13 All Best-Estimate Lifetime DSM-IV Diagnoses

	Total N=100
DSM-IV Diagnosis	N
Major Depressive Disorder	30
Recurrent	*24*
Single	*6*
Dysthymic Disorder	2
Depression NOS	13
Bipolar I Disorder	4
Cyclothymic Disorder	1
Panic Disorder	9
With Agoraphobia	*3*
Without Agoraphobia	*6*
Generalized Anxiety Disorder	1
Anxiety Disorder NOS	6
Unknown	8
Unaffected	37

NOS; not otherwise specified.

Table 4.14 Summary of all Lifetime DSM-IV Diagnoses

	Total N=100
	N
Any DSM-IV Mood Disorder	50
Any DSM-IV Anxiety Disorder	16

4.3.3 History of Suicidal Thoughts and Suicide Attempts

The frequencies and percentages of individuals with a history of suicidal thoughts and suicide attempts are summarised in Table 4.15 and illustrated in Figure 4.6. Individuals are only included in one category so for example an individual with a past history of suicidal thoughts who had also made a suicide attempt is only included in the category for suicide attempt. Thirteen individuals had made a suicide attempt in their lifetime.

Brief descriptions of the suicide attempts made by thirteen individuals are summarised in Table 4.16. One individual, classified as having severe DD, made a suicide attempt during an episode of depression, which was in reaction to a worsening of the symptoms of their DD. For the remaining twelve individuals there was no subjective or objective temporal relationship between their suicide attempts and the symptoms of DD.

Table 4.15 History of Suicidal Thoughts and Suicide Attempts

	Total N=100
	N
Multiple suicide attempts likely to result in death	1
Suicide attempt likely to result in death	3
Suicide attempt unlikely to result in death	9
Suicidal Thoughts	18
Unsure	6
None	63

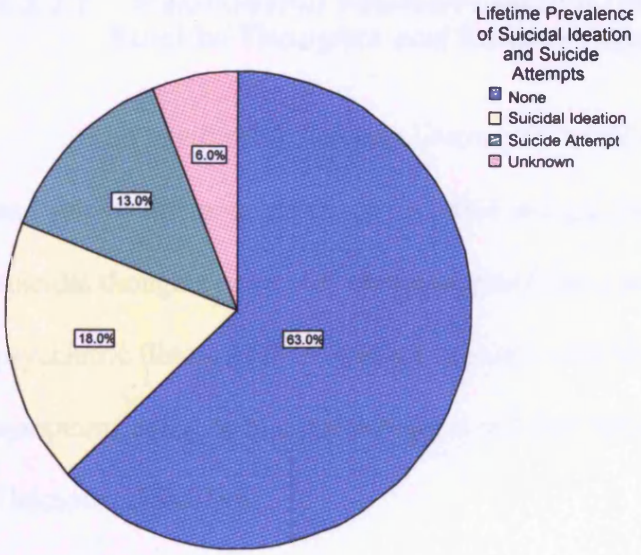


Figure 4.6 Summary of History of Suicidal Ideation and Suicide Attempts - Frequencies

Table 4.16 Descriptions of Suicide Attempts by Thirteen Individuals

Study ID	Description
DAR4-1	Multiple overdoses over a 20-year period. Medical notes report attempts to be impulsive and in response to domestic difficulties. Described as having an emotionally unstable personality disorder.
DAR70-1	One serious overdose following marital problems. Described as having a cyclothymic personality.
DAR85-1	Impulsive suicide attempts over a month period following a relationship breakdown.
DAR55-1	Multiple overdoses over a 15-year period. Described in medical notes as having somatoform disorder.
DAR46-1	Two overdoses following domestic difficulties.
DAR39-1	Impulsive overdose following a situational crisis.
DAR19-1	Overdose on one occasion.
DAR18-1	Diagnosis of bipolar disorder. Admitted to hospital on a section following an overdose, mood described as being elated at the time.
DAR7-1	Overdose as a teenager following a relationship breakdown.
DAR30-1	Multiple overdoses often in response to domestic situations. Described in medical notes as having a dependent personality.
DAR58-1	Overdose in late teens.
DAR52-1	Suicide attempt during episode of depression in reaction to a flare up of DD.
DAR17-1	Overdose during an episode of depression in relation to a number of stressful life events.

4.3.3.1 Relationship between Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

The relationship between lifetime DSM-IV diagnosis and history of suicidal thoughts and suicide attempts are shown in Table B-2 pg. 298. All of the individuals with a history of suicidal thoughts or suicide attempts either had a positive DSM-IV lifetime diagnosis of psychiatric illness or there was a suggestion of a history of psychiatric illness but not enough symptoms could be elicited to make a definite lifetime DSM-IV diagnosis (classified as Unknown DSM-IV).

4.3.4 Lifetime Treatment for Psychiatric Illness

Table 4.17 summaries the types of treatment individuals had received for psychiatric illness in their lifetime. Individuals who had received more than one type of treatment, for example had consulted their GP for symptoms of depression, received antidepressants and been referred to secondary psychiatric services, are included in more than one treatment category. Almost half of the sample (49 individuals) had at some stage in their lives consulted their GP for psychiatric symptoms. Forty-six individuals on at least one occasion in their lives had been offered or received antidepressant, anxiolytic or hypnotic medication by their GP for treatment of psychiatric symptoms. Five individuals in the sample had been treated with a mood stabilizer, these included the four individuals in the sample with diagnosis of Bipolar I Disorder and another individual with a lifetime diagnosis of Major Depressive Disorder who had taken multiple overdoses over a 15 year period and had an extensive psychiatric history. The four individuals with Bipolar I Disorder had also been treated with antipsychotic medication on at least one occasion. Twenty-five individuals had been referred by their GP to secondary psychiatric services. Nine individuals had received psychiatric inpatient treatment

for episodes of depression (3 individuals), suicide attempts (3 individuals), manic episodes (2 individuals) and both episodes of mania and depression (1 individual).

Table 4.17 Lifetime Treatment for Psychiatric Illness

	Total N=100
	N
Consulted GP for physical symptoms thought by GP to be related to symptoms of anxiety or depression	2
GP consultation for psychiatric symptoms	49
Offered/received antidepressants, anxiolytics or hypnotics for psychiatric symptoms	46
Received mood stabilisers	5
Received antipsychotic medication	4
Referred by GP to psychiatric services	25
Psychiatric inpatient	9

Individuals who had received more than one type of treatment are included in more than one category.

4.3.5 Key Psychiatric Clinical Variables

The 55 individuals with a lifetime DSM-IV diagnosis of any psychiatric illness were also given additional ratings on key psychiatric clinical variables namely age of onset of psychiatric illness, number of episodes of affective illness and longest duration of affective episodes. In some cases there was not enough information regarding individuals' psychiatric history to make a rating on these variables (this is reflected in the N columns in the tables).

4.3.5.1 Age of Onset of Psychiatric Illness

Descriptive data for the age of onset of psychiatric illness causing impairment of 39 individuals is summarised in B-3 pg. 298. The median age of onset of the 39 individuals with any lifetime psychiatric illness and the 35 individuals with a lifetime depressive disorder was 25 years and ranged from 14 to 58 years. The median age of onset of the four individuals with a lifetime history of bipolar disorder was 23 years and ranged from 15 to 52 years.

4.3.5.2 *Number of Episodes of Affective Illness*

Descriptive data for the number of episodes of affective illness among individuals with a lifetime DSM-IV diagnosis of a mood disorder are summarised in Table B-4 pg. 299. The 30 individuals with a diagnosis of Major Depressive Disorder had a median of four episodes of depression (range; 1 to 20 episodes). One female diagnosed with Major Depressive Disorder had experienced two episodes of sub clinical mania following the births of both of her children. A further individual with Major Depressive Disorder developed a hypomanic episode after being treated with antidepressant medication. Two individuals with a lifetime diagnosis of Dysthymic Disorder had both experienced one chronic period of depressed mood that did not reach criteria for an episode of major depression. Nine of the 13 individuals with a lifetime diagnosis of Depression NOS had a median of two episodes of depression (range; 1 to 130 episodes). It was not possible to determine the number of episodes in the other four cases. Three of the four individuals with Bipolar I Disorder had a median of 10 episodes of depression (range; 1 to 50 episodes) and six episodes of mania (range; 1 to 20 episodes). It was not possible to determine the number of episodes in the other case of Bipolar I Disorder.

4.3.5.3 *Longest Duration of Affective Illness*

Descriptive data for the length of the longest episode of affective illness in weeks among individuals with a lifetime DSM-IV diagnosis of a mood disorder is summarised in B-5 pg. 299. It was possible to determine the length of the longest episode of depression in 29 individuals with a lifetime diagnosis of Major Depressive Disorder. The median length of the longest episode of depression was 26 weeks and ranged from two weeks to 260 weeks (five years). The two individuals with a lifetime diagnosis of Dysthymic Disorder had experienced chronic periods of depression lasting 312 weeks (six years) and 780 weeks (15 years) respectively. The median length of the longest episode of depression among nine individuals with Depression NOS was three weeks and ranged from four days to 130 weeks (2 ½ years).

For two individuals with Bipolar I Disorder the median length of the longest episode of depression was 14 weeks (range; 12 to 15 weeks) and the median length of the longest episode of mania was five weeks (range; 2 to 50 weeks).

4.3.6 Section Summary

This section has described the prevalence of lifetime psychiatric symptoms in the sample including lifetime DSM-IV diagnosis, history of suicidal thought and attempts and treatment for psychiatric illness. The following section describes the prevalence of other neuropsychiatric features in the sample.

4.4 Other Neuropsychiatric Features

4.4.1 Lifetime Prevalence of Epilepsy

Three individuals in the sample had a lifetime diagnosis of epilepsy, described in Table 4.18. One individual with a lifetime diagnosis of tonic-clonic epilepsy was currently receiving treatment for epilepsy. Two individuals with a lifetime diagnosis of idiopathic epilepsy and petit mal epilepsy respectively were not currently receiving any treatment for epilepsy.

Table 4.18 Individuals with a Lifetime Diagnosis of Epilepsy

Type of Epilepsy	Details	Investigations
Tonic-Clonic Epilepsy	Tonic clonic seizures starting around the age of eight. Currently being treated with anti-epileptic medication. Also absence seizures starting in childhood.	EEGs dramatically abnormal with frequent generalised epileptic discharges (from medical notes).
Idiopathic Epilepsy	Admitted at the age 18 for investigations of epilepsy. Grand mal fits observed. Treated with anti-epileptic medication for 40 years, stopped 12 months ago after not having a fit for 15 years.	EEG showed no specific epileptiform pattern. Further EEG showed a generalised excess of slow rhythms (from medical notes).
Petit Mal Epilepsy	Age 6-13; absence/petite mal episodes- lasting for a few minutes a couple of times a week.	Investigated and diagnosed by a neurologist (reported by participant).

4.4.2 Lifetime Prevalence of Investigations for Blackouts and Brief Periods of Loss of Consciousness

Thirteen individuals had had investigations for blackouts, periods of loss of consciousness or fainting episodes, summarised in Table 4.19. In eleven of these cases individuals either reported they were unsure of the results of the investigations or they and/or their medical notes reported no neurological abnormalities were found. A CT revealed some mild atrophic changes in an individual aged 40 who received neurological investigations after collapsing. An individual with a lifetime diagnosis of bipolar disorder was given investigations for an episode of loss of consciousness aged 55, which revealed a possible aneurysm or medial sphenoid ridge meningoma.

4.4.3 Summary of all Neurological Investigations and Treatments

In total thirty-six individuals had been referred to a neurologist and had neurological investigations or had been treated for a neurological condition, summarised in Table 4.20. Four individuals who had been treated or investigated for two different neurological conditions are included in the table twice. This included an individual who had petit mal epilepsy in childhood who also had viral meningitis aged 43, a 51 year old individual who had

been given a CT scan to investigate memory problems and an audiogram for hearing difficulties, a 58 year old individual who had a CT scan to investigate headaches and had also had an audiogram to investigate hearing problems and a 39 year old individual who had had investigations for migraines and problems with peripheral vision. Sixty-one individuals had consulted their GP for possible neurological symptoms, summarised in Table 4.21. Twenty-three individuals who had consulted their GP for two or more possible neurological symptoms on separate occasions are included in the table more than once.

Table 4.19 Investigations for Blackouts, Periods of Loss of Consciousness or Fainting Episodes

Study ID	Details	Investigations
DAR72-1	*Query of Temporal Lobe Epilepsy* GP notes report *'temporal lobe epilepsy or vasovagal attacks'.*	At interview reported having brain scans to investigate blackouts.
DAR17-1	*Query of Petit Mal Epilepsy* Reported periods of loss of concentration/daydreaming at school.	At interview reported having an EEG – unsure of results.
DAR27-1	*Query of Epileptic Fit* GP notes report *'query epileptic fit'.* Reported having unusual falls/blackouts.	Unsure.
DAR18-1	*Query of Epileptic fit* GP notes report *'loss of consciousness which may have been grand mal in nature'.*	From notes: EEG was suggestive of focal activity from the left posterior temporal region. CT scan showed an increased density lesion. *Thought to be either an aneurysm or medial sphenoid ridge meningoma.*
DAR99-1	Reported fainting episodes since teenage years occurring anywhere.	Reported having EEG and CT scans.
DAR91-1	Hospital notes report a history of headache and fainting episodes and one episode of *'transient loss of consciousness lasting 3-4 minutes no associated convulsions or incontinence'.*	From hospital notes: EEG and head CT within normal range.
DAR55-1	Reported having one blackout.	Reported investigations, including brain scans, were normal.
DAR52-2	Reported a 2 week period of blackouts whilst being treated in hospital for a flare of her DD.	Reported blood tests and brain scans were all normal.
DAR92-1	Reported fainting on a few occasions, first age 15.	Reported CT scan taken following one episode was normal.
DAR48-1	From GP notes: investigations after collapsing, having headaches at the time.	From GP notes: CT scan showed some atrophic changes.
DAR28-1	Reported fainting attacks since the age of 11 also recorded in medical notes.	In medical notes: CT scan for syncopal attacks normal.
DAR4-1	Reported periods of collapsing with associated with numbness one side of the body and slurred speech, episodes recorded in medical notes.	From medical notes: neurologist reported no abnormal neurological signs.
DAR23-1	Faint feelings and light-headedness recorded in GP notes.	Referred to a neurologist - no neurological symptoms found.

Table 4.20 Investigations of Neurological Symptoms by a Neurologist and/or Neurological Investigations

Type of neurological condition investigated by neurologist or neurological procedure	Frequency
Epilepsy	3
Blackouts, periods of loss of consciousness or fainting episodes	13
Headaches	6
Hearing Problems	9
Other*	9
Total**	**40**

* Viral encephalitis; viral meningitis; other meningitis; CT scan or peripheral vision; head MRI investigations for slight paralysis; investigations for left sided weakness following 3 possible strokes; CT scan poor memory; halting gait; loss of feeling in leg whilst driving.
**Four individuals who had been treated or investigated for two different neurological conditions are included in the table twice.

Table 4.21 GP Consultation for Neurological Symptoms

Type of neurological condition	Frequency
Epilepsy	3
Fits, Faints, blackouts	24
Headaches	33
Hearing Problems	14
Other*	13
Total**	**87**

* Viral encephalitis; viral meningitis; other meningitis; CT scan or peripheral vision; head MRI investigations for slight paralysis; investigations of left sided weakness following 3 possible strokes; CT scan poor memory; halting gait; loss of feeling in leg whilst driving; dizziness; hand shaking; positional vertigo; twitching legs.
**23 individuals who had consulted their GP for two or more possible neurological symptoms are included in the table more than once.

4.4.4 Learning Difficulties

The frequencies of individuals diagnosed with dyslexia and individuals who received extra help at school are summarised in Table 4.22. Five individuals reported that they had been given a diagnosis of dyslexia and a further nine individuals reported that they received extra help at school for problems with reading and/or spelling and/or maths. This included an individual aged 29 who had attended a school for children with behavioural and learning difficulties and an individual aged 21 with a lifetime diagnosis of tonic-clonic epilepsy who was diagnosed with a language area based learning difficulty at the age of 11.

Fifty-nine individuals returned the Adult Dyslexia Checklist (ADC) (previously described in Chapter 3 section 3.2.6.2 pg. 43). Eleven individuals (18.6%) gave nine or more YES responses on the questionnaire indicating difficulty. The numbers of individuals with a diagnosis of dyslexia, receiving extra help at school and scoring nine or more YES responses on the ADC are shown in Table 4.23. In total 21 individuals had either a diagnosis of dyslexia, received extra help at school or scored nine or more on the ADC or a combination of the three.

Table 4.22 Individuals Diagnosed with Dyslexia and/or Reported Receiving Extra Help at School

	Frequency
Diagnosis of dyslexia	5
Received extra help at school	13

Four Individuals diagnosed with dyslexia and received extra help at school are included in the table twice.

Table 4.23 Summary of Measures of Learning Difficulties

Measure of Learning Difficulties	Frequency
Diagnosis of dyslexia only	1*
Received extra help at school only	7 (includes 4*)
≥ 9 YES responses on the ADC only	7
Diagnosis of dyslexia AND received extra help at school only	2 (includes 1*)
Diagnosis of dyslexia AND ≥ 9 YES responses on the ADC only	0
Received extra help at school AND ≥ 9 YES responses on the ADC only	2
Diagnosis of dyslexia AND received extra help at school AND ≥ 9 YES responses on the ADC	2
TOTAL	**21**

n* = number of individuals not returning the Adult Dyslexia Checklist. ADC= Adult Dyslexia Checklist.

4.4.5 Section Summary

This section has described the lifetime prevalence of additional neuropsychiatric features in the sample including epilepsy, neurological investigations and treatments and learning difficulties. The following section describes the IQ scores of the sample.

4.5 IQ Scores

Eighty-five individuals completed the two-subset version of the Weschsler Abbreviated Scale of Intelligence (WASI) (previously described in chapter 3 section 3.2.7 pg. 44). The descriptives and distributions of IQ scores are summarised in Table 4.24 and Table 4.25 & Figure 4.7 respectively. The reasons for the remaining 15 individuals not completing the IQ measure are reported in Table 4.26. The mean IQ score was 100, which is within the average range of IQ. Seventy-eight individuals (92%) had IQ scores within the range classified as being average. Two individuals (2.4%) had scores within the superior IQ range. Four individuals (4.7%) had scores within the borderline category and one individual had a score within the extremely low category.

Table 4.24 IQ Scores –Descriptives

N	Mean	Std. Deviation	95% CI	Range
85	100.38	13.143	97.54-103.21	67-125

Table 4.25 IQ Scores-Distributions

IQ Score	Classification	Frequency	%
120-129	Superior	2	2.4
110-119	High Average	18	21.2
90-109	Average	48	56.5
80-89	Low Average	12	14.1
70-79	Borderline	4	4.7
69 and below	Extremely Low	1	1.2

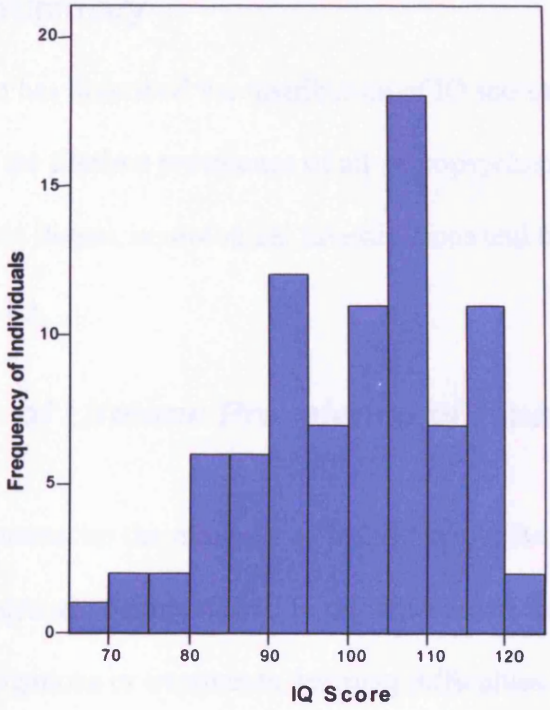


Figure 4.7 Distribution of IQ Scores

Table 4.26 Reasons Fifteen Individuals did not complete the IQ Measurement

	N
English not first language	2
Time constraints/not feasible	7
Not happy to complete	3
Unable to concentrate due to slightly high mood	1
Unable to concentrate due to current medication	1
Too anxious	1
Total	**15**

4.5.1 Relationship of IQ with Age and Gender

There was a significant positive correlation between IQ and age at interview (r=0.270, n=85, p=0.012). Descriptive data for the IQ scores of males and females are summarised in Table B-6 pg. 299. There was no significant difference between the mean IQ scores of males and females (t=0.111, df=83, p=0.912).

4.5.2 Section Summary

This section has described the distribution of IQ scores in the sample. The following section summaries the lifetime prevalence of all neuropsychiatric features in the sample including psychiatric illness, neurological investigations and treatments, learning difficulties and below average IQ.

4.6 Summary of Lifetime Prevalence of Neuropsychiatric Features

Table 4.27 summaries the numbers of individuals in the sample with a lifetime history of any DSM-IV diagnosis, being referred to psychiatric services, being referred for neurological investigations or treatments, learning difficulties and having a below average IQ (<80). Fifty-two individuals had either been referred to secondary psychiatric services and/or been referred to a neurologist at some point in their life. In total 78 individuals in the sample had a lifetime history of *any* of the lifetime neuropsychiatric phenotypes examined in this study.

Table 4.27 Frequencies of Individuals with Specific Lifetime Neuropsychiatric Features and any Neuropsychiatric Phenotype

Lifetime Neuropsychiatric Feature	N
Any lifetime DSM-IV disorder	55
Referred to psychiatric services	25
Referred for neurological investigations or treatments	36
Learning difficulties	21
Below average IQ (<80)	6
Referred to psychiatric services OR referred for neurological investigations or treatments	52
*Any neuropsychiatric phenotype	78

* Number of individuals with a lifetime history of any DSM-IV disorder OR being referred to psychiatric services OR being referred for neurological investigations or treatments OR learning difficulties OR IQ <80.

The following section describes the prevalence of family history of neuropsychiatric features including psychiatric illness and epilepsy in the sample.

4.7 *Family History of Neuropsychiatric Features*

4.7.1 Family History of Psychiatric Illness and Epilepsy in 1st Degree Relatives

The numbers of individuals having first-degree relatives with a history of psychiatric illness and epilepsy are displayed in Table 4.28. In total 43 individuals reported they knew of a first-degree relative who had received treatment for a psychiatric illness or had experienced psychiatric symptoms causing significant impairment. This included thirteen individuals with multiple (two or more) first-degree relatives with a history of psychiatric illness. Six individuals had a first-degree relative with a diagnosis of epilepsy and one individual had two first-degree relatives diagnosed as having epilepsy.

Table 4.28 Family History of Psychiatric Illness and Epilepsy

	No First Degree Relative with Known History	1st Degree Relative with History	Multiple 1st Degree Relatives with History
Psychiatric Illness	57	30	13
Epilepsy	93	6	1

Figures are the numbers/ percentages of the 100 individuals with DD.

The numbers of individuals having first-degree relatives with a history of specific psychiatric illnesses are summarised in Table 4.29. The most common type of psychiatric illness in first-degree relatives was major depression. Thirty-two individuals had a family history of major depression. Two individuals reported that they had a first degree relative with a diagnosis of bipolar disorder, and a further two individuals gave descriptions of their relatives' psychiatric symptoms that were thought to be bipolar disorder, these are described in Table 4.30. Three individuals reported they had a first-degree relative with a diagnosis of schizophrenia. Three individuals had a first degree relative with a history of a severe psychiatric illness including episodes of psychosis and other psychiatric illnesses requiring admission to a psychiatric hospital. Eight individuals had a first-degree relative with other

psychiatric illnesses including anxiety disorders alcohol misuse/dependence and obsessive-compulsive disorder.

The 43 individuals with a first-degree relative with a history of psychiatric illness had a total of 64 first-degree relatives with a positive psychiatric history. Of these 64 relatives, 34 (53.1%) also had a diagnosis of DD, 23 (35.9%) did not have DD and in seven cases (10.9%) it was unknown whether or not they had DD (summarised in Table B-7 pg. 300). The seven individuals with a first-degree relative with a history of epilepsy had a total eight first-degree relatives with epilepsy. Of these eight relatives six (75%) had DD, one (12.5%) did not have DD and in one case (12.5) it was unknown whether or not they had DD (summarised in Table B-8 pg. 300).

Table 4.29 Family History of Psychiatric Illness - by Diagnosis

Diagnosis in 1st Degree Relatives of Individuals with DD	No First Degree Relative with Known Illness	1st Degree Relative with Illness	Multiple Affected 1st Degree Family Members
Major Depression	68	22	10
Bipolar Affective Disorder	96	4	0
Schizophrenia	97	3	0
Other Severe Psychiatric Disorder	97	2	1
Other Psychiatric Illness	92	6	2

Figures are the numbers/percentages of the 100 individuals with DD.
Individuals are only included positively in the table once for each of their relatives e.g. an individual with a 1st degree relative with schizophrenia and substance abuse is only rated as 1st degree relative with schizophrenia, an individual with one first degree relative with schizophrenia and another relative with substance abuse problems is rated as 1st degree relative with schizophrenia and 1st degree relative with other psychiatric illness.

Table 4.30 Descriptions of Family Members with a Definite or Possible History of Bipolar Disorder

Diagnosis of Index Individual	Family Member	DD	Description
Unaffected	Mother	No	Admitted to a psychiatric hospital aged 40 and 50, described as being 'high' with grandiose ideation.
Bipolar I	Mother	Yes	Described as seeing a psychiatrist for depression and anxiety, in daughters' medical notes described as having a bipolar illness.
Unaffected	Sister	Yes	Diagnosis of Bipolar Disorder.
Unaffected	Sister	No	Has seen a psychiatrist and has periods when her behaviour is very out of character. On lithium.

Examples of families with multiple members with neuropsychiatric features are displayed in Figure 4.8 to Figure 4.11.In family one (Figure 4.8) the index individual was a female who had a hypomanic episode following antidepressant treatment, she reported that her mother who also had DD had been admitted to a psychiatric hospital on numerous occasions. She had three sons with a diagnosis of heroin abuse/dependence. Two had a diagnosis of DD and had both suffered from depression and also blackouts and photoflash epilepsy respectively. Her younger son in his early 20s who did not have DD had an episode of psychosis following a period of sleep deprivation. In family two (Figure 4.9) four siblings all with DD also all had a history of neuropsychiatric features including, depression, bipolar disorder, an episode of psychosis following childbirth and a form of childhood epilepsy. Their father who also had DD had experienced depression and the same type of childhood epilepsy as his son. In family three (Figure 4.10) four siblings had a history of depression and/or suicide attempts. Three of these individuals also had DD. Their father who had DD also had a history of depression. The 30-year-old daughter of the index individual had a diagnosis of DD and no history of psychiatric illness. The index individual in family four (Figure 4.11) was a female in her late 50's with a history of depression and anxiety her medical notes also reported a short period of paranoia which did not reach delusional intensity. She reported that her sister who she did not think had DD had also experienced symptoms of anxiety. She was unsure whether her father had DD although remembered him seeing a dermatologist, thought that he had had a possible breakdown. One adult son without DD had received treatment for anxiety and depression and had made suicide attempts. Her other son with DD had a diagnosis of schizophrenia and depression and had also made suicide attempts. She was unsure whether her adult daughter had DD but felt she had probably suffered from depression.

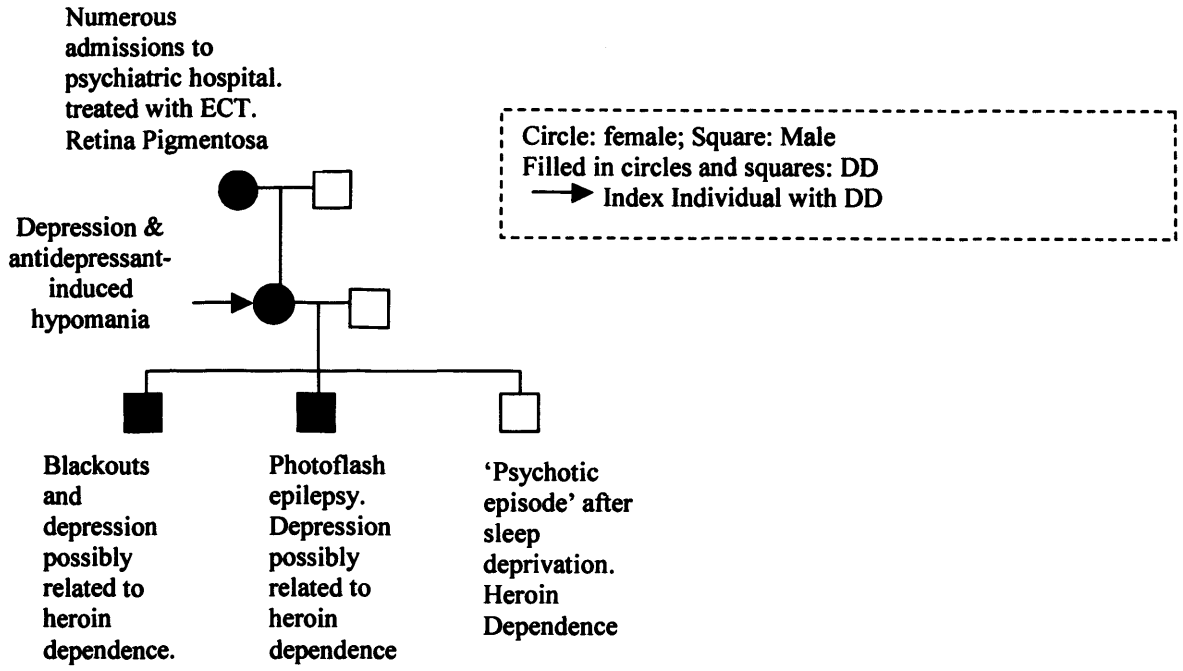


Figure 4.8 Family One: Multiple Members with DD and Neuropsychiatric Illness

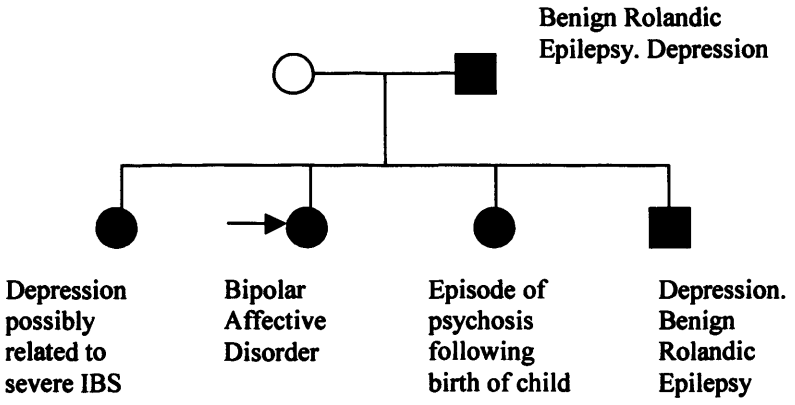


Figure 4.9 Family Two: Multiple Members with DD and Neuropsychiatric Illness

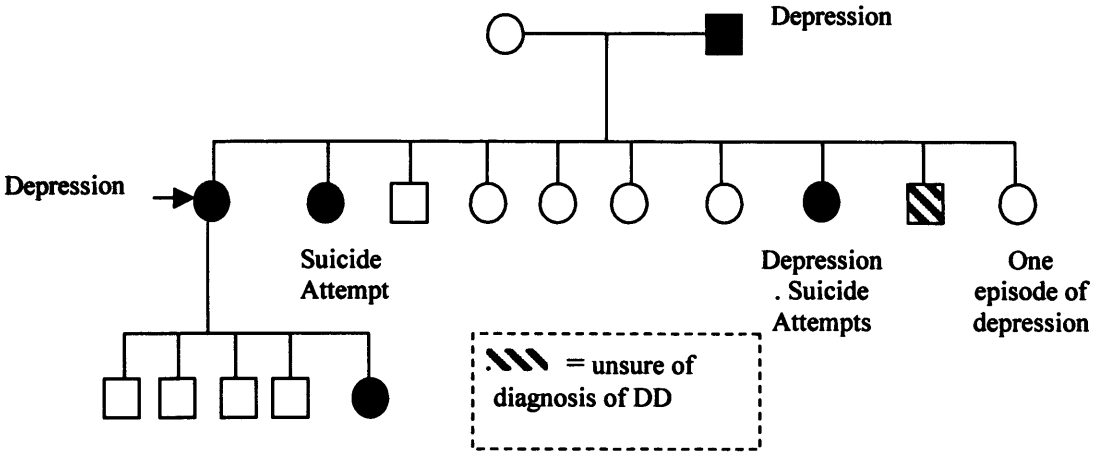


Figure 4.10 Family Three: Multiple Members with DD and Neuropsychiatric Illness

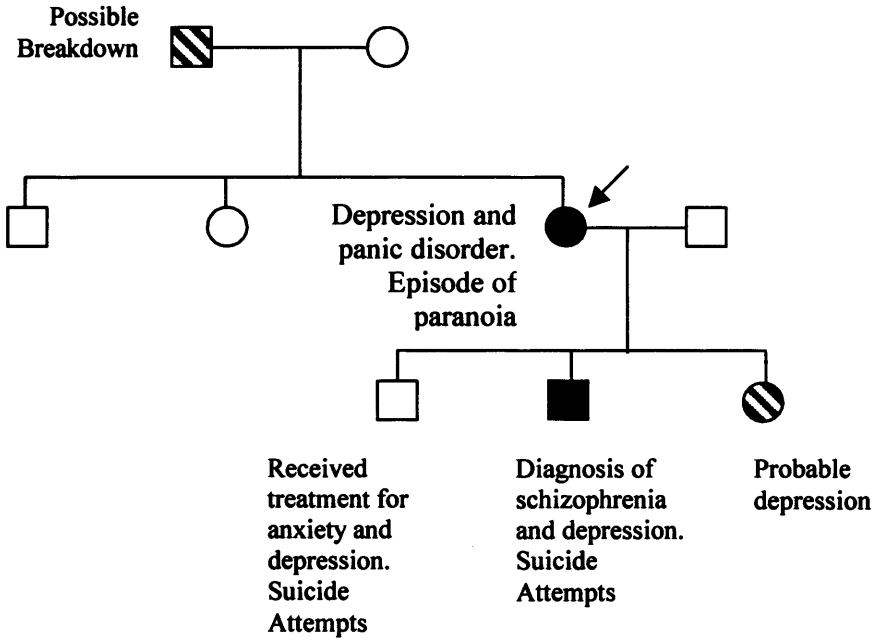


Figure 4.11 Family Four: Multiple Members with DD and Neuropsychiatric Illness

4.7.2 Section Summary

This section has described the prevalence of family history of neuropsychiatric features in the sample including psychiatric illness and epilepsy. The following section investigates the relationships between lifetime neuropsychiatric features, IQ scores and family history of psychiatric illness among individuals in the sample.

4.8 Relationship between Lifetime Neuropsychiatric Features, IQ Scores and Family History of Psychiatric Illness

The relationships between lifetime DSM-IV diagnosis of psychiatric illness, history of investigations for blackouts/loss of consciousness, lifetime diagnosis of epilepsy, family history of psychiatric illness, below average IQ score and history of learning difficulties among individuals in the sample is displayed in Table B-9 pg 300. Three significant relationships are printed in bold and the frequencies and percentages of these are displayed in Table B-10 to Table B-11 pg. 301. Significantly more individuals with a history of investigations for blackouts/loss of consciousness had a lifetime DSM-IV diagnosis of psychiatric illness (85% vs. 53%; χ^2=4.411, df=1, p=0.036) and a family history of psychiatric illness (69% vs. 40%; χ^2=3.781, df=1, p=0.052). Significantly more individuals with learning difficulties had a below average IQ score (45% vs. 12%; Fisher's, p=0.003).

4.8.1 Section Summary

This section has described the relationships between lifetime neuropsychiatric features, IQ scores and family history of psychiatric illness among individuals in the sample. The following section investigates subjective and objective relationships between the clinical features of DD and the presence and severity of lifetime psychiatric symptoms observed in the sample.

4.9 Relationship between DD Clinical Features and Lifetime Psychiatric Features

This section investigates the relationship between DD clinical features and the presence and severity of lifetime psychiatric features in the sample. Individual's subjective thoughts regarding the relationship between the psychiatric features they had experienced and

the features of DD are reported in section 4.9.1. The relationship between age of onset of DD and age of onset of psychiatric symptoms and illness is reported in section 4.9.2. Relationships between the clinical features of DD, including age of onset and duration, severity, symptoms (pain and malodour), impact on quality of life and family history of DD, and lifetime psychiatric features, including history of a lifetime DSM-IV diagnosis, history of suicidal thoughts and/or attempts and key psychiatric variables (age of onset of psychiatric illness, number of episodes of depression and longest episode of depression), are investigated in sections 4.9.3 to 4.9.7. A summary of all the trends and significant relationships found is presented in section 4.9.8.

4.9.1 Subjective Relationship between DD and Psychiatric Features

Individuals who reported a history of psychiatric illness were asked whether they felt there had been a lifetime and/or temporal relationship between the symptoms of their DD and the occurrence of episodes of psychiatric illness. A positive *lifetime* relationship indicated individuals felt that all the episodes of psychiatric illness they had experienced were due to having DD. A positive *temporal* relationship indicated that individuals had experienced episodes of psychiatric illness that they felt were due to the symptoms of DD although individuals did not attribute all episodes of psychiatric illness as being due to having DD.

As it has been shown in 4.3.1 pg. 61, 50 individuals in the sample had a DSM-IV lifetime diagnosis of mood disorder. All of these individuals had a history of depressive symptoms. The frequencies and percentages of individuals reporting subjective lifetime and/or temporal relationships between episodes of depression and the symptoms of DD are summarised in Table B-12 pg. 301 and displayed in Figure 4.12. Three individuals (six percent) with lifetime DSM-IV diagnoses of Major Depressive Disorder (Recurrent), Dysthymic Disorder and Depression Not otherwise Specified respectively reported a *lifetime* relationship indicating that they felt all the episodes of depression they had experienced were

directly caused by the symptoms of DD. Nineteen individuals (38%) reported a *temporal* relationship indicating that they felt some episodes of depression they had experienced were caused by the symptoms of DD, although they had experienced other episodes of depression that they did not relate to having DD.

Fifteen individuals in the sample had a DSM-IV lifetime diagnosis of anxiety disorder. One of these individuals with a DSM-IV diagnosis of panic disorder reported a possible lifetime relationship and definite temporal relationship between panic episodes and the symptoms of DD. All other individuals did not relate their symptoms of anxiety to the symptoms of DD.

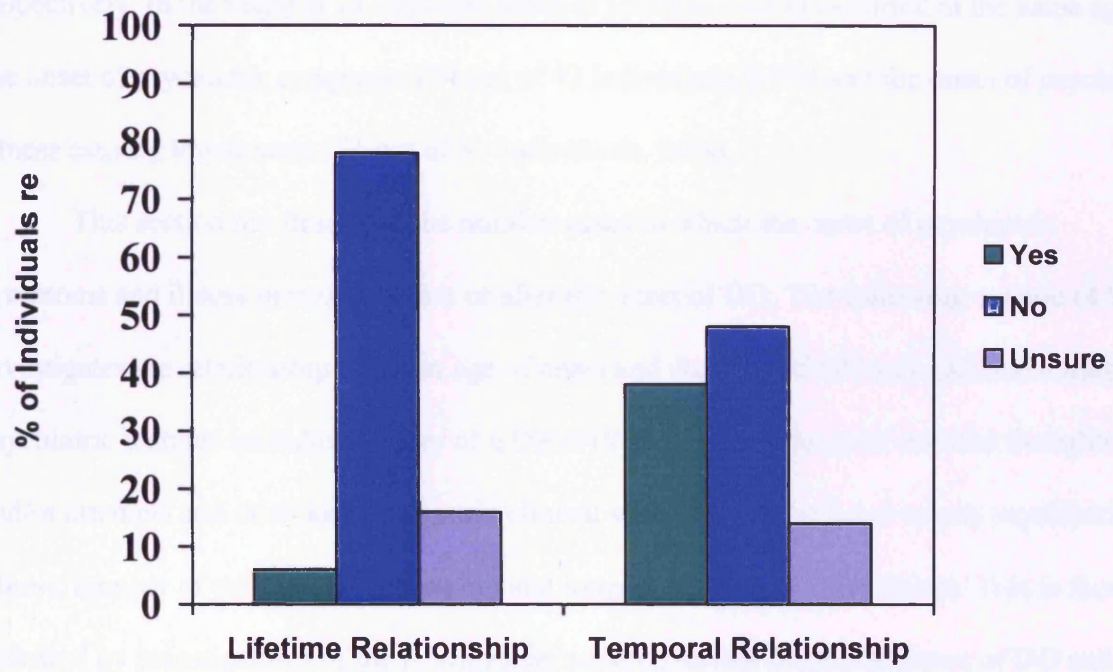


Figure 4.12 Percentages of Individuals Reporting a Lifetime and Temporal Relationship between Episodes of Depression and the Symptoms of DD

4.9.2 Age of Onset of DD and Age of Onset of Psychiatric Symptoms and Illness

To investigate the relationship between age of onset of DD and the age of onset of psychiatric illness, the number of individuals for which the onset of DD preceded or occurred at the same age as the onset of psychiatric illness was compared with the number of individuals in whom the onset of psychiatric illness preceded the onset of DD. Of the 55 individuals with a DSM-IV diagnosis, the age of onset of DD was known for 53 individuals. Of these individuals, the age of onset of any psychiatric symptoms and the age of onset of any psychiatric illness causing impairment could be determined for 42 and 37 individuals respectively. In the majority of cases the onset of DD preceded or occurred at the same age as the onset of psychiatric symptoms (34 out of 42 individuals, 81%) and the onset of psychiatric illness causing impairment (31 out of 37 individuals, 84%).

This section has described the number cases in which the onset of psychiatric symptoms and illness occurred before or after the onset of DD. The following section (4.9.3) investigates the relationship between age of onset and duration of DD and additional lifetime psychiatric features including history of a DSM-IV diagnosis, history of suicidal thoughts and/or attempts and other key psychiatric clinical variables (age of onset of any psychiatric illness, number of episodes of depression and longest duration of depression). This is then followed by investigations of the relationship between further clinical features of DD and the presence and severity of lifetime psychiatric features in sections 4.9.4 to 4.9.7.

4.9.3 Age of Onset and Duration of DD - Relationship with Psychiatric Features

4.9.3.1 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

Descriptives for the age of onset and duration of DD among individuals with and without a lifetime DSM-IV diagnosis and among individuals with and without a history of suicidal thoughts or attempts are shown in Table B-13 pg. 302. Individuals with and without a lifetime DSM-IV diagnosis did not significantly differ in their median age of onset of DD (13 vs. 14 years; U=840.5, n=90, p=0.250) or the median duration of their DD (31 vs. 30 years; U=911.5, n=90, p=0.571). Similarly, individuals with a history of no suicidal thoughts or attempts, suicidal thoughts and suicide attempts did not significantly differ in their median age of onset (14 vs. 14 vs. 14.5 years; H= 0.783, df =2, p= 0.676) or the median duration of their DD (29 vs. 36 vs. 32 years; H= 2.262, df= 2, p= 0.323).

4.9.3.2 Key Psychiatric Clinical Variables

Correlations between the age of onset and duration of DD and key psychiatric clinical variables (age of onset of any psychiatric illness, number of episodes of depression and longest duration of depression) are shown in Table B-14 pg. 302. There were no significant correlations between age of onset and duration of DD and any of the key psychiatric clinical variables.

4.9.4 DD Severity - Relationship with Psychiatric Features

4.9.4.1 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

Comparisons of the percentages of individuals with a lifetime DSM-IV diagnosis and a history of suicidal thoughts or attempts among individuals with mild, moderate and severe

DD are shown in Table B-15 pg. 303. There was no significant difference in the percentage of individuals with a positive DSM-IV diagnosis among individuals with mild, moderate and severe DD (50% vs. 63% vs. 60%; χ^2=1.047, df=2, p=0.592). There was also no significant difference in the percentages of individuals with a history of suicidal thoughts and attempts among individuals with mild, moderate and severe DD (χ^2= 6.79, df=4, p=0 .143). However, there was a trend for the prevalence of suicidal thoughts, but not suicide attempts, to increase with DD severity (see Figure 4.13).

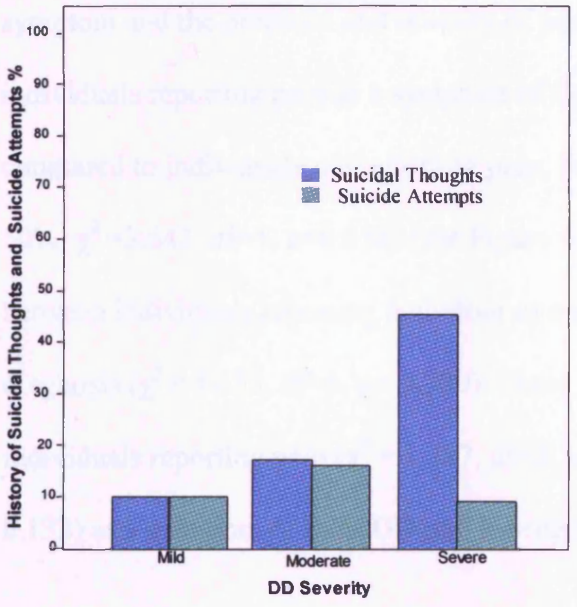


Figure 4.13 Percentage of Individuals with a History of Suicidal Thoughts and Suicide Attempts among Individuals with Mild, Moderate and Severe DD

4.9.4.2 Key Psychiatric Clinical Variables

Descriptives for key psychiatric clinical variables among individuals with mild, moderate and severe DD are shown in Table B-16 pg. 303. Individuals with mild, moderate and severe DD did not significantly differ in their median age of onset of psychiatric illness (H= 0.229, df =2, p = 0.892), the median number of episodes of depression (H=0.030, df =2, p =0.985) or the median longest duration of depression they had experienced (H=2.525, df =2 p =0.283).

4.9.5 Symptoms of DD - Relationship with Psychiatric Features

4.9.5.1 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

Comparisons of the percentages of individuals with a lifetime DSM-IV diagnosis and a history of suicidal thoughts or attempts among individuals who reported pain and malodour as being a symptom of their DD are shown in Table B-17 pg. 304. As almost all individuals in the sample reported itching was a symptom of their DD, the relationship between this symptom and the presence and severity of psychiatric features was not investigated. More individuals reporting pain as a symptom of their DD had a lifetime DSM-IV diagnosis compared to individuals not reporting pain, but this did not quite reach significance (70% vs. 50%; χ^2 =3.643, df=1, p=0.056) (see Figure 4.14). There was no significant relationship between individuals reporting malodour as a symptom of DD and having DSM-IV lifetime diagnosis (χ^2 = 1.035, df=1, p= 0.309). There was no significant relationship between individuals reporting pain (χ^2 = 1.887, df=2, p= 0.389) or malodour (χ^2 = 4.043 df=2, p= 0.132) as a symptom of their DD and having a history of suicide thoughts or suicide attempts.

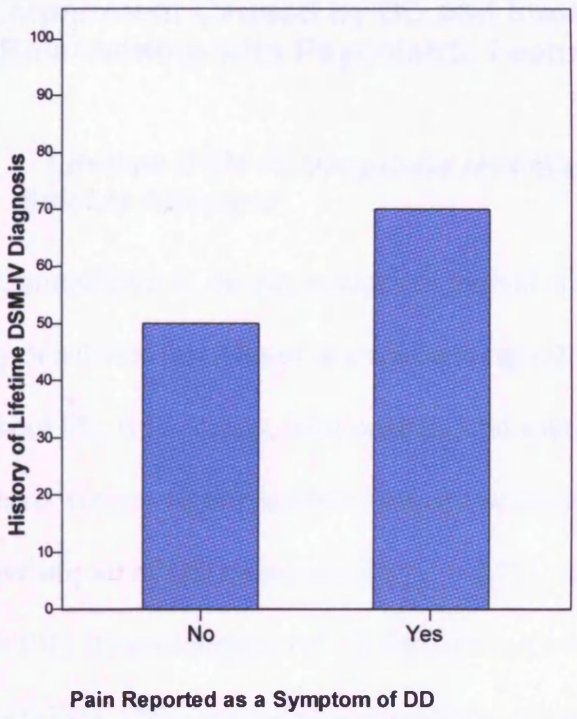


Figure 4.14 Percentage of Individuals with a Lifetime DSM-IV Diagnosis among Individuals Not Reporting and Reporting Pain as a Symptom of Their DD

4.9.5.2 Key Psychiatric Clinical Variables

Descriptives for key psychiatric variables among individuals not reporting and reporting pain and malodour as a symptom of their DD are shown in Table B-18 pg. 304. Individuals reporting and not reporting pain and malodour as symptoms of their DD did not significantly differ in their age of onset of psychiatric illness, the number of episodes of depression or the longest duration of depression they had experienced.

4.9.6 Impairment Caused by DD and Impact on Quality of Life-Relationship with Psychiatric Features

4.9.6.1 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

Comparisons of the percentages of individuals with a lifetime DSM-IV diagnosis and a history of suicidal thoughts or attempts among individuals reporting an impact of DD on their school life, work/career, relationships, and social activities are shown in Table B-19 pg. 305. There were no significant relationships between lifetime DSM-IV diagnosis and the subjective impact of DD on school life ($\chi^2 = 2.771$, df=3, p= 0.428), work/career ($\chi^2 = 1.778$, df=2, p= 0.411), relationships ($\chi^2 = 2.689$, df=2, p= 0.261) and social activities ($\chi^2 = 2.916$, df=2, p= 0.233). There were also no significant relationships between lifetime history of suicidal thoughts and suicide attempts and the subjective impact of DD on school life ($\chi^2 =$ 4.325 df=6, p= 0.653), relationships ($\chi^2 = 4.585$, df=4, p= 0.337) and social activities ($\chi^2 =$ 6.161, df=4, p= 0.193). There was a significant relationship between lifetime history of suicidal thoughts and suicide attempts and the subjective impact of DD on work/career ($\chi^2 =$ 11.595, df=4, p= 0.021). Further 2x2 chi-square analyses revealed that a significantly higher proportion of individuals reporting DD had a mild impact on their work/career had made a suicide attempt compared to individuals reporting no impact of DD on their work/career (36.4% vs. 7.1% Fisher's exact test, p= 0.025), shown in Figure 4.15.

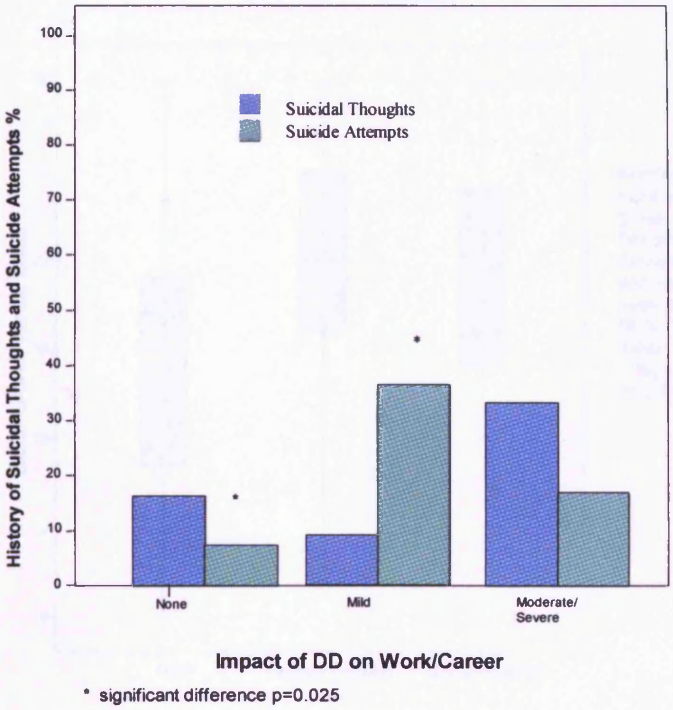


Figure 4.15 Percentage of Individuals with a History of Suicidal Thoughts and Suicide Attempts among Individuals Reporting Varying Degrees of Impact of DD on their Work/Career

Descriptives for DLQI last and worst week scores among individuals with and without a DSM-IV lifetime diagnosis and among individuals with no history of suicidal thoughts or suicide attempts, a history of suicidal thoughts and a history of suicide attempts are summarised in Table B-20 pg. 306. Individuals with and without a lifetime DSM-IV diagnosis did not significantly differ in their median scores on the DLQI-Last week (5 vs. 3; U= 846.000, n= 91, p = 0.215) or DLQI-Worst week (16.5 vs. 14; U= 841.500, n = 91, p = 0.203). Individuals with a history of no suicidal thoughts or attempts, suicidal thoughts and suicide attempts did not significantly differ in their median scores on the DLQI-Last week (H= 2.146, df =2, p = 0.342) but did significantly differ in their median scores on the DLQI – Worst week (H = 7.120, df = 2, p = 0.028). Further Mann-Whitney U tests indicated that individuals with a history of suicidal thoughts had significantly higher median scores on the DLQI-Worst week than individuals with no history of suicidal thoughts/suicide attempts (U = 333, n = 80, p = 0.017), shown in Figure 4.16.

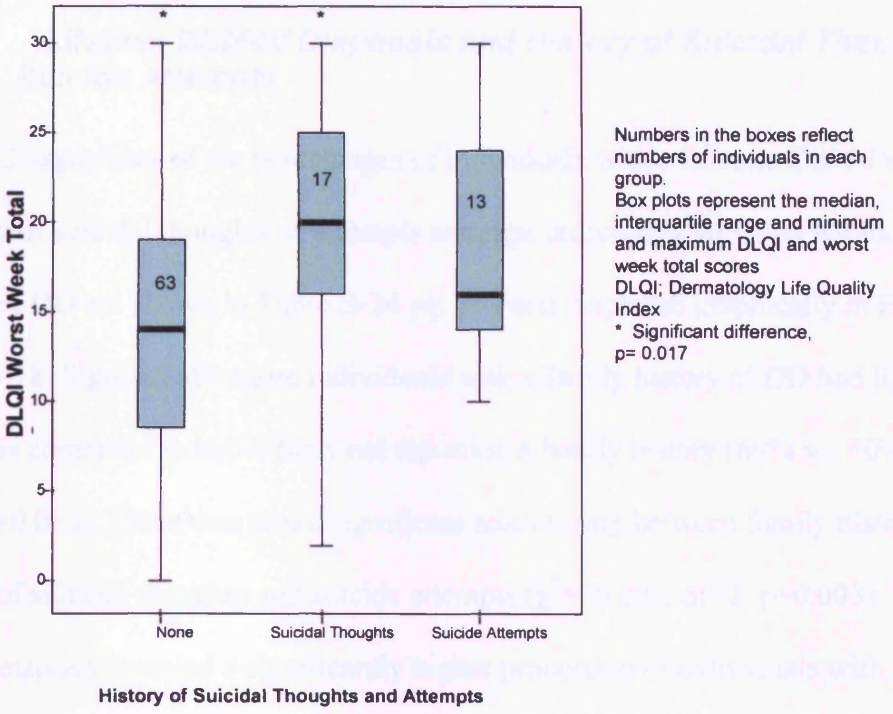


Figure 4.16 DLQI - Worst Week Scores among Individuals with and without a History of Suicidal Thoughts and Attempts

4.9.6.2 Key Psychiatric Clinical Variables

Descriptives for key psychiatric variables among individuals reporting the impact of having DD on their school life, work/career, relationships and social activities as being none, mild, moderate or not applicable are shown in Table B-21 pg. 307 and Table B-22 pg. 308. In all of these four domains, individuals subjectively reporting the impact of having DD as being none, mild, moderate, severe or not applicable did not significantly differ in their age of onset psychiatric illness, the number of episodes of depression or the longest duration of depression they had experienced. Correlations between DLQI last week and worst week scores and key psychiatric variables are shown in Table B-23 pg. 309. There was no significant correlation between DLQI last week and worst week scores and any of the key psychiatric variables.

4.9.7 Family History of DD - Relationship with Psychiatric Features

4.9.7.1 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

Comparisons of the percentages of individuals with a lifetime DSM-IV diagnosis and a history of suicidal thoughts or attempts amongst individuals with and without a family history of DD are shown in Table B-24 pg. 309 and displayed graphically in Figure 4.17 and Figure 4.18. Significantly more individuals with a family history of DD had lifetime DSM-IV diagnosis compared to individuals not reporting a family history (69% vs. 40%, χ^2=6.175, df=1, p=0.013). There was also a significant relationship between family history of DD and history of suicidal thoughts and suicide attempts (χ^2=10.803, df=2, p=0.003). Further 2x2 chi-square analyses revealed a significantly higher proportion of individuals with a family history of DD had a lifetime history of suicidal thoughts compared to individuals with no family history of DD (27% vs. 0%; χ^2=9.503, df=1, p=0.002).

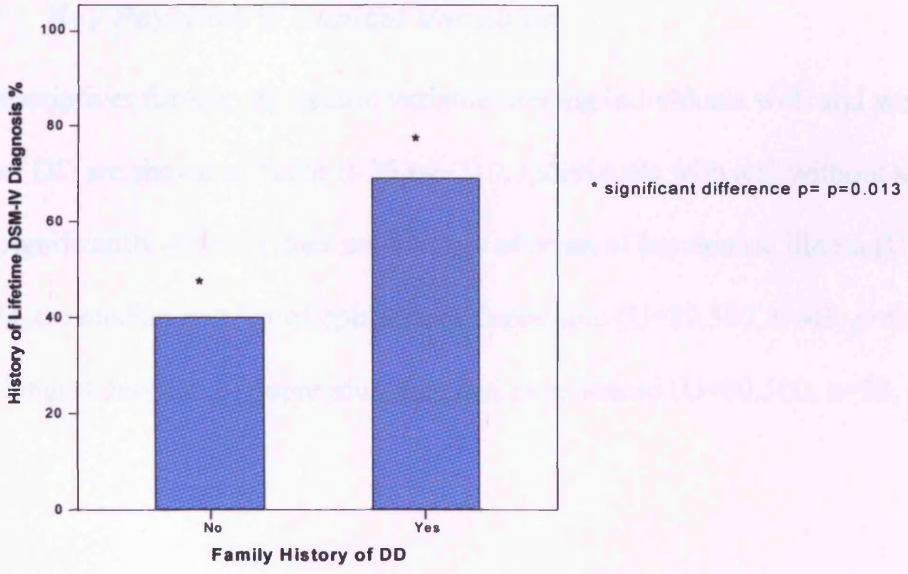


Figure 4.17 Percentage of Individuals with a Lifetime DSM-IV Diagnosis among Individuals with and without a Known Family History of DD

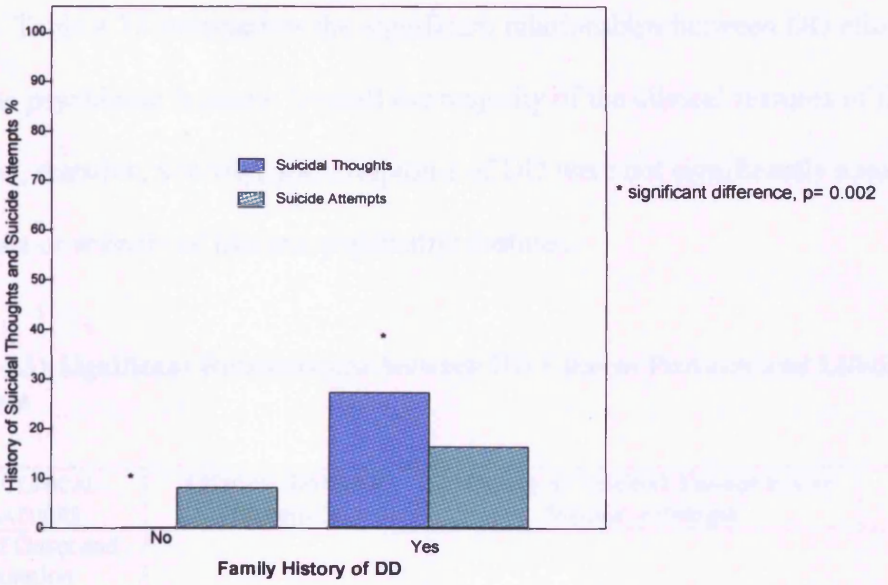


Figure 4.18 Percentage of Individuals with a History of Suicidal Thoughts and Suicide Attempts among Individuals with and without a Known Family History of DD

4.9.7.2 Key Psychiatric Clinical Variables

Descriptives for key psychiatric variables among individuals with and without a family history of DD are shown in Table B-25 pg. 310. Individuals with and without a family history did not significantly differ in their median age of onset of psychiatric illness (U=74, n=37, p=0.433), the median number of episodes of depression (U=89.500, n=41, p=0.562) or the median longest duration of depression they had experienced (U=80.500, n=39, p=0.470).

4.9.8 Section Summary

Table 4.31 summarises the significant relationships between DD clinical features and lifetime psychiatric features. Overall the majority of the clinical features of DD, including age of onset, duration, severity, and symptoms of DD were not significantly associated with the presence or severity of lifetime psychiatric features.

Table 4.31 Significant Relationships between DD Clinical Features and Lifetime Psychiatric Features

DD CLINICAL FEATURES	Lifetime DSM-IV Diagnosis	History of Suicidal Thoughts and Suicide Attempts	Key Psychiatric Clinical Variables
Age of Onset and Duration	-	-	-
Severity	-	-	-
Symptoms (pain and malodour)	**	-	-
Subjective Impairment and Impact on Quality of Life	-	A significantly higher proportion of individuals reporting a mild impact of DD on their work/career had a history of suicide attempts compared to individuals reporting no impact of DD on their work/career. Individuals with a history of suicidal thoughts had significantly higher scores on the DLQI-Worst week than individuals with no history of suicidal thoughts or suicide attempts.	-
Family History	A significantly higher proportion of individuals with a family history of DD had a lifetime psychiatric illness compared to individuals with no family history of DD	A significantly higher proportion of individuals with a family history of DD had a lifetime history of suicidal thoughts compared to individuals with no family history of DD.	-

** Relationship almost reached significance; a higher proportion of individuals reporting pain a as a symptom of their DD had a lifetime DSM-IV diagnosis compared to individuals not reporting pain (70% vs. 50%; χ^2=3.643, df=1, p=0.056). DLQI; Dermatology Life Quality Index.

The following section investigates the relationship between the clinical features of DD and history of learning difficulties and IQ scores.

4.10 Relationship between DD Clinical Features and Learning Difficulties and IQ scores

This section investigates the relationship between DD clinical features, including age of onset, severity and family history of DD and history of learning difficulties and IQ scores.

4.10.1 Age of Onset and Duration of DD - Relationship with Learning Difficulties

Descriptives for the age of onset of DD among individuals with and without learning difficulties are summarised in Table B-26 pg. 310. Individuals with and without learning difficulties did not significantly differ in their median age of onset of DD (13 vs. 14 years; U= 605.0, n=96, p = 0.244).

4.10.2 DD Severity-Relationship with Learning Difficulties

There was no significant difference in the percentage of individuals with learning difficulties among individuals with mild, moderate and severe DD (23% vs. 19% vs. 27%; χ^2=0.403, df=2, p=0.805) (see Table B-27 pg. 310).

4.10.3 Subjective Impact of DD on School Life- Relationship with Learning Difficulties

There was no significant difference in the percentage of individuals with learning difficulties among individuals reporting the impact of DD on their school life as not applicable, none, mild or moderate/severe (15% vs. 24% vs. 27% vs. 24%; χ^2 = 1.277, df=3, p= 0.758) (see Table B-28 pg 311).

4.10.4 Family History of DD-Relationship with Learning Difficulties

There was no significant difference in the percentage of individuals with learning difficulties among individuals reporting and not reporting a family history of DD although

there was a trend for a higher percentage of individuals reporting a family history of DD to have learning difficulties (24% vs. 15%; χ^2=0.745, df=1, p=0.388) (see Table B-29 pg 311.).

4.10.5 Age of Onset of DD- Relationship with IQ Scores

There was a significant positive correlation between age of onset of DD and IQ Scores (rho= 0.240, n=82, p=0.030). As previously reported, there was a positive correlation between IQ score and age at interview (r=0.270, n=85, p=0.012). When controlling for age at interview there was no significant correlation between age of onset of DD and IQ scores (r=0.117, df=79, p=0.299).

4.10.6 DD Severity- Relationship with IQ Scores

Descriptives of IQ scores among individuals with mild, moderate and severe DD are summarised in Table B-30 pg. 311. Individuals with mild, moderate and severe DD did not significantly differ in their median IQ scores (107 vs. 102 vs. 92; H=5.256, df=2, p=0.072) although there was a trend for IQ scores to decrease with an increase in DD severity, which almost reached significance, shown in Figure 4.19.

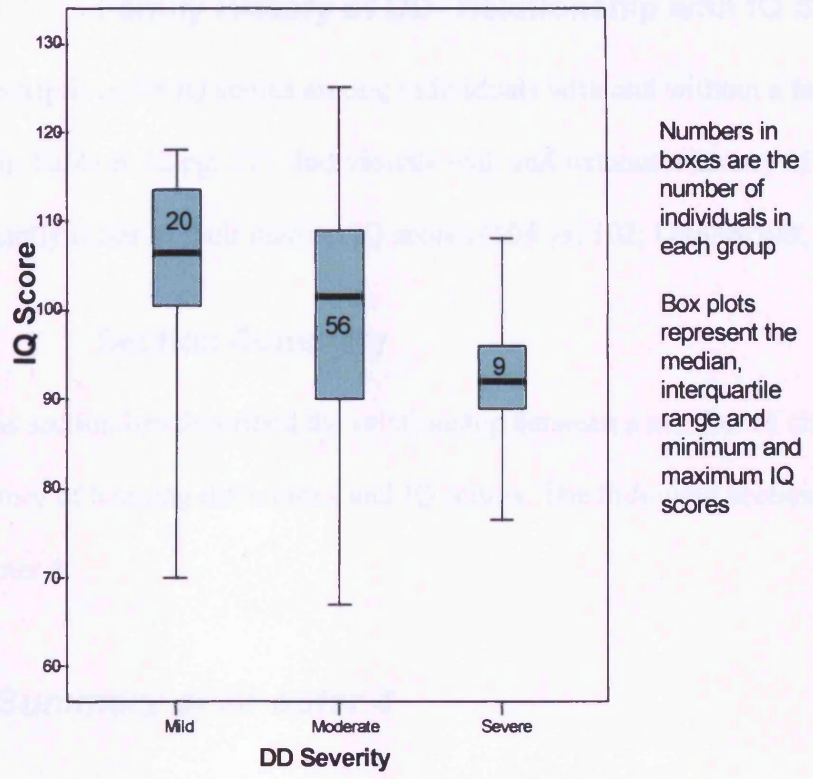


Figure 4.19 IQ Scores among Individuals with Mild, Moderate and Severe DD

4.10.7 Subjective Impact of DD on School Life - Relationship with IQ Scores

Descriptives of IQ scores among individuals reporting the impact of DD on their school life as not applicable, none, mild or moderate/severe are summarised in Table B-31 pg. 311. Individuals reporting the impact as being not applicable, none, mild or moderate/severe did not significantly differ in their median IQ scores (105 vs. 105 vs. 99.5 vs., 96; H=2.955, df=3, p=0.399). However, there was a trend for IQ scores to decrease with an increase in the reported impact of DD on school.

4.10.8 Family History of DD- Relationship with IQ Scores

Descriptives for IQ scores among individuals with and without a family history of DD are shown in Table B-32 pg. 312. Individuals with and without a history of DD did not significantly differ in their median IQ scores (104 vs. 102; U=636.500, n=79, p=0.935).

4.10.9 Section Summary

This section has described the relationship between a number of clinical features of DD and history of learning difficulties and IQ scores. The following section provides a summary of Chapter 4.

4.11 Summary of Chapter 4

This chapter has described the demographic, clinical and neuropsychiatric features of 100 unrelated individuals with DD. The main findings of the chapter are summarised below.

Clinical Features of DD

- 33% of individuals had been admitted to hospital on at least occasion for treatment of DD, individuals reported an impact of DD on their school life (28%), relationships (30%), work and career (35%) and social activities (53%).
- Individuals with moderate and severe DD reported the symptoms of DD had a greater impact on quality of life than individuals with a mild form of the disorder.
- 53% of individuals reported a definite family history of DD whereas 26% reported no family history of the disorder.

Lifetime Prevalence of Neuropsychiatric Illness

The second part of the chapter reported the lifetime prevalence of a number of neuropsychiatric features among individuals in the current sample. These figures are summarised in Table 4.32. Seventy eight percent of the sample had a lifetime history of *any* of the lifetime neuropsychiatric phenotypes measured in the current study, 55% had a lifetime diagnosis of any DSM-IV disorder, four percent had a lifetime diagnosis of Bipolar I Disorder, 13% of the sample had made a suicide attempt and 25% had been referred to psychiatric services.

Table 4.32 Lifetime Prevalence of Neuropsychiatric Features in 100 Unrelated Individuals with DD

Neuropsychiatric Phenotype	% Of Sample
Any neuropsychiatric phenotype	78
Any DSM-IV disorder	55
Any DSM-IV mood disorder	50
Major Depressive Disorder	30
Bipolar I Disorder	4
Suicide attempt	13
Suicidal thoughts	18
Referred by GP to secondary psychiatric services	25
Admitted to a psychiatric hospital	9
Referred to a neurologist and had neurological investigations or had been treated for a neurological condition	36
Epilepsy	3
Investigations for blackouts, periods of loss of consciousness or fainting episodes	13
Learning Difficulties	21
Below average IQ (<80)	6

Family History of Neuropsychiatric Features

The numbers of individuals in the current sample with first-degree relatives with a history of psychiatric illness and epilepsy have been reported. Comparisons of the prevalence of DD among first-degree relatives with a psychiatric illness and epilepsy found:

- Of 64 first-degree relatives with a positive psychiatric history, a higher percentage were reported to have DD (53.1%) than were not (35.9%).
- Of eight first-degree relatives with a diagnosis of epilepsy a higher percentage were reported to have DD (75%) than were not (12.5%).

Relationship between Lifetime Neuropsychiatric Features, IQ Scores and Family History of Psychiatric Illness

Investigations of the relationships between the presence of lifetime neuropsychiatric features, below average IQ scores and family history of psychiatric illness among individuals in the current sample have been described. The significant findings were:

- More individuals with a history of investigations for blackouts/loss of consciousness had a lifetime DSM-IV diagnosis of psychiatric illness and a family history of psychiatric illness.
- More individuals with learning difficulties had a below average IQ score

Relationships between DD Clinical Features and Lifetime Neuropsychiatric Features and IQ Scores

The final part of the chapter reported the findings of numerous investigations of relationships between the clinical features of DD, the presence and severity of lifetime neuropsychiatric features and IQ scores. Main findings included:

- Six percent of individuals felt that all the episodes of psychiatric illness they had experienced were due to the symptoms of DD and 38% of individuals felt at least one episode of psychiatric illness was due to the symptoms of DD.
- In 81% of cases the onset of DD preceded or occurred at the same age as the onset of psychiatric symptoms.

- Overall the majority of the clinical features of DD, including age of onset, duration, severity, and symptoms of DD were not significantly associated with the presence or severity of lifetime neuropsychiatric features or IQ scores. However, significant relationships and trends were observed and are summarised in Table 4.33.

The following chapter (Chapter 5) discusses these findings.

Table 4.33 Summary of the Significant Relationships and Trends Between DD Clinical Features and Lifetime Neuropsychiatric Features and IQ Scores

DD CLINICAL FEATURES	Lifetime DSM-IV Diagnosis of Psychiatric Illness	History of Suicidal Thoughts and Suicide Attempts	Key Psychiatric Clinical Variables	Learning Difficulties	IQ
Age of Onset and Duration	-	-	-	-	-
DD Severity	-	*Trend for the prevalence of suicidal thoughts, but not suicide attempts, to increase with DD severity.*	-	-	*Trend for IQ scores to decrease with an increase in DD severity.*
Symptoms (Pain, Malodour)	*Trend for more individuals reporting pain as a symptom of their DD to have a lifetime DSM-IV diagnosis.*	-	-		
Subjective Impairment and Impact on Quality of Life	-	A significantly higher proportion of individuals reporting a mild impact of DD on their work/career had a history of suicide attempts compared to individuals reporting no impact of DD on their work/career. Individuals with a history of suicidal thoughts had significantly higher scores on the DLQI-Worst week than individuals with no history of suicidal thoughts or suicide attempts.	-	-	-
Family History	A significantly higher proportion of individuals with a family history of DD had a lifetime psychiatric illness compared to individuals with no family history of DD.	A significantly higher proportion of individuals with a family history of DD had a lifetime history of suicidal thoughts compared to individuals with no family history of DD.	-	*Trend for higher proportion of individuals reporting a family history of DD to have learning difficulties.*	-

Significant relationships are shown in normal text. Trends almost reaching significance are shown in *italics.*

5 DEMOGRAPHICS, CLINICAL AND NEUROPSYCHIATRIC FEATURES IN 100 INDIVIDUALS WITH DD: DISCUSSION

This chapter will discuss the results presented in Chapter 4. These results and discussions relate to the first aim of the thesis, which was to conduct a systematic investigation of the neuropsychiatric characteristics in a sample of 100 unrelated individuals with DD using a battery of standardised neuropsychiatric measures. After a brief discussion of the demographics of the sample, the clinical features of DD in the individuals in the present study are compared to those described in previous case series studies of DD. This is followed by a comparison of the lifetime neuropsychiatric features in the current sample to the reported rates in previous case series studies of DD as well as the reported rates in the general population and among individuals with other skin conditions. The prevalence of DD among first-degree relatives with a reported history of neuropsychiatric illness is then briefly examined. This is followed by a discussion of the investigations into whether specific types of neuropsychiatric features appear to co-occur in individuals with DD. The findings of the investigations into the relationships between the clinical features of DD and the presence and severity of lifetime neuropsychiatric features are then discussed along with the extent to which these findings provide support for the contribution of psychosocial and pleiotropic (mutations in the *ATP2A2* gene have effects in the skin and brain) factors in the presence of neuropsychiatric features in DD.

5.1 Demographics

Although DD is reported to be equally prevalent among males and females, participants in the current study were predominantly female (71%). Although this may be partly due to a response bias (females may be more likely to respond to an invitation to take part in a research project (Rosenthal & Rosnow, 1991)) both the systematic and non-systematic

methods of recruitment are likely to have also contributed. The main systematic method of recruitment of participants was via one consultant dermatologist, Dr Sue Burge, who sent invitation letters to a higher number of females (62%), which is likely to have contributed to the predominance of females in the current sample. The proportion of males and females among the 60 individuals sent invitation letters from the DD support group is unknown although the majority of individuals who responded were female (79%). Since females are more likely to engage in self-help behaviours concerning health issues (Galdas *et al.*, 2005) it is likely that a higher number of females contact, and remain in contact with, the DD support group.

Eighty-eight percent of the sample reported that they had married/lived as married (lived with a partner for at least one year) and the majority (86%) were currently living with others, mainly with a spouse and/or children. This suggests that, in the sample as a whole, having DD does not appear to impair individuals' ability to form and maintain long -term relationships. This is in contrast to an early case series study carried out in Denmark in which the authors reported statistically more unmarried individuals among individuals with DD than in the normal population (Svendsen & Albrectsen, 1959).

A large proportion of individuals (41%) in the present study reported having no qualifications. This figure is over twice as high as the percentage of individuals in the UK population of working age (males 16-64, females 16-59) reported to have no qualifications (15%) (Office for National Statistics, 2003). The present study included individuals over working age (males above 64 and females above 59), which were the age groups with the highest proportion of individuals with no qualifications (see Table 4.2 pg. 52). However, excluding these individuals does not affect the proportion of individuals in the current sample with no qualifications (40%). A recruitment bias may also account for these differences since individuals with formal qualifications may be more likely to currently be in full time

employment and be less likely to participate in a research study due to other commitments. However, the finding that a large proportion of the current study had no formal qualifications is consistent with the findings of a cases series study of DD carried out in Slovenia where the authors highlighted that over half the individuals in their sample had only finished a primary or vocational school (Miljkovic *et al.*, 2005).

5.2 DD Clinical Features and Impact on Quality of Life

The distribution of the age of onset of DD in the present sample matches that reported in a previous large UK case series study of 163 individuals with DD (Burge & Wilkinson, 1992) with approximately 75% of individuals in both studies reporting the symptoms of their DD started before the age of 20. Since a proportion of the individuals in the present sample were also part of this previous UK case series study, similar findings may be expected. Epidemiological studies of DD carried out in Denmark (Svendsen & Albrectsen, 1959) and Slovenia (Miljkovic *et al.*, 2005) have also reported that age of onset DD occurred before the age of 19 in the majority of cases (92% and 67% respectively). The proportions of individuals with mild, moderate and severe DD in the present study and the study conducted by Burge & Wilkinson (1992) are also similar with the majority (two thirds) of individuals being classified as having moderate DD. Conversely, in the case series study of DD conducted in Slovenia, which used the same criteria for classifying DD severity, the majority of individuals were found to have mild DD. However, in all three studies, individuals classified as having severe DD made up the smallest proportion. These comparisons suggest the general clinical features of DD in the current sample are representative of those commonly observed in DD.

5.2.1 Impairment and Impact on Quality of Life

The high frequencies of individuals reporting itching (92%), pain (54%) and malodour (60%) as symptoms of their DD in the current sample indicates the suffering experienced by

the majority of individuals with DD. The proportions of individuals reporting itching and malodour are similar to the proportions of individuals reporting these symptoms in the UK case series sample of DD collected by Burge & Wilkinson (1992). However, the percentage of individuals reporting pain in the current sample is twice as high as the figure reported in their study. The reasons for this are unclear, although the criteria used by Burge & Wilkinson to positively rate the symptom of pain may have been more stringent, though this is not reported. In the current sample, impairment in social activities was the aspect of individuals' lives most frequently reported as being affected by having DD (see Figure 4.4 pg. 57). Similarly, 63% of individuals in the Burge & Wilkinson study stated that having DD had been a social handicap. One third of individuals in the current study reported DD had caused impairment in their work/career, although only half this number in the Burge and Wilkinson study reported their employment prospects had been affected by DD. The reason for this difference is likely to be that the figure in the current study included individuals reporting a mild impact of DD on their work/career.

Individuals' scores on the Dermatology Life Quality Index (DLQI) indicated that the majority of individuals in the current study felt that their DD had caused little impact on their everyday life during the past week (Table 4.8 pg. 58). The median score on the questionnaire (4 out of 30) was identical to the median score found in a previous study in which the DLQI was completed by 137 individuals with DD Harris *et al.* (1996). In the current sample, scores on the questionnaire significantly increased with DD severity, with individuals with moderate and severe DD reporting the symptoms of DD having a greater impact on quality of life over the past week than individuals with a mild form of the disorder (see Figure 4.5 pg. 60). This trend was also observed by Harris *et al.* (1996). However, the wide range of DLQI scores among individuals classified as having mild, moderate and severe DD suggests that other factors, in addition to the severity of the disorder, may also contribute to the subjective impact

the symptoms of DD have on individuals' everyday lives. For the purposes of the current study, the DLQI was also used to measure impairment in the week when individuals felt the symptoms of DD had been at their worst. This slightly modified version of the measure was used to investigate possible relationships between impact of quality of life caused by DD and the presence of neuropsychiatric features, discussed later in this chapter, and there are therefore no comparison data for scores on this version of the questionnaire.

Lewis & Finlay (2004) reviewed the use of the DLQI in 37 different skin conditions and calculated a 'mean of means' DLQI score for each skin condition from previous published studies. Table 5.1 summaries the DLQI scores for the four skin conditions in which the DLQI has been the most widely used, atopic eczema, acne, psoriasis and vitiligo. The mean DLQI scores found across studies of atopic eczema (12.2) and acne (11.9) are considerably higher than the median score of 4 found in the current study and by Harris *et al.* (1996) suggesting in general these conditions may cause more impairment than DD. However, some of these studies have been carried out in an inpatient setting where the symptoms of these skin conditions will be more severe. This is reflected in the range of the mean DLQI scores across the studies; for example in atopic eczema the range of mean scores across the studies range from 4.5 to 21.4 out of the possible total score of 30.

Table 5.1 DLQI Scores in other Dermatological Conditions

Skin Condition	Number of studies (number of individuals)	DLQI Scores Range of Means Across Studies	DLQI Scores Mean of Means Across Studies
Atopic Eczema	15 (1409)	4.5-21.4	12.2
Acne	8 (838)	4.3-17.7	11.9
Psoriasis	13 (2468)	1.7-18.2	8.8
Vitiligo	5 (856)	4.8-15	5.6

DLQI; Dermatology Life Quality Index. Figures reported by Lewis & Finlay (2004).

5.2.2 Family History of DD

Although DD is autosomal dominantly inherited with high penetrance, only half of the individuals in the current study reported a known family history of DD. This finding is consistent with the findings of other case series studies of DD. Of the 163 individuals, from 107 families interview by Burge & Wilkinson (1992), 46 individuals (47% of the families) reported no family history of DD. Similar rates of individuals reporting no family history of DD were reported in the case series studies carried out by Munro (1992) (43%) and Zeglaoui *et al.* (2005) (58%). Munro (1992) examined family members of individuals reporting no family history of DD and found that around half of these individuals actually did have a family history. Munro suggested that the remaining cases where family history was not found could be explained either by new cases of DD arising as a result of spontaneous *de novo* mutations in the gene for DD (*ATP2A2* had not yet been identified as the DD gene) or cases where information regarding parentage was incorrect. Another possibility not discussed in the literature is that a proportion of individuals may have the clinical features of DD caused by factors other than having a mutation in *ATP2A2* i.e. be phenocopies. Such individuals would be unlikely to report a family history of DD. In Chapters 8 and 9 of this thesis, the detection of mutations in *ATP2A2* among individuals reporting and not reporting a family history of DD is described and will indicate whether or not this last suggestion could be a possibility.

5.3 Investigations of a Population-Level Association Between DD and Neuropsychiatric Features

Seventy-eight percent of the sample had a lifetime history of *any* of the lifetime neuropsychiatric phenotypes measured in the current study. In addition, just over half of the sample had either been referred to secondary psychiatric services and/or been referred to a neurologist at some point in their life. These figures appear to suggest a potential population-

level association between DD and neuropsychiatric features. However, a number of the features measured are not uncommon in the general population. The following sections compare the lifetime prevalence of neuropsychiatric features in the current sample to the reported rates of these features in previous case series studies of DD (see Chapter 2, Table 2.2 pg. 12 for a brief description of these studies) as well as the reported rates of these features in the general population and among samples of individuals with other skin conditions. Such comparisons will give an indication of whether certain neuropsychiatric phenotypes do appear to occur more commonly in DD than in other populations.

5.3.1 Lifetime Prevalence of Psychiatric Disorders

Over half (55%) of the individuals in the present study had a lifetime diagnosis of a psychiatric illness according to the diagnostic criteria DSM-IV and one quarter of the sample had been referred to secondary psychiatric services at some point in their life. This finding is consistent with a small number of case series studies that have reported a high prevalence of psychiatric illness among individuals with DD (Ringpfeil *et al.*, 2001; Svendsen & Albrectsen, 1959). However, the prevalence of psychiatric illness is much higher than that previously reported in two large case series studies carried out in the UK in which very small numbers of cases of psychiatric illness were reported (Burge & Wilkinson, 1992; Munro, 1992). Out of 163 individuals interviewed by Burge & Wilkinson (1992), two individuals had been treated for depression and another had a diagnosis of bipolar affective disorder (BPAD) and of the 75 individuals interviewed by Munro (1992) one individual and an additional affected family member had been admitted to a psychiatric hospital. A likely explanation for this finding is that in the current study a standardised semi-structured interview was used to elicit the presence of lifetime psychiatric symptoms whereas in the other two previous UK case series studies this was assessed on recording individuals' general medical history and treatment. A further explanation could be a bias in the present study towards including

individuals with a history of psychiatric illness. However, almost all the participants in the current study were recruited via a dermatologist or the DD support group i.e. on the basis of having DD rather than a psychiatric illness. In addition, a proportion of individuals in the current sample were also in the study conducted by Burge & Wilkinson (1992). Of the participants in the current study recruited via Dr Sue Burge, 41% were found to have a lifetime diagnosis of a psychiatric illness. This suggests that methodological differences between the studies, rather than a recruitment bias, accounts for the greater prevalence of psychiatric illness in the current study. One third of participants in the current study were recruited via the DD support group. It is possible that individuals in contact with the group have suffered more as a result of having DD and are more likely to have experienced psychiatric symptoms than individuals with DD not in contact with the group. Of the participants recruited via the DD support group, 71% had a lifetime diagnosis of a psychiatric illness, which is higher than the prevalence of psychiatric illness among individuals who were recruited via a dermatologist (46%). This indicates that recruiting participants via the DD support group may have biased the sample to contain a higher proportion of individuals with a psychiatric illness. However, the majority of participants in the study were recruited via dermatologists and almost half of these participants had a history of psychiatric illness.

In the current sample a significantly higher percentage of females than males had a history of psychiatric illness (65% vs. 31%). This finding is consistent with that observed in large epidemiological studies of psychiatric illness (Alonso *et al.*, 2004; Kessler *et al.*, 1994). There are a number of social and biological factors that are likely account for this finding, including the fact that males may be less likely to recognise and/or report the symptoms of psychiatric illness.

To my knowledge there are no published data relating to the lifetime prevalence of psychiatric illness or contact with psychiatric services in the UK general population that use

methodology directly comparable to that used in this study. In 2000, the Office for National Statistics (www.statistics.gov.uk) carried out a survey of psychiatric morbidity among 8800 adults aged between 16-74 in private households in Great Britain (Singleton *et al.*, 2001). However, the survey only reports past week prevalence rates of psychiatric illness. A widely reported study of lifetime prevalence rates of psychiatric disorders is the US National Comorbidity Survey (NCS) (Kessler *et al.*, 1994). However, this study was carried out in the US over 10 years ago and the psychiatric diagnoses were made according to an earlier edition of the diagnostic criteria DSM (DSM-III-R) making comparisons with the lifetime prevalence of psychiatric illness in the current sample less meaningful. A more closely matched sample to the current sample is that collected by the European Study of the Epidemiology of Mental Disorders (ESEMeD) (Alonso *et al.*, 2004). This recent study investigated lifetime and 12-month prevalence of mood, anxiety and alcohol disorders in 21,425 individuals across six European countries (Belgium, France, Germany, Italy, the Netherlands and Spain) according to DSM-IV diagnostic criteria. In the following sections the prevalence of psychiatric disorders in the current sample will be compared to the prevalence rates reported in these two large epidemiological studies.

5.3.1.1 Mood Disorders

Half of the individuals in the present study had a lifetime diagnosis of a mood disorder (see Table 4.14 pg. 63). This figure is over twice as high as the total lifetime prevalence of mood disorders reported in the ESEMeD (14%) and NCS (19.3%). However, these two surveys had an equal proportion of males and females whereas the present sample was predominantly female (71%). Since the current study and these two epidemiological studies found females to have a higher prevalence of mood disorders, the predominance of female participants will inflate the prevalence of mood disorders in the current sample. However, the lifetime prevalence of mood disorders among females only in the ESEMeD (18.2%) and NCS

(23.9%) remain lower than the 50% total lifetime prevalence observed in the current sample. Across the ESEMeD and NCS epidemiological studies the mid-point between the average total lifetime prevalence (16.7%) and average female only lifetime prevalence (21%) is 19%. Since the proportion of women in both these studies was approximately equal, this mid-point between the prevalence of mood disorders in the total samples (comprising of 50% females) and the prevalence in the female only samples (comprising of 100% females) is an estimate of the prevalence of mood disorders in these studies if the proportion of women was 75%. This figure is a more appropriate population prevalence of mood disorders to compare to the prevalence of mood disorders in the present sample (comprising of 71% females), as it accounts for the higher percentage of females in the current sample. Using this value, the prevalence of mood disorders in the present study is significantly higher than the population prevalence reported in the two large epidemiological studies (50% vs. 19%; binomial test, p= <0.0001).

The most common psychiatric illness observed in the present sample was major depression with a prevalence of 30%. This finding is consistent with a previous case series study of DD, in which depression was reported as the most commonly observed neuropsychiatric phenotype (Ringpfeil *et al.*, 2001). This percentage is again higher than the lifetime prevalence of major depression reported in the ESEMeD (total; 12.8%, females; 16.5%) and NCS (total; 17.1%, females; 21.3%). Based on these figures, the most appropriate population prevalence of major depression to compare to the prevalence of major depression in the current sample would be 17% i.e. the mid-point between the average total lifetime prevalence and average female only lifetime prevalence. Using this value, the prevalence of major depression in the present study is significantly higher than the population prevalence reported in the two epidemiological studies (30% vs. 17%; binomial test, p=0.0005).

Four percent of individuals in the present study had a diagnosis of bipolar disorder. Four further individuals showed evidence of mood bipolarity including an individual diagnosed with cyclothymic disorder, another described in their medical notes as having a cyclothymic personality, a female reporting sub-clinical high mood after the births of both her children and an individual who developed hypomanic symptoms after taking antidepressants. The prevalence of bipolar disorder in the current study is higher than that reported in the previous case series studies of unrelated individuals with DD, in which only one case of bipolar disorder has been reported (Burge & Wilkinson, 1992). It is possible that bipolar disorder may have been under-reported in these studies particularly the older case series studies, where individuals with bipolar disorder may have been described as having other conditions such as depression, schizophrenia, "emotional problems" or "unrealistic thinking". This is highlighted in the case of a male described as being depressed by Medansky & Woloshin (1961) who is also reported to have "spoken at considerable length about his success in business and the big deals behind which he was the driving force". This description sounds indicative of the presence of manic symptoms, although the authors do not comment on this.

The lifetime prevalence of mania in the NCS is 1.6% (1.7% in females) (the ESEMeD does not report prevalence figures for bipolar disorder). These figures are similar to other reported lifetime prevalence rates of bipolar disorder. Bebbington & Ramana (1995) reviewed studies reporting the epidemiology of bipolar disorder and found the lifetime prevalence to be fairly consistent across the studies and suggested the lifetime prevalence of bipolar disorder for males and females was between 1-1.5%. The most appropriate population prevalence of bipolar disorder to compare to the prevalence of bipolar disorder in the current sample would therefore be 1.5%. Using this value, the prevalence of bipolar disorder in the present study is significantly higher than the population prevalence (4% vs. 1.5%; binomial test, $p=0.05$), although this finding would not remain significant after corrections for multiple testing.

The findings of the current study together with previous case series studies are consistent with the existence of a modest population association between bipolar disorder and DD.

5.3.1.2 Anxiety Disorders

In the current study 16% of the sample had a lifetime diagnosis of any anxiety disorder. This is similar to the lifetime prevalence of anxiety disorders reported in the ESEMeD (total; 13.6%, females, 17.5%) and lower than that reported in the NCS (total; 24.9%, females; 30.5%). Based on these figures, the most appropriate population prevalence of anxiety disorders to compare to the prevalence of anxiety disorders in the current sample would be 22%. Using this value, the prevalence of anxiety disorders in the present study is actually significantly lower than the population prevalence reported in the two epidemiological studies (16% vs. 22%; binomial test, p=0.03), although this finding would not remain significant after corrections for multiple testing. This finding suggests that there is not a non-specific increased rate of all psychiatric disorders in the current sample.

5.3.1.3 Suicidal Thoughts and Suicide Attempts

Thirteen individuals in the current sample had made a suicide attempt and a further 18 reported a lifetime history of suicidal thoughts. Therefore, in total, almost one third of the sample (31%) had a lifetime history of suicidal thoughts. This finding is consistent with previous studies reporting a high prevalence of suicidal ideation and attempts among individuals with DD (Denicoff *et al.*, 1990; Ringpfeil *et al.*, 2001). Similar to the findings of Denicoff *et al.*,(1990), that most individuals did not relate their suicidal ideation to their DD, only one individual in the current study had made a suicide attempt in reaction to a worsening of the symptoms of their DD, whereas for the remaining twelve individuals there was no subjective or objective temporal relationship between their suicide attempts and the symptoms of DD.

The survey of psychiatric morbidity among adults in Great Britain carried out by the Office for National Statistics (Singleton *et al.*, 2001) collected data on lifetime suicidal thoughts and suicide attempts in 8450 individuals, which were published in a separate report (Meltzer *et al.*, 2002). The lifetime prevalence of suicide attempts (total; 4.4%, females only; 5.3%) and suicidal thoughts (total; 14.9%, females only; 17.1%) in this general population survey is much lower than the prevalence observed in the current DD sample. Just over half the individuals in the psychiatric morbidity survey were women. To take into account the higher percentage of women in the current study, population prevalence figures of 4.9% for suicide attempts and 16% for suicidal ideation were used to compare to the prevalence of suicidal thoughts and attempts observed in the current study. Using these values, the prevalence of suicide attempts (13% vs. 4.9%; binomial test, p=0.0008) and suicidal ideation (31% vs. 16%; binomial test, p=<0.0001) in the current study are significantly higher than that observed in the UK general population.

A summary of the prevalence of mood disorders, major depression, bipolar disorder, suicide attempts and suicidal ideation in the present study compared to prevalence of these features reported in general population samples are summarised in Table 5.2 and Table 5.3. These comparisons indicate that the prevalence of mood disorders, specifically major depression, suicide attempts and suicidal thoughts are significantly higher in individuals with DD than in the general population.

Table 5.2 Comparison of the Lifetime Prevalence of Mood and Anxiety Disorders in the Current Study to the Prevalence Reported in Two Major Epidemiology Studies

	Lifetime Prevalence %				
	Current DD	**ESEMeD Total (Female only)**	**NCS Total (Female only)**	***Most Appropriate Comparison**	**Binomial Test (P)**
Diagnostic Criteria	DSM-IV	DSM-IV	DSM-III-R	-	
Any Mood Disorder	50	14 (18.2)	19.3 (23.9)	19	**<0.0001**
Major Depression	30	12.8 (16.5)	17.1 (21.3)	17	**0.0005**
Bipolar I Disorder	4	-	1.6 (1.7)	1.5**	**0.05**

ESEMeD; European Study of the Epidemiology of Mental Disorders (Alonso *et al.*, 2004), NCS; National Comorbidity Survey (Kessler *et al.*, 1994), DSM; Diagnostic and Statistical Manual of Mental Disorders. DD, Darier's Disease.
*The mid point between the average total prevalence and average female prevalence across the two epidemiological studies to take into account the predominance of females (71%) in the current sample. ** Taking into account the prevalence bipolar disorder commonly reported in other epidemiological studies.

Table 5.3 Comparison of the Lifetime Prevalence of Suicidal Thoughts and Attempts in the Current Study to the Prevalence Reported in the Office for National Statistics Survey of Psychiatric Morbidity Among Adults in Great Britain

	Lifetime Prevalence %			
	Current DD	**ONS Psychiatric Morbidity Survey Total (Female only)**	** **Most Appropriate Comparison**	**Binomial Test (P)**
Suicidal attempts	13	4.4 (5.3)	4.9	**0.0008**
Suicidal thoughts*	31	14.9 (17.1)	16	**<0.0001**

ONS; Office For National Statistics Survey of Psychiatric Morbidity (Meltzer *et al.*, 2002), DD, Darier's Disease, F; lifetime prevalence in females only.
* Figures include individuals with a lifetime history of suicide attempts.
**The mid point between the total prevalence and female prevalence to take into account the predominance of females (71%) in the current sample.

5.3.2 Psychiatric Co-morbidity in Other Skin Conditions

Literature relating to psychiatric co-morbidity in other skin disorders indicates that there is a high prevalence of psychiatric disorders among dermatology patients. It is commonly reported that the overall prevalence of psychiatric co-morbidity in dermatology patients with conditions including acne, alopecia, psoriasis and vitiligo, is around 30% with depression and anxiety being commonly observed (Gupta & Gupta, 2003; Picardi *et al.*, 2000). However, for a number of reasons, it is not meaningful to compare the prevalence of psychiatric illness found in the current sample of individuals with DD to the prevalence reported among individuals with other skin conditions in the literature. Firstly, in the current sample, lifetime prevalence of psychiatric illness was measured whereas the studies in the literature have assessed point prevalence. Secondly, the reported overall prevalence of psychiatric co-morbidity in dermatology patients has come from studies conducted in both outpatient and inpatient dermatology settings whereas all of the individuals in the current study were outpatients (although one third had been a dermatology inpatient at some point in their lives). Finally, most of the studies reporting psychiatric co-morbidity in dermatology patients have assessed the presence of psychiatric symptoms using brief self-rated measures/general screening questionnaires whereas the presence of these symptoms in the present sample was assessed using a standardised clinical semi-structured interview.

The prevalence of suicidal ideation among psoriasis (5.5%) and acne (5.6%) patients has been reported to be twice as high as that reported among general medical patients (Gupta & Gupta, 1998). This study measured the point prevalence of suicidal ideation in both inpatients and outpatients using a self-rated measure. The findings therefore cannot be compared to the lifetime prevalence of suicidal ideation observed in the current sample of individuals with DD.

A limitation of the present study is the lack of a control sample. To further support the finding of an increased prevalence of psychiatric illness in DD, a control sample of individuals with another skin disorder of a similar severity to DD needs to be collected and assessed using a standardised psychiatric clinical interview. Collecting a control sample of individuals with another skin condition was considered when the current study was first being designed however, due to time constraints this was not possible. The skin condition used as a control sample would have to be selected carefully, as complex relationships are likely to exist between psychiatric and dermatologic symptoms in a number of skin conditions. As well as psychiatric symptoms being a consequence of having a skin disorder, psychosocial stress is also reported to play a role in the exacerbation and onset of a number of skin conditions such as psoriasis and atoptic dermatitis (Gupta & Gupta, 2003).

5.3.3 Epilepsy and Other Neurological Conditions

Three individuals in the current sample had a lifetime diagnosis of three different types of epilepsy (tonic-clonic, idiopathic and petit mal) giving an overall prevalence of 3% (30/1000). This prevalence of epilepsy is consistent with a number of other case series studies of DD, for example, Burge & Wilkinson (1992) reported the prevalence of epilepsy in their sample as 42.9 / 1000. A review article of the epidemiology of the epilepsies (Sander & Shorvon, 1996) indicates that the reported lifetime prevalence rates of epilepsy in the general population are very variable, ranging from 1.5-57/1000 depending on the definitions used, with the average prevalence being 10.3/1000 (1.3%). Using this value, the prevalence of epilepsy in the current study is not significantly higher than in the general population (3% vs. 1.3%; binomial test, $p=0.1$). The findings of the current study and the previous case series studies do not support a population-level association between DD and epilepsy, although the prevalence of epilepsy in these studies is higher than the reported population prevalence of epilepsy. These findings

could suggest that a subgroup of individuals/families with DD may have an increased susceptibility to developing epilepsy.

Over one third of individuals in the current study had been referred to a neurologist and/or had neurological investigations and almost two thirds had consulted their GP for possible neurological symptoms, most common being blackouts/periods of loss of consciousness (24% consulted GP, 13% referred for investigations), hearing problems (14% consulted GP, 9% referred for investigations) and headaches (33% consulted GP, 6% referred for investigations). This rate seems high but it is not clear whether the prevalence of these 'soft' neurological symptoms is higher than in the general population. In a large community based study in the UK investigating the incidence and lifetime prevalence of neurological disorders, migraine, tension headache and ear conditions were all excluded due to resource constraints (MacDonald *et al.*, 2000). The finding that thirteen percent of individuals in the current sample had received investigations for blackouts, periods of loss of consciousness or fainting episodes is noteworthy. Whether these episodes have an underlying neurological cause in any of the reported cases remains unclear, although the majority of investigations carried out on these individuals at the time of these episodes were reported to be normal. It is also possible that in a proportion of these individuals the episodes of loss of consciousness may be part of a somatization disorder.

Lifetime history of the presence of neurological symptoms and disorders in the current study was assessed using a brief checklist of questions. Future studies could carry out a more detailed assessment of the presence of neurological features in individuals with DD including the administration of specific neurological tests such as audiograms and neuroimaging.

5.3.4 Lifetime History of Learning Difficulties

Five percent of the current sample reported that they had been given a diagnosis of dyslexia. It is not meaningful to compare this figure to the reported prevalence of dyslexia in the general population since it is unlikely that older participants in the current sample with dyslexia would have ever been given a formal diagnosis. Thirteen percent of the sample reported they had received extra help at school although this also likely to be dependent on participants' age and this figure may be an underestimate of individuals in the sample who required extra help at school.

In an attempt to overcome these difficulties, 59 individuals completed the self-rated Adult Dyslexia Checklist (ADC) with 19% having a score on the questionnaire indicating difficulty. The checklist was posted to individuals after they had been visited. Therefore it is possible that individuals with greater difficulties with reading and/or spelling may have been less likely to return the questionnaire. When this questionnaire was previously completed by 679 adults in the UK, including 32 with known dyslexia, 10% had scores indicating difficulty (The British Dyslexia Association). Comparison of these figures indicates that twice as many individuals in the current study had scores indicating difficulty than in the sample of 679 UK adults. However, that sample was predominantly comprised of degree course, access course and A-level students and is therefore not a very suitable comparison group.

One fifth of individuals in the current sample were classified as having learning difficulties (individuals who had a diagnosis of dyslexia and/or received extra help at school and/or had a high score in the ADC reflecting difficulty). These findings do not indicate a population level association between DD and learning difficulties. This is consistent with the findings of the majority of previous case series studies of DD, which have reported relatively small numbers of individuals with learning difficulties. However, in a case series study in Slovenia, the majority of individuals were noted to have an impairment of memory and

capacity to concentrate (Miljkovic *et al.*, 2005). Similarly, an early study conducted in Denmark suggested that individuals with DD differed from 'normal' in terms of their mental development (Svendsen & Albrectsen, 1959). It is not clear how these impairments were assessed in these two studies. The current study is limited by the methods used to measure learning difficulties. Individuals' responses to the questions regarding dyslexia and receiving extra help at school are likely to be dependent on their age group. As previously discussed, a high percentage of individuals in the current sample reported having no qualifications. There are a number of possible reasons for this finding, including the possibility that individuals with DD may be impaired in specific aspects of learning. This needs further investigation using standardised neuropsychological measures such as memory and attention tests.

5.3.5 Normative IQ Scores

The mean IQ score of the 85 individuals who completed the Wechsler Abbreviated Scale of Intelligence (WASI) measure was 100, which is within the average range of IQ. Just six percent of individuals in the current study had a below average IQ. Only one previous small case series study of DD has measured IQ (Medansky & Woloshin, 1961). In this study, two individuals out of the five assessed were found to have a below average IQ, although it is difficult to make comparisons to such a small sample.

A comparison of the distribution of IQ scores in the current sample to the normative sample published in the WASI manual is displayed in Table 5.4 The distribution of IQ scores in the current DD sample is almost identical to the distribution reported in the normative sample, which is reported to be highly representative of the English speaking US population. This comparison is limited by the normative sample coming from the US population (a specific UK normative sample is not available for the WASI) and also by the fact that almost half the sample consisted of children. Despite these limitations, the findings of the current study suggest that individuals with DD do not differ from the general population in terms of

their general level of intellectual functioning. It is, however, possible that individuals with a lower level of intellectual functioning may be less likely to respond to an invitation to take part in a research study.

Table 5.4 Comparison of the Distribution of IQ Scores in the Current Study to the Distribution in the Published Normative Sample

		%	
IQ Score	**Classification**	**Current DD Sample**	**Published Normative Sample**
130 and above	Very Superior	0	2.0
120-129	Superior	2.4	7.3
110-119	High Average	21.2	15.6
90-109	Average	56.5	50.0
80-89	Low Average	14.1	15.8
70-79	Borderline	4.7	6.8
69 and below	Extremely Low	1.2	2.5

IQ scores measured using the Wechsler Abbreviated Scale of Intelligence (WASI)

5.4 Family History of Neuropsychiatric Features

Almost half the individuals in the current sample (43%) reported that they had at least one first-degree relative with a history of psychiatric illness that had required treatment and/or caused significant impairment. Seven individuals in the sample had a family history of epilepsy in their first-degree relatives. The first-degree relatives with a history of psychiatric illness and epilepsy were more commonly reported as having DD than not having DD. This was most apparent for epilepsy where 75% of the first-degree relatives with a diagnosis of epilepsy were also reported to have DD. Similarly, of the first-degree relatives with a positive psychiatric history, a higher percentage were reported to have DD (53.1%) than were not (35.9%).

Previous family studies of DD have consistently reported a greater prevalence of neuropsychiatric features in family members with DD than in family members without DD. The examples of four families with multiple members with DD and neuropsychiatric illness displayed in the present thesis (Figure 4.8-Figure 4.11 pg. 80) also show an increased prevalence of neuropsychiatric features among family members with DD, although no cases

of complete co-segregation between DD and neuropsychiatric features were observed. A limitation of the current study is that information regarding family history was obtained from the index individual and the majority of first-degree relatives were not seen to confirm the absence or presence of a history of neuropsychiatric features or a diagnosis of DD. However, it is more likely that an affected first-degree relative with mild DD may have been reported as being unaffected than a true unaffected relative being reported as having DD.

There are a number of explanations for the observed increased prevalence of neuropsychiatric features among individuals with DD compared to their relatives unaffected by DD. The increased prevalence of psychiatric illness in individuals with DD compared to their unaffected relatives may reflect a psychological reaction to having a chronic disorder. However, in a family displaying complete co-segregation between DD and mood disorders interviewed by Craddock *et al.* (1994b), family members did not relate their mood symptoms to their skin disorder. In addition, psychosocial factors would not account for the more severe neuropsychiatric features being reported more commonly among first-degree relatives with DD. For example, in the family described by Getzler & Flint (1966) neuropsychiatric features including 'schizophrenic episodes' and 'depressive psychosis' were reported more commonly among family members with DD. Such severe psychiatric symptoms are unlikely to result from a psychological reaction to having a skin disorder. It is possible that in this family a gene predisposing individuals to these psychiatric symptoms was in linkage disequilibrium with the DD gene. Another possible explanation is that mutations in *ATP2A2,* in addition to causing DD, have effects in the brain and cause individuals with DD to have an increased susceptibility to developing neuropsychiatric features. The prevalence of neuropsychiatric features in a sample of individuals with DD and their first-degree relatives, unaffected by DD, will be investigated and discussed in further detail later in this thesis in Chapters 10 to 12.

5.5 Relationship between Lifetime Neuropsychiatric Features, IQ Scores and Family History of Psychiatric Illness

Investigations into whether certain neuropsychiatric features appear to occur more commonly together in individuals with DD revealed that significantly more individuals with a history of investigations for blackouts/loss of consciousness also had a lifetime DSM-IV diagnosis of psychiatric illness and a family history of psychiatric illness compared to individuals without a history of having such investigations. It is possible that in these individuals the psychiatric features and blackouts/episodes of loss of consciousness have a shared pathogenic mechanism therefore providing support for the hypothesis that mutations in *ATP2A2* have pleiotropic effects in the skin and brain. However, it is also possible that in some individuals the episodes of loss of consciousness may be part of the psychiatric condition, for example may reflect a severe panic attack, which is not recognised by the individual or may be part of a somatization disorder. It is not possible to establish the cause for the co-occurrence of psychiatric illness and episodes of loss of consciousness in the individuals in the current sample. Neuroimaging investigations on these individuals would help to determine whether there is a potential biological cause for the co-occurrence of these symptoms. Significantly more individuals with learning difficulties had a below average IQ score compared to individuals without learning difficulties. This is an expected finding since the two measures are likely to be measuring the same neuropsychiatric phenotype in these individuals.

5.6 Relationship between DD Clinical Features and Lifetime Neuropsychiatric Features

Relationships, both subjective and objective, between the clinical features of DD and the presence and severity of lifetime neuropsychiatric features in the current sample were investigated to help determine the extent to which psychosocial and pleiotropic factors may play a role in the presence of neuropsychiatric features in DD.

Only a small percentage (6%) of individuals in the current sample felt that all the episodes of psychiatric illness they had experienced were due to the symptoms of DD. This finding is consistent with previous studies in which individuals with DD have not related their psychiatric symptoms to their DD (Craddock *et al.*, 1994b; Denicoff *et al.*, 1990) and suggests that in many individuals with DD, the additional presence of psychiatric symptoms may be due to factors other than a psychological reaction to having a chronic skin disorder. It is, however, possible that individuals may attribute the psychiatric symptoms they have experienced to other life events such as relationship problems, which could have resulted from them having DD, although this is difficult to assess. Over one third of the current sample (38%) did report that they felt at least one episode of psychiatric illness, which in all cases was an episode of depression, was directly due to the symptoms of DD. This highlights the possibility that episodes of depression experienced by individuals with DD can be a direct consequence of the symptoms of DD. In the majority of cases (81%) the onset of DD preceded or occurred at the same age as the onset of psychiatric symptoms. This finding was expected since the symptoms of DD generally appear between the ages of 11 and 20 and would therefore be expected to occur before the emergence of psychiatric symptoms. This finding highlights the potential for psychosocial factors to play a large role in the development of psychiatric symptoms in DD although this does not exclude the possibility that other factors may play an important role. Overall, the majority of the other clinical features of DD, including age of onset and duration, severity, impairment and impact on

quality of life were not significantly associated with the presence or severity of lifetime neuropsychiatric features. The significant findings from these investigations, and the relationships that almost reached significance, are summarised in Table 4.33 pg. 103. A limitation of these investigations is the small group sizes, which limit the power to detect significant relationships.

A number of relationships that would be expected if psychosocial factors alone accounted for the occurrence of neuropsychiatric features in DD were not observed. For example, the reported impact on quality of life caused by DD was not found to be associated with a presence of a lifetime history of psychiatric illness or with measures of the severity of psychiatric illness including numbers of episodes of depression. DD severity was also not found to be associated with these psychiatric features, although these findings need to be examined in larger samples.

5.6.1.1 Relationships between DD Severity, Impact on Quality of Life and History of Suicidal Thoughts and Suicide Attempts

Significant relationships were observed between the impairment and impact on quality of life caused by DD and history of suicidal thoughts and suicide attempts. Significantly more individuals reporting DD to have caused mild impact on their work/career had a history of suicide attempts compared to individuals reporting no impact (Figure 4.15 pg. 91). However, this significant finding should be treated with caution for a number of reasons. Firstly, if a true relationship existed it would be expected that individuals reporting a moderate/severe impact on their work/career due to DD would have a greater prevalence of history of suicide attempts than individuals reporting a mild impact, although this was not observed. Secondly, the reported impact of DD on work/career did not appear to be correlated with other measures of the presence or severity of lifetime psychiatric features.

Individuals with a history of suicidal thoughts reported a significantly greater impact on quality of life during the worst week of their DD (measured by the DLQI) compared to individuals with no history of suicidal thoughts or attempts (see Figure 4.16 pg. 92). Due to the cross sectional design of the current study, it is not possible to establish whether history of suicidal thoughts may be primary or secondary to the reported impact of DD on quality of life. It is likely that individuals whose quality of life has been severely affected by DD would experience suicidal thoughts in reaction to their skin disorder but it is also possible that individuals with history of suicidal thoughts may be more likely to retrospectively report that DD has had a greater impact on their life. Evidence for suicidal thoughts being experienced as a direct consequence of the symptoms of DD is supported by the observed trend for the prevalence of history suicidal thoughts, to increase with DD severity (Figure 4.13 pg. 87). This trend was not observed for suicide attempts. The relationship between quality of life and suicidal thoughts in DD requires further investigation in a larger number of individuals and would ideally be investigated longitudinally. The presence of an association between quality of life and suicidal thoughts would have implications for the management of DD and the potential use of quality of life measures to identify individuals with DD who are at risk of experiencing suicidal thoughts.

History of suicide attempts was not observed to be associated with DD severity or the impact of DD on quality of life. The small sample number of individuals in the present study with a history of suicide attempts means that power to detect significant relationships would be limited. However, the observation that in all but one of these cases there was no subjective or objective temporal relationship between the suicide attempt and the symptoms of DD supports the notion that factors other than a direct reaction to the symptoms of DD are likely to explain the observed increased prevalence of suicide attempts in DD.

5.6.1.2 *Increased Prevalence of Psychiatric Illness Among Individuals Reporting Pain as a Symptom of DD*

A non-significantly higher prevalence of history of psychiatric illness was observed in individuals reporting pain as a current or past symptom of their DD (Figure 4.14 pg. 89). This finding is difficult to interpret, although experiencing pain as a result of DD could cause individuals to become depressed, individuals with a past or current history of depression may be more likely to retrospectively report that their DD had been painful in the past. It is possible that, due to the number of relationships investigated in the current study, this finding could be due to chance. This is supported by the fact that pain as a symptom of DD was not observed to be associated with any of the measures of severity of psychiatric symptoms, such as the number of episodes of depression individuals had experienced.

5.6.1.3 *Increased Prevalence of Psychiatric Illness and Suicidal Thoughts Among Individuals Reporting a Positive Family History of DD*

Individuals reporting a definite or probable family history of DD had a significantly higher prevalence of history of psychiatric illness (Figure 4.17 pg. 93) and suicidal thoughts (Figure 4.18 pg. 94) compared to individuals reporting a negative family history of DD. A similar trend was also observed for suicide attempts (Figure 4.18 pg. 94). An explanation for this observation is that having another family member with DD, especially severe DD, may cause individuals to experience depression and possibly suicidal thoughts particularly if they are concerned that their DD will become as severe as their relatives' symptoms. Having a child with DD might also lead to feelings of guilt that they have passed on the disorder, which may lead to the development of symptoms of depression. It is also possible that the observed prevalence of psychiatric symptoms among individuals reporting a family history of DD could be providing support for the pleiotropy hypothesis that mutations in *ATP2A2* may confer susceptibility to psychiatric illness. Certain mutations that may lead to a more severe, easily recognised, form of DD in families, may also have effects in the brain and contribute to

individuals developing psychiatric symptoms. It is also possible that a proportion of individuals not reporting a family history of DD may not have a mutation in *ATP2A2* i.e. they may be phenocopies of DD. This possibility is investigated and discussed further in Chapters 8 and 9.

A non-significant trend was observed for the prevalence of learning difficulties to be higher among individuals reporting a positive family history of DD. However, the difficulties encountered in measuring learning difficulties in the present study, discussed above, limits the conclusions that can be drawn from this finding. No relationship between IQ scores and family history of DD were observed in the sample.

5.6.2 Relationship between DD Severity and IQ Scores

A trend for IQ scores to decrease with an increase in DD severity was observed (Figure 4.19 pg. 98). This relationship almost reached significance despite the small number of individuals classified as having severe DD. This association could support the role of psychosocial factors, since having severe symptoms of DD from an early age may affect school attendance and performance leading to a reduced level of intellectual functioning in adulthood. This is supported by the finding that there was also a trend for IQ scores to decrease with an increase in the subjective reported impact of DD on school. However, the trend for IQ scores to decrease with DD severity could also be providing support for pleiotropy hypotheses in that certain mutations in *ATP2A2* may lead to the presence of both severe DD and severe neuropsychiatric features including a reduced level of intellectual functioning.

5.7 Summary, Limitations and Suggestions for Further Research

This chapter has discussed the findings of the first systematic investigation into the neuropsychiatric phenotype in DD using a battery of standardised neuropsychiatric measures. A comparison of the prevalence of neuropsychiatric features in the current sample to available data regarding the prevalence of these features in the general population, suggests a potential population-level association between DD and mood disorders, specifically major depression, suicide attempts and suicidal thoughts. This finding is limited by the fact that there are no reported lifetime figures for the prevalence of these features in other skin conditions, highlighting the need for a control sample of individuals with a skin disorder of similar severity to be assessed using a similar battery of standardised neuropsychiatric measures. The prevalence of bipolar disorder and epilepsy in the sample is non-significantly higher than the prevalence in the general population suggesting that a subset of individuals/families with DD may have an increased risk of developing these disorders.

It is difficult to compare the apparently high prevalence of soft neurological symptoms in the sample to the prevalence of these features in the general population. This needs to be investigated in future studies using specific neurological tests. Similarly, difficulties were encountered in assessing true prevalence of learning difficulties in the current sample, which requires further investigations using standardised neuropsychological measures. However, the findings of the current study do suggest individuals with DD do not differ from the general population in terms of their general level of intellectual functioning.

Despite the fact that the majority of individuals in the current study were recruited on the basis of having DD, it is possible that there could be a recruitment bias towards individuals who have experienced psychiatric symptoms as a consequence of their DD, since these individuals may be in more regular contact with a dermatologist and/or the DD support group. Conversely, individuals with more severe neuropsychiatric features, including severe

psychiatric illness and learning difficulties may be less likely to be in regular contact with dermatologists and/or respond to an invitation to take part in a research study. Such individuals may be underrepresented in this current sample.

The investigations of the relationships between the clinical features of DD and the presence and severity of lifetime neuropsychiatric features indicate that psychosocial factors alone cannot account for the observed increased prevalence of mood disorders, suicidal thoughts and suicide attempts observed in the current sample. In addition, the increased prevalence of psychiatric illness and history of suicidal thoughts and attempts among individuals reporting a positive family history DD and the trend for IQ scores to decrease with an increase in DD severity potentially provides support for the pleiotropy hypothesis that mutations in *ATP2A2* may confer susceptibility to neuropsychiatric features in individuals with DD. A limitation of these investigations is the small group sizes, which limits the power to detect significant relationships. There is also the possibility that due to the number of relationships examined, some of the significant findings may have been due to chance. Possible explanations for the significant relationships observed have been discussed, although due to the cross-sectional design of the study it is not possible to make any definite causal inferences. These findings require replication in larger samples of individuals with DD, ideally in a longitudinal study. Over all, the high prevalence of mood disorders, suicidal thoughts and suicide attempts observed in the current sample highlights the need for assessment and recognition of psychiatric symptoms in DD, particularly in individuals reporting a positive family history of DD.

The following chapters (Chapter 6 to 9) are concerned with addressing the second main aim of the thesis, which is to investigate possible genotype-phenotype correlations between the type and/or locations of pathogenic mutations detected in the *ATP2A2* gene and neuropsychiatric features observed in this current sample. Evidence for such correlations

would support the hypothesis that mutations in the *ATP2A2* gene may have pleiotropic effects in the skin and brain and therefore confer susceptibility to neuropsychiatric features in individuals with DD.

6 GENOTYPE-PHENOTYPE CORRELATIONS BETWEEN MUTATIONS DETECTED IN *ATP2A2* AND NEUROPSYCHIATRIC PHENOTYPES: INTRODUCTION

This chapter describes the DD causing mutations in *ATP2A2* that have previously been reported in the literature. This is followed by a description of the functional domains of the sarco/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2) protein, which is encoded by the *ATP2A2* gene. Finally, previous findings of studies investigating possible relationships between the type and/or location of mutations within *ATP2A2* and the presence of neuropsychiatric features are discussed.

6.1 Mutations Reported in ATP2A2 In the Literature

The *ATP2A2* gene spans 21 exons and has three splice variants (a, b and c), which are a result of alternative splicing of exon 20. To date, 143 *ATP2A2* mutations have been reported in 175 unrelated individuals in the literature (Chao *et al.*, 2002; Dhitavat *et al.*, 2003b; Dode *et al.*, 2003; Foggia *et al.*, 2006; Godic *et al.*, 2004; Ikeda *et al.*, 2003; Jacobsen *et al.*, 1999; Jones *et al.*, 2002; Onozuka *et al.*, 2004; Racz *et al.*, 2005; Racz *et al.*, 2004; Ren *et al.*, 2006; Ringpfeil *et al.*, 2001; Ruiz-Perez *et al.*, 1999; Sakuntabhai *et al.*, 1999a; Sakuntabhai *et al.*, 2000; Sakuntabhai *et al.*, 1999b; Takahashi *et al.*, 2001; Wada *et al.*, 2003; Wang *et al.*, 2006; Yang *et al.*, 2004; Yang *et al.*, 2001). These reported mutations are distributed across the gene. The types of mutations found and their approximate percentages are summarised in Table 6.1. A brief description/definition of each mutation type is given below.

Nonsense mutations: a single base substitution that leads to a premature termination codon (PTC) that specifies termination of protein synthesis. These mutations are expected to have a dramatic effect on the protein.

Frameshift mutations: small deletions or insertions of bases leading to a shift in the reading frame of the triplet codon system. These mutations can potentially lead to a PTC and are expected to have a dramatic effect on the protein.

Missense mutations (non-synonymous mutations): a single base substitution leads to an amino acid change. This change can either be *conservative,* where the amino acids have similar properties or *non-conservative* where they do not. Non-conservative changes result in a greater change in the protein properties and have a greater potential to be pathogenic.

In-frame mutations: small deletions or insertions of bases that do not shift the reading frame of the triplet codon system. This leads to the deletion or insertion of amino acids. These mutations are likely to result in a change in the properties of a protein but are not expected to be as damaging as frameshift and nonsense mutations.

Splice site mutations: a single base substitution in the splice donor (first few bases in an intron) or splice acceptor (last few bases in an intron) sequences, which flank exons. This may lead to retention of large segments of intronic DNA or to entire exons being spliced out and can therefore result in a change in the properties of a protein.

***Other*:** include large insertions and deletions of bases, which are likely to have a dramatic effect on the protein structure and function.

Table 6.1 Frequencies of Types of Mutations Previously Reported in *ATP2A2*

Mutation Type	Number	%
Nonsense	14	10
Frameshift (insertion/deletion)	33	23
Missense	71	50
In-frame (insertion/deletion)	9	6
Splice Site	12	8
Other	4	3
Total	**143**	**100**

The majority of the 143 mutations reported in the literature to date have been found to be unique within a single family (83%). Twenty-five mutations (17%) have been reported more

than once in apparently unrelated individuals. Four separate missense mutations have been reported in three or more apparently unrelated families.

6.1.1 Mutation Detection Rates in ATP2A2

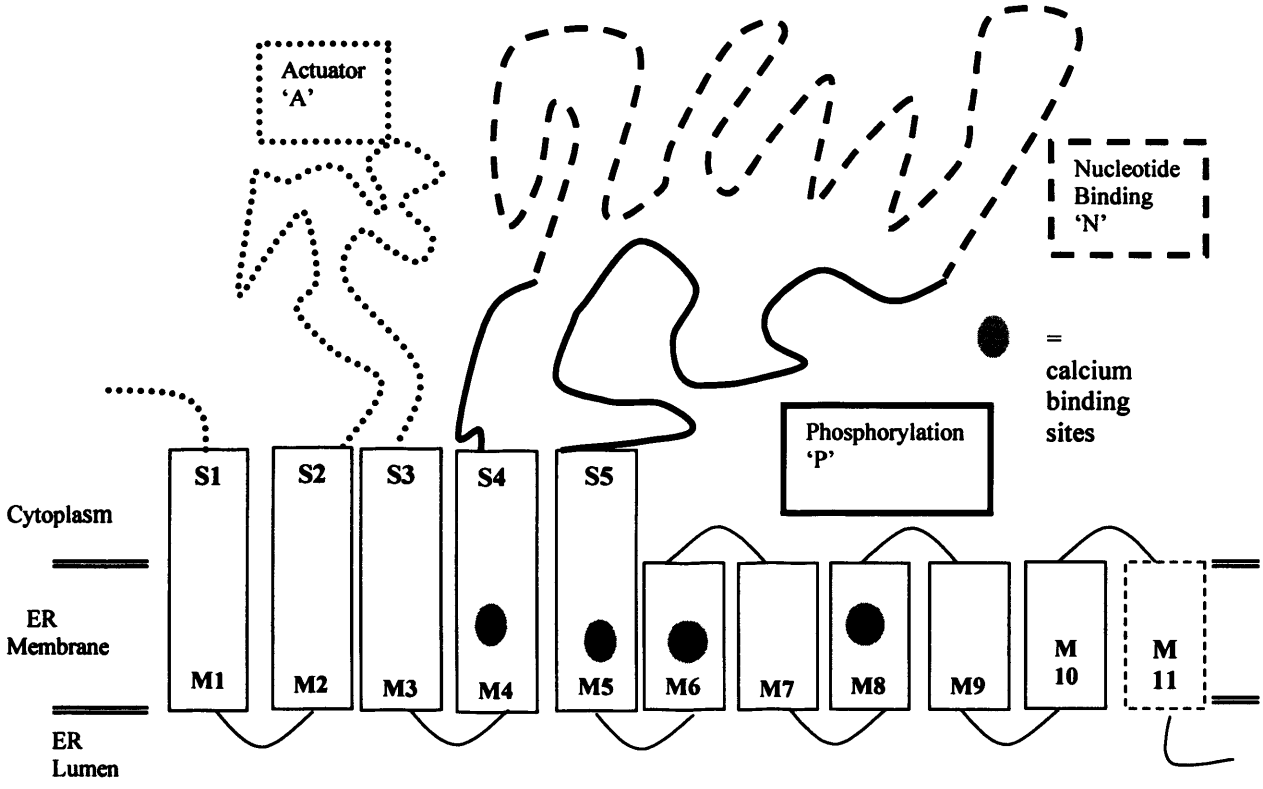
Reported disease-causing mutation detection rates in larger scale mutation screens of the *ATP2A2* gene (10 or more unrelated individuals with DD) range from 50% (Ikeda *et al.*, 2003) to 70% (Onozuka *et al.*, 2004). Explanations for these findings have included DNA quality and/or technical reasons (Ikeda *et al.*, 2003), undetected mutations being located in an unscreened region of the *ATP2A2* gene (Ringpfeil *et al.*, 2001) and the screening strategies used not being able to detect large scale mutations (Ruiz-Perez *et al.*, 1999). A further explanation for a DD causing mutation not being detected in individuals is that they could have clinical features similar to DD caused by factors other than a pathogenic mutation in *ATP2A2* i.e. be a phenocopy of DD.

6.2 Domain Structure of SERCA2

As previously described in Chapter 2, *ATP2A2* encodes three isoforms of the SERCA2 protein (a, b and c), which are identical up to amino acid 993 and differ in their carboxyl termini and expression (Table 2.1 pg. 7). Both the SERCA2a and SERCA2b isoforms have been found to be present in the epidermis although the SERCA2b isoform predominates (Ruiz-Perez *et al.*, 1999; Tavadia *et al.*, 2004). The major role of the SERCA2b isoform in the epidermis is supported by a mutation causing DD being found in a region of the protein specific to the 2b isoform (Dhitavat *et al.*, 2003b). SERCA2b has also been found to be highly expressed in the brain with the highest levels being found in the Purkinje neurons of the cerebellum and the pyramidal cells of the hippocampus (Baba-Aissa *et al.*, 1998).

SERCA2b is 1042 amino acids in length and contains five major domains: the M domain which is composed of 11 transmembrane domains (M1-M11), the S domain, which is

composed of five stalk domains (S1-S5) and three cytoplasmic domains; the Actuator (A) domain, the Nucleotide ATP-binding (N) domain and the Phosphorylation (P) domain. A schematic diagram of the functional domains of the SERCA2b protein, based on the schematic diagram of the protein published by Dode *et al.* (2003), is shown in Figure 6.1. SERCA2b differs from SERCA2a (997 amino acids) and SERCA2c (999 amino acids) by having a proposed additional 11th transmembrane domain and a carboxyl terminus that extends into the endoplasmic reticulum (ER) lumen (Verboomen *et al.*, 1994).



ER; endoplasmic reticulum, S*n;* stalk domains, M*n;* transmembrane domains

Figure 6.1 Schematic Diagram of the Functional Domains of SERCA2b

6.2.1 M Domain

The SERCA2 protein has seven Ca^{2+} binding amino acids within four of the transmembrane domains (M4, M5, M6 and M8) which form two Ca^{2+} binding sites (Toyoshima *et al.*, 2000). Site 1 contains amino acids from M5, M6 and M8 and site 2 contains amino acids from M4 and M6. The SERCA2 protein alternates between two conformational states, E1 and E2, resulting in two Ca^{2+} ions being transported from the cytoplasm into the ER against a concentration gradient (MacLennan *et al.*, 1997). In the E1 state the Ca^{2+} binding sites are of high affinity and facing the cytoplasm. After ATP binding, the pump is phosphorylated by ATP allowing the protein to undergo a series of conformational changes resulting in the E2 state in which the Ca^{2+} binding sites are of low affinity and facing the ER lumen.

6.2.2 A, N and P Domains

The cytoplasmic section of SERCA2 contains three separate functional domains, described below.

Actuator (A) domain is involved in transferring the energy from ATP hydrolysis to Ca^{2+} transport. It is also thought to act as a gating mechanism, making bound Ca^{2+} ions inaccessible from either side of the ER membrane before they are released in the ER lumen (Toyoshima & Mizutani, 2004).

Nucleotide ATP-binding (N) domain contains amino acids critical for binding to ATP allowing phosphorylation of the P domain.

Phosphorylation (P) domain is split into two parts, separated by the N domain, contains the amino acid phosphorylated by ATP and additional amino acids critical for ATP hydrolysis.

A 3-D representation of the SERCA2 protein published in a review article by Foggia & Hovnanian (2004) is shown in Figure 6.2.

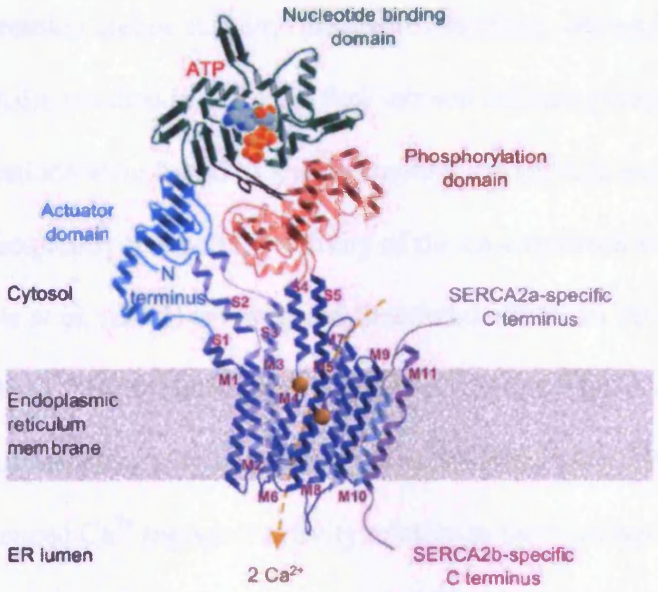


Figure 6.2 3-D Schematic Diagram of the Domain Structure of SERCA2 published in Foggia & Hovnanian (2004)

6.3 Functional Studies of Effects of ATP2A2 Mutations

As individuals with DD are heterozygous for mutations in *ATP2A2,* one normal allele continues to encode a normal SERCA2b protein. Recent functional studies have investigated the effects of 56 specific mutations in *ATP2A2* on the functioning of 'mutant' SERCA2b proteins. These studies have found that the majority of mutations result in a SERCA2b protein with very limited or loss of Ca^{2+} transport activity due to a lack of protein expression and/or function (Ahn *et al.*, 2003; Dode *et al.*, 2003; Miyauchi *et al.*, 2006; Sato *et al.*, 2004). However, a small number of exceptions have been found, described below. Overall the functional studies suggests that the expression of the normal wild type SERCA2b protein from the single normal allele is not sufficient to provide normal function (haploinsufficiency).

Ahn *et al.* (2003) examined the expression and function of 12 SERCA2b proteins resulting from DD causing mutations. Ten out of the 12 proteins displayed decreased expression and/or stability. Only one mutation, located in the 7th transmembrane (M7) domain, resulted in a protein that showed calcium pumping activity (V843F). In addition, five mutations were found to lead to 'mutant' SERCA2b pumps that interacted with and subsequently reduced the activity of the co-expressed wild-type normal SERCA2b pumps. Dode *et al.* (2003) investigated functional effects of 10 missense mutations in SERCA2b. Eight of the mutations were found to result in a SERCA2b protein with no significant Ca^{2+} transport. One unique mutation, located in the M8 to M9 cytosolic loop, was found to have an enhanced Ca^{2+} transport activity relative to the wild type SERCA2b and had reduced sensitivity to inhibition by lumenal Ca^{2+} (S920Y). Sato *et al.* (2004) examined the expression and function of three SERCA2b proteins resulting from DD causing missense mutations in *ATP2A2*. All three proteins were found to exhibit nearly normal protein expression and Ca^{2+} transport function. One mutation, located in the stalk 4 (S4) domain, was found to result in a SERCA2b protein with markedly reduced sensitivity to the feedback inhibition by ER lumenal Ca^{2+} (L321F). It is postulated by the authors that this would bring about an abnormally elevated Ca^{2+} level in the ER lumen which could in turn produce a strong feedback inhibition of the wild type SERCA2b pump.

6.4 Genotype-Phenotype Studies

A small number of studies have been conducted to investigate genotype-phenotype correlations between mutations detected in *ATP2A2* and the neuropsychiatric features observed in individuals with DD. Such relationships would provide strong evidence that mutations in *ATP2A2* do have pleiotropic effects in the skin and brain. Jacobsen *et al.* (1999) investigated genotype-phenotype correlations in 19 unrelated individuals with DD. Seventeen distinct mutations were identified with two pairs of individuals being found to have the same

mutation. The 10 individuals in the study with a lifetime history of neuropsychiatric features were all examined by a research psychiatrist and diagnoses were made according to standard diagnostic criteria. The neuropsychiatric phenotypes observed included, cyclothymic disorder, schizoid personality disorder NOS, bipolar I disorder, major depression and epilepsy. Genotype-phenotype correlations were observed. A predominance of missense mutations was found in individuals with DD and neuropsychiatric features compared to individuals with DD only (70% vs. 38%). The mutations in individuals with a neuropsychiatric phenotype also showed a non-random clustering in the last third of the gene, all being located in exons 13-19. Interestingly, two apparently unrelated individuals in the study with a lifetime diagnosis of a psychiatric disorder were found to have the same missense mutation in the ATP binding 'N' domain of SERCA2 (C560R). One of these individuals had bipolar I disorder and the other individual had a diagnosis of schizoid personality disorder NOS and suffered from a form of psychosis. A further missense mutation in the 7^{th} transmembrane (M7) domain of the SERCA2b protein (V843F) was found in two unrelated individuals with neuropsychiatric features. One of these individuals had epilepsy and the other had experienced blackouts as well as an episode of major depression. Although the study had a relatively small sample size, the findings did provide evidence of genotype-phenotype correlations between mutations in *ATP2A2* and the occurrence of neuropsychiatric features in DD. This was highlighted in particular by unrelated individuals with the same mutation being found to have similar neuropsychiatric phenotypes.

Three further studies have failed to find any relationship between the type and/or location of mutations in *ATP2A2* and the presence of neuropsychiatric features. Ringpfeil *et al.* (2001) investigated genotype-phenotype correlations in 14 families/individuals with DD in whom 14 distinct mutations were identified. Neuropsychiatric features were assessed by medical and family history and considered significant if the disorder resulted in medical care or

consultation of a specialist. The authors reported no obvious genotype-phenotype correlations. However, three families with the most severe clinical features of DD also had severe neuropsychiatric features including "severe emotional problems", suicide attempts and depression. Two of these families both had an in-frame deletion mutation each eliminating one amino acid in the Stalk 1 (S1) domain (Del L41 and Del P42). A further family classified as having severe DD had members with variable neuropsychiatric features including seizure disorder, depression and schizophrenia. This family had a missense mutation in the ATP binding 'N' domain (F487S). Sakuntabhai *et al.* (1999a) investigated genotype-phenotype associations in 22 families/individuals with DD including five families reported in Sakuntabhai *et al.* (1999b). Information regarding neuropsychiatric features was obtained as part of the general medical history. The authors reported that neuropsychiatric abnormalities were not associated with a specific type of mutation and varied among affected family members within the same family. Ruiz-Perez *et al.* (1999) identified 40 different mutations in 47 families with DD. To investigate genotype-phenotype correlations between the mutations detected and the neuropsychiatric features observed, the authors focused on six individuals/families with the clearest evidence of associated neuropsychiatric disorders. This included one individual with notably low intelligence, an individual with schizophrenia and four families where one or more family members had neuropsychiatric features including learning or behavioural difficulties, history of fits and schizophrenia. The authors found that these families had different types of mutations and concluded that although neuropsychiatric features in individuals with DD did not appear to be associated with certain types of mutations they could be an intrinsic, but inconsistent consequence of mutations within the *ATP2A2* gene.

One limitation of the studies not finding associations between mutations in *ATP2A2* and the presence of neuropsychiatric features in individuals with DD is that neuropsychiatric

diagnoses were made based on questions regarding general medical history and treatment rather than on systematic and standardised assessment by a trained researcher. In addition, investigations of genotype-phenotype correlations were carried out in studies with small sample sizes.

Site-directed mutagenesis studies have also provided support for mutations in *ATP2A2* having pleiotropic effects in the skin and brain by highlighting that specific mutations may be associated with the presence of neuropsychiatric features. Sato *et al.* (2004) found a unique missense mutation (L321F) in the stalk 4 (S4) domain which led to a SERCA2b protein with markedly reduced sensitivity to feedback inhibition by ER Ca^{2+}. This mutation was found in a female and her daughter both with severe DD who both also had severe neuropsychiatric disorders and behaviour problems. The authors suggested that this mutation could be having pleiotropic effects in the skin and brain. A further mutation resulting in reduced sensitivity to ER lumenal Ca^{2+}, located in the M8 to M9 cytosolic loop (S920Y), was reported by Dode *et al.* (2003). Interestingly, this mutation has been reported in a family with moderate to severe DD and a member with neuropsychiatric problems which included epilepsy and low-normal IQ (Jacobsen *et al.*, 1999; Sakuntabhai *et al.*, 1999a). Ahn *et al.* (2003) suggest that the finding that certain mutations in *ATP2A2* result in SERCA2b pumps that can interact with and reduce the activity of the wild-type protein, could account for both the variable clinical features observed in individuals with DD and the presence of neuropsychiatric features.

Of the 143 DD causing mutations in *ATP2A2* reported in the literature to date, approximately one quarter have been reported in individuals who have additional neuropsychiatric features. At present, no clear relationship has emerged between presence of neuropsychiatric features and the type and/or location of mutations in *ATP2A2*. This in part may be due to small sample sizes and the lack of detailed information reported regarding the neuropsychiatric phenotypes and/or how this information has been collected. Previous case

series studies of DD have been undertaken by dermatologists untrained in psychiatric assessment and the presence of neuropsychiatric features has been based on recording general medical history and treatment.

6.5 Further Aims of the Thesis

In addition to investigating the prevalence of neuropsychiatric features in 100 individuals with DD (described and discussed in Chapters 3 to 5) a further aim of the thesis was to carry out investigations into the relationship between mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed. The observation of relationships between the type and/or location of mutations in *ATP2A2* and the presence and/or severity of specific neuropsychiatric phenotypes would provide strong evidence that mutations in *ATP2A2* have pleiotropic effects in the skin and brain. The advantages of the current study compared to previous studies are that genotype-phenotype correlations were assessed in a larger sample of individuals and the neuropsychiatric features were assessed in detail systematically using standardised measures.

A further aim of the current study was to compare the clinical and neuropsychiatric features in individuals in whom a DD causing mutation *was* and *was not* detected. Differences between the two groups could suggest that a proportion of individuals in the sample have a skin condition with very similar clinical features to DD caused by factors other than a pathogenic mutation in *ATP2A2*.

The following chapter (Chapter 7) describes methods used to investigate genotype-phenotype correlations in a large sample of unrelated individuals with DD.

7 GENOTYPE-PHENOTYPE CORRELATIONS BETWEEN MUTATIONS DETECTED IN *ATP2A2* AND NEUROPSYCHIATRIC PHENOTYPES: METHODS

This chapter describes the methods used to investigate correlations between the type and/or locations of pathogenic mutations detected in the *ATP2A2* gene and the neuropsychiatric phenotypes observed in a sample of unrelated individuals with DD. The collection of DNA samples is described followed by the techniques and processes involved in DNA extraction (carried out by two members of the psychiatric genetics laboratories at Birmingham and Cardiff Universities) and the detection of variants in the *ATP2A2* gene (carried out by the laboratory at the Wales Gene Park). The subsequent analysis I carried out on the Gene Park data is then described. This included the identification of pathogenic mutations within *ATP2A2* and comparison of these to the distribution of previously reported *ATP2A2* mutations. The comparisons made between individuals in whom a pathogenic mutation was and was not detected are then described. Finally, the approaches to investigating possible genotype-phenotype correlations between the type and location of the pathogenic mutations detected within *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD are described. Additional information relating to this chapter can be found in section C of the Appendix starting at pg. 313, and will be referenced throughout the chapter.

7.1 DNA Collection and Extraction

7.1.1 DNA Collection

Venous whole blood samples or Oragene™ (Genoteck Inc.) saliva samples were collected from 93 index individuals with DD. Samples were also obtained from 11 additional affected family members (from 11 separate families- including two cases where no DNA could be collected from the index individual) and 17 unaffected family members (from 17

separate families). The frequencies of blood and saliva samples collected are summarised in Table 7.1.

Table 7.1 Frequencies of Blood and Saliva Samples Collected

	Blood Samples	Oragene Saliva Samples	Total
Index Individuals	88	5	93
Affected Family Members	8	3	11
Unaffected Family Members	8	9	17
Total	104	17	121

7.1.2 Known Pathogenic Mutations in *ATP2A2*

Pathogenic mutations in *ATP2A2* were already known for eight of the index individuals with DD in the present study. This information was provided by Dr Susan Burge, the Consultant Dermatologist who was involved in the recruitment of research participants. DNA was collected again from seven of these individuals in the present study for quality control purposes (see section 7.3.3)

7.1.3 DNA Extraction

DNA extraction of the blood and saliva samples was carried out by Fiona Middle (Molecular Psychiatry Group Laboratory, Birmingham University) and Detelina Grozeva (Psychiatric Genetics Laboratory, Cardiff University). The DNA extraction protocols followed for the two types of samples are provided in Appendix C.i pg. 313.

7.2 Detection of Variants in ATP2A2 - Wales Gene Park

The detection of variants in *ATP2A2* was carried out by the Wales Gene Park (http://www.walesgenepark.co.uk). The Wales Gene Park is an enterprise set up to advance genetics research and technology in Wales. The gene park has close links with Cardiff University and has a genomic facility that offers services to researchers with large-scale genomic projects. Two plates of extracted DNA samples were sent to the Gene Park, prepared

by Detelina Grozeva and Dr. Elaine Green. The following sections describe briefly the three sequential techniques carried out by the Wales Gene Park to detect variants in *ATP2A2.*

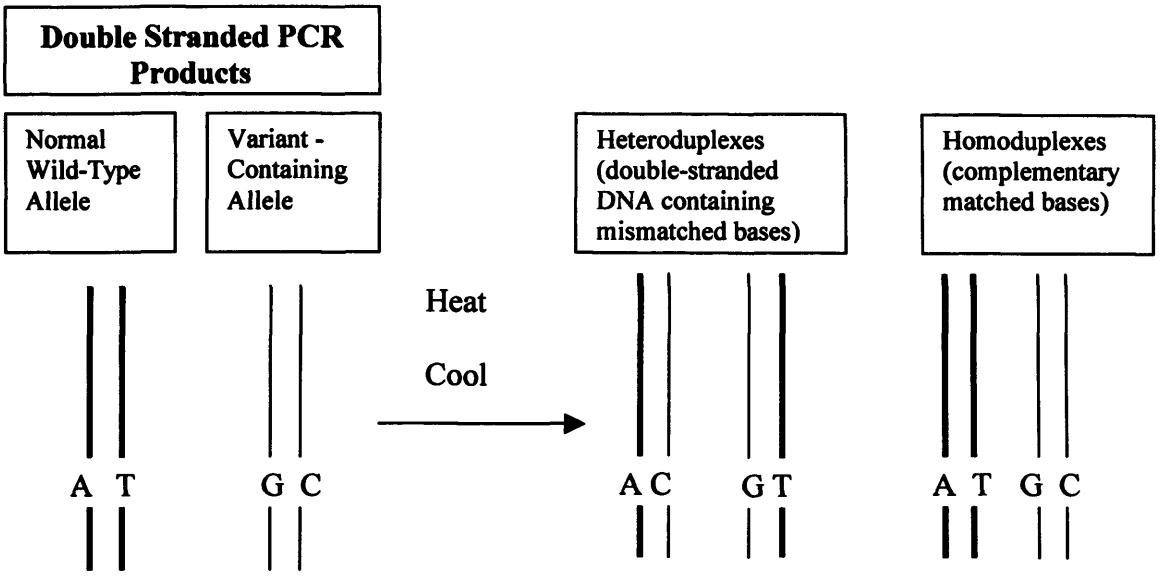
7.2.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a method of selectively amplifying small target fragments of DNA (usually a few hundred base pairs in length). This technique produces multiple copies (millions) of small fragments of DNA (PCR products), which can then be used in subsequent techniques used to detect DNA variants.

The Wales Gene Park attempted to amplify all 21 exons of *ATP2A2* by PCR. The gene was divided up into fragments according to the exons apart from exon 8, a large exon, which was cut into two fragments and two fragments each of which contained two small exons, 2&3 and 12&13 respectively. No PCR products could be generated for exon 1 therefore variants in this exon could not be detected in the present study. A further small number of fragments failed PCR amplification in some samples (an average of 3.6% of fragments across the two plates, excluding exon 1) although no more than four fragments failed in an individual sample.

7.2.2 Denaturing High Performance Liquid Chromatography (DHPLC) Analysis

Denaturing High Performance Liquid Chromatography (DHPLC) is a screening technique used to identify fragments of DNA that are likely to contain sequence variants. The first process in this technique involves heating double-stranded PCR products causing them to be denatured into single strands that are then slowly cooled allowing them to re-anneal into double-stranded products. A basic principle behind the technique is that if an individual has one normal allele and one variant-containing allele at a gene locus, the single strands of DNA re-anneal to form both *homoduplexes* and *heteroduplexes,* shown in Figure 7.1.



Individual in this case is heterozygous - has one normal allele containing the base A (and its paired base T) and one variant-containing allele which contains the base G (and its paired base C) at the same site. Heating and then cooling the double-stranded DNA leads to the formation of both homoduplexes (double-stranded DNA containing matched paired bases) and heteroduplexes (double stranded-DNA containing mismatched paired bases).
PCR; polymerase chain reaction.

Figure 7.1 Example of the Formation of Heteroduplexes in an Individual Containing a DNA Variant

Double-stranded homoduplexes have complementary sequences forming matched bases (i.e. A-T and G-C) whereas heteroduplexes have mismatched bases (i.e. A-C and G-T). Due to the mismatched bases, heteroduplexes are not as stable to heat as homoduplexes and denature at a lower temperature. DHPLC analysis involves heating the homoduplexes and heteroduplexes at a specified temperature, heteroduplexes, which will denature more completely, can then be distinguished from homoduplexes using high performance liquid chromatography. Therefore the DHPLC screening technique detects heteroduplexes in PCR products indicating the likely presence of a DNA variant. PCR products showing heteroduplexes can then be sequenced to identify the variant, described below. In the present study, excluding exon 1, 96.4% (100-3.6% PCR amplification fails) of the DNA length of the *ATP2A2* gene was successfully screened for DNA variants using DHPLC analysis.

7.2.3 DNA Sequencing

Sequencing is the final stage of detecting DNA variants and involves identifying the complete DNA sequence in a fragment of DNA. Automated sequencing was carried out using an ABI 3100 automated sequencer. This is based upon Sanger dideoxynucleotide sequencing, with the four ddNTPs being labelled with four different fluorophors. Each fluorophor has a different emission wavelength when excited by the laser in the ABI 3100. The relative size of each terminated sequencing product is then determined by electrophoresis. Sequences are then compared to the known established human gene sequence in order to detect variants. It is expensive and labour intensive to sequence all fragments of a gene in a large number of individuals therefore sequencing is only carried out on PCR fragments showing heteroduplexes during DHPLC analysis i.e. fragments that have been identified as being likely to contain a DNA variant.

7.3 Further Mutational Analysis on Data Obtained From the Gene Wales Park

The Wales Gene Park provided a report listing the variants identified in *ATP2A2* and the samples/individuals in whom they were detected, an example of the format of the data is shown in Table 7.2. Information was also provided indicating the small number of fragments that failed PCR amplification and the samples/individuals in whom these fails occurred, an example of the format of these data is provided in Table 7.3.

Table 7.2 Format of Wales Gene Park Data Reporting Variants Detected in *ATP2A2*

Mutation ID	Fragment	Mutation (AA)	Region	SNP seq	Sample
167 A>G	X2_3	Q56R	Coding	GCTGGAACTTGTGATTGAGC **R**	13
219+179 G>T	X2_3		Intronic	CAAAACACTTGAAGCCATTG **K**	27
1000C>T	X8B	R334.	Stop	CAAAGAAAAATGCCATTGTT **Y**	1 and 18

Table 7.3 Format of Wales Gene Park Data Reporting Fragments which Failed PCR Amplification

	FRAGMENT																			
SAMPLE/INDIVIDUAL	2_3	4	5	6	7	8A	8B	9	10	11	12_13	14	15	16	17	18	19	20A		
1																	F			
2		F																F		
3																		F		
4												F						F		

PCR; Polymerase Chain Reaction, F; fragment failed PCR amplification.

The following sections describe the analyses I carried out on the data obtained from the Wales Gene Park. This firstly involved identifying which of the variants detected by the Wales Gene Park were likely to be pathogenic mutations.

7.3.1 Exclusion of Known Non-Pathogenic Polymorphisms

In order to identify which of the variants detected by the Wales Gene Park were likely to be pathogenic mutations it was important to initially identify and subsequently exclude any variants that were known non-pathogenic polymorphisms. All DNA variants identified by the Wales Gene Park were checked against a database of known single nucleotide polymorphisms (SNPs) in the human genome (http://www.ncbi.nlm.nih.gov/SNP/index.html). This database also contains known information on the population allele frequency of common polymorphisms. DNA variants were also checked against known non-pathogenic polymorphisms in *ATP2A2* previously reported in the literature.

7.3.2 Identification of Pathogenic Mutations

After excluding any non-pathogenic polymorphisms it was necessary to decide which of the remaining variants were likely to be pathogenic mutations. This was achieved by ranking the variants according to how likely they were to be pathogenic. Mutations were ranked into nine categories ranging from the mutation types thought to be most likely to be causing DD to variants thought to be least likely to be causing DD, shown in Table 7.4. In order to decide

whether each of the non-synonymous amino acid changes (missense mutations) detected were likely to be conservative or non-conservative, a program SIFT (Sorting Intolerant From Tolerant- website http://blocks.fhcrc.org/sift/SIFT.html) was used. This is a sequence-homology-based programme that predicts whether an amino acid change will affect protein function. The program predicts the impact of all possible 20 amino acid changes at each amino acid position in a protein. Amino acid changes are predicted as being either not tolerated i.e. non-conservative and likely to affect protein function or tolerated i.e. conservative and not likely to affect protein function. An example of the SIFT program output is shown in Table 7.5.

A cut-off was decided above which mutations were thought to be highly likely to be pathogenic and below which mutations were thought to be less likely to be pathogenic. Mutation types already reported in the literature as being pathogenic mutations down to and including splice-site mutations were thought to be highly likely to be pathogenic. The three remaining mutation types, including conservative non-synonymous amino acid changes, synonymous amino acid changes (silent mutations) and intronic mutations were thought to be less likely to be pathogenic and were not included in any of the subsequent analyses.

Table 7.4 Ranking DNA Variants Detected in *ATP2A2*

Rank	Mutation Type
1	Reported in literature as a pathogenic mutation
2	Large insertion/deletions
3	Nonsense or Frameshift
4	Non-synonymous amino acid change (Missense): Non conservative
5	In-frame insertion/deletion
6	Splice site
7	Non-synonymous amino acid change (Missense): Conservative
8	Synonymous amino acid change (Silent)
9	Intronic

Table 7.5 Example of SIFT Program Output

Predict Not Tolerated	Amino Acid Position	Predict Tolerated
d h g n e c s w r k y p q t a f v i	1M	L M
c w f m i y l v h r g t n p a k S	2E	D Q E
	3N	w c m p i g H f v R Q L s Y k e T N A D

SIFT; Sorting Intolerant From Tolerant.
Example output predicts the impact of all possible 20 amino acid changes at three amino acid positions in a protein e.g. at position 1 of the protein, the amino acid change M1L is predicted as being tolerated, whereas the change M1V is predicted as not being tolerated.

7.3.3 Quality Control

It was important to check the quality of the data regarding the detection of DNA variants within *ATP2A2* and the subsequent identification of pathogenic mutations. This was carried out in a number of ways including:

- Comparing the frequencies of polymorphisms detected in the present study to the previously reported frequencies of these polymorphisms - it would be expected that the frequencies should be similar.
- Confirming that the pathogenic mutations already known for a small number of individuals in the study via Dr Susan Burge were identified in the same individuals in the current mutation detection.
- Confirming that, where DNA samples were collected from an index individual and an affected family member, the pathogenic mutation identified in the index individual was also detected in the affected family member.
- Confirming that, where DNA samples were collected from an index and an unaffected family member, the pathogenic mutation identified in the index individual was not detected in the unaffected family member.

7.3.4 Comparison of Type and Location of Mutations Detected Compared to Previously Reported Mutations in ATP2A2

The type and location of the pathogenic mutations detected in the present sample were compared to the distribution of *ATP2A2* mutations previously reported in the literature. The main purpose of this comparison was to ensure that the distribution of the pathogenic mutations detected in the present study was roughly similar to the distribution of previously reported mutations and therefore a representative sample of mutations.

7.4 Comparison of the Clinical Features of DD and Neuropsychiatric Phenotype In Individuals in whom a Pathogenic Mutation was and was not Detected

Before investigations into genotype-phenotype correlations were carried out, comparisons were made between index individuals with DD in whom a pathogenic mutation *was* and *was not* detected. Individuals in the two groups were compared on a number of clinical features of DD and neuropsychiatric features, summarised in Table 7.6. These have all previously been described in Chapter 3.

Table 7.6 Clinical Features of DD and Neuropsychiatric Features Compared in Individuals with DD in whom a Pathogenic Mutation *was* and *was not* Detected

Clinical Features of DD
Positive Family History of DD
DD Severity
DLQI -Worst Week Ever
Neuropsychiatric Phenotypes
Lifetime DSM-IV Diagnosis
BADDS Mania Dimension
BADDS Depression Dimension
History of Suicide Attempts
Contact with Psychiatric Services
History of Learning Difficulties
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes

DLQI; Dermatology Life Quality (described previously in Section 3.2.2.5 pg. 38), BADDS; Bipolar Affective Disorder Dimensional Scale (described previously in Section 3.2.5 pg. 42).

7.5 *Investigations of Genotype-Phenotype Correlations*

The following sections describe the approaches carried out to investigate possible genotype-phenotype correlations between the type and location of the pathogenic mutations detected within *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD. The neuropsychiatric phenotypes used in the genotype-phenotype correlation investigations are summarised in Table 7.7.

Table 7.7 Neuropsychiatric Phenotypes used in Genotype-Phenotype Correlation Investigations

Neuropsychiatric Phenotype	Further Details
BADDS Mania Dimension	Score from 0-100 reflecting the lifetime presence and severity of manic symptoms.
BADDS Depression Dimension	Score from 0-100 reflecting the lifetime presence and severity of depressive symptoms.
Age of onset of psychiatric Illness	Age of onset of any psychiatric illness causing impairment (years).
Any Lifetime DSM-IV Diagnosis	Lifetime history of any DSM-IV psychiatric disorder (yes or no).
History of Suicide Attempts	Lifetime history of suicide attempts (yes or no).
History of Psychiatric Contact	Lifetime history of contact with secondary psychiatric services (yes or no).
Learning Difficulties	Lifetime history of learning difficulties defined as having a diagnosis of dyslexia and/or receiving extra help at school and/or scoring 9 or more YES responses on the Adult Dyslexia Checklist. (yes or no).
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes	Lifetime history of investigations for blackouts, loss of consciousness or fainting episodes (yes or no).
Personal History of Bipolar Disorder and/or 1st Degree Relatives with DD and Bipolar Disorder	The small number of index individuals with bipolar disorder would make genotype-phenotype correlation investigations uninterpretable. Therefore this phenotype was extended to include individuals with a 1st degree relative with DD and bipolar disorder.
Diagnosis of Epilepsy and/or 1st Degree Relatives with DD and a Diagnosis of Epilepsy	The small number of index individuals with epilepsy would make genotype-phenotype correlation investigations uninterpretable. Therefore this phenotype was extended to include individuals with a 1st degree relative with DD and epilepsy.

BADDS; Bipolar Affective Disorder Dimension Scale, DSM-IV; Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

7.5.1 Schematic Diagram of the SERCA2b Protein

The pathogenic mutations identified in the present study were mapped onto schematic diagrams of the SERCA2b protein. The structure of the protein and its functional domains were based on the schematic diagram of the protein published by (Dode *et al.*, 2003) and the domain structure of the protein provided by the Swiss-Prot Protein Database, a biological database of protein sequences (http:www.expasy.org). In each diagram the mutations were highlighted according to the absence or presence of a specific neuropsychiatric phenotype. In the case of the BADDS Mania score, this was converted into a categorical variable by highlighting mutations where individuals had BADDS mania scores greater or equal to 10 as a marker of mood bipolarity. The aim of this approach was to investigate whether clustering of mutations within the SERCA2b protein could be observed for any specific neuropsychiatric phenotype.

7.5.2 Mutation Type

As described in Chapter 6, different types of mutations are likely to have varying degrees of impact on protein structure and function. Therefore, the neuropsychiatric phenotypes of individuals with frameshift, in-frame (insertion and deletions), missense, nonsense, other (large insertions and deletions) and splice-site mutations were compared.

7.5.3 Mutation Location within Functional Domains of SERCA2b

As described in Chapter 6, the SERCA2 protein contains three cytoplasmic domains: Actuator (A), Nucleotide ATP-binding (N) and Phosphorylation (P) as well as 11 transmembrane domains (M1-M11). The seven essential Ca^{2+}-binding amino acids are located within four of the transmembrane domains (M4, M5, M6 and M8). The transmembrane regions M4 and M5 are connected to the stalk regions S4 and S5 respectively (see Figure 6.1 pg. 137). It is possible that mutations within the S4 and S5 regions could have an impact on

the Ca^{2+}-binding functioning of the M4 and M5 transmembrane regions. Therefore, in the present analysis, the stalk regions S4 and S5 were included as part of the collective Ca^{2+}-binding domains. The neuropsychiatric phenotypes of individuals with mutations in the A, N, P and Ca^{2+}-binding domains respectively were compared to the neuropsychiatric phenotypes of individuals not having mutations in these domains.

7.5.4 Mutation Location within *ATP2A2*

It is likely that the location of a mutation within a gene will influence the extent to which it has an impact on protein structure and function. For example, mutations at the beginning of a gene are more likely to have a detrimental impact than those occurring towards the end. However, as discussed in the previous chapter, investigations of genotype-phenotype correlations in 19 individuals with DD conducted by Jacobsen *et al.* (1999) found mutations among individuals with DD and neuropsychiatric features clustered in the last third of the gene. Therefore in the current study, the neuropsychiatric phenotypes of individuals with mutations located in the first third, middle third and last third of *ATP2A2* were compared. The three sections were determined by dividing the total number of nucleotide bases within the *ATP2A2* gene (3126) into three.

7.6 Statistical Analysis

Data were extracted from the study Access database into the statistical package SPSS version 12.0.1 (SPSS Inc., 2003). Normality of the data was assessed using the Kolmogorov-Smirnov Test. The majority of the data were not normally distributed so non-parametric statistical tests were used, outlined below. Statistical tests were considered significant at the $p<0.05$ level, (two tailed).

Continuous data:

- Differences between two groups were assessed using Mann-Whitney U tests.
- Differences between 3+ groups were assessed using Kruskal-Wallis Analysis of Variance. If a significant difference was found *post hoc* comparisons were performed on each pair of groups using Mann-Whitney U tests.

Categorical data

- Relationships between two categorical variables were assessed using 2x2 and 2x3 chi-square tests. In cases where 20% or more of the cells in a chi-square table had an expected count of less than five, Fisher's Exact tests (2x2 tables) and exact significance tests for Pearson's chi-square (2x3 tables) were used. For 2x3 chi-square tables, if a significant relationship was found *post hoc* comparisons were performed on each pair of variables using 2x2 chi-square tests.

Due to the modest size of the sample and limited power, corrections were not routinely made for multiple testing. Therefore, any significant findings were treated with caution and an emphasis was placed on looking for trends firstly, between the prevalence of neuropsychiatric features amongst individuals in whom a pathogenic mutation *was* and *was not* detected and secondly, between the neuropsychiatric phenotypes of individuals with specific mutation types and/or with mutations located in specific locations of the *ATP2A2* gene.

The following chapter (Chapter 8) reports the results of the investigations carried out into the relationship between mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD.

8 GENOTYPE-PHENOTYPE CORRELATIONS BETWEEN MUTATIONS DETECTED IN ATP2A2 AND NEUROPSYCHIATRIC PHENOTYPES: RESULTS

This chapter reports investigations carried out into the relationship between mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD. First, the detection of non-pathogenic polymorphisms and pathogenic mutations in the sample is described. The type and location of mutations detected are then compared to the distribution of *ATP2A2* mutations previously reported in the literature. This is followed by a comparison of the clinical features of DD and neuropsychiatric phenotypes in individuals in whom a pathogenic mutation *was* and *was not* detected. Finally, genotype-phenotype correlations between the type and location of the pathogenic mutations detected and the neuropsychiatric phenotypes observed are investigated. Additional information relating to this chapter can be found in section D of the Appendix starting at pg. 316, and will be referenced throughout the chapter.

8.1 Mutation Detection in Index Individuals with DD

93 unrelated index individuals with DD, 11 additional affected and 17 unaffected family members were screened for variants in 20 of the 21 exons of *ATP2A2* including intron-exon boundaries (described in Chapter 7 section 7.2 pg. 146). As no PCR product could be generated for exon 1, this exon was not screened.

8.1.1 Frequencies of Non-Pathogenic Polymorphisms

The frequencies and percentages of previously reported non-pathogenic polymorphisms detected in the present sample are summarised in Table 8.1. One silent polymorphism in exon 15 (A724A) was detected in 10 index individuals (11%), 2 affected family members (18%) and one

unaffected individual (6%). A further silent polymorphism in exon 15 (S765S) was identified in one index individual. The most common polymorphism located in intron 18 was detected in 34 of the 93 index individuals (37%), 3 affected individuals (27%) and 5 unaffected individuals (29%). The frequencies of this polymorphism in the present study are broadly consistent with the previously reported frequency of this polymorphism (16%).

Table 8.1 Frequencies of Previously Reported Non-Pathogenic Polymorphisms

	Polymorphism					
Sample Type	2172G→A Exon 15 A724A		2295G→A Exon 15 S765S		2741+54G→A Intron 18	
	N	%	N	%	N	%
Index (n=93)	10	11	1	1	34	37
Affected (n=11)	2	18	0	0	3	27
Unaffected (n=17)	1	6	0	0	5	29

8.1.2 Quality Control

Pathogenic mutations causing DD were already known for seven individuals in the present sample. Four of these mutations were detected in the same individuals in the current mutation detection. The three other mutations that were not detected were either located in exon 1, for which no PCR product could be generated or were located in a DNA fragment which failed PCR amplification in those particular individuals (as described in section 7.2.1 pg. 147). Eighteen variants detected in the current sample had previously been reported in the literature, including the four mutations mentioned above.

In the case of 12 index individuals, a variant was detected that was not detected in their unaffected relative without DD. In five cases a variant was not detected in either the index individual or their unaffected relative. Seven variants detected in seven index individuals were also detected in an affected relative. There was one case where a variant was detected in an index

individual but not their affected relative. In this case the DNA fragment in which the variant was detected failed PCR amplification in the affected relative. In one case a DNA variant that was not detected in an index individual was detected in their affected relative, this is discussed later in the chapter. In four families, a variant was detected in an index and affected relative but not in an unaffected relative. These figures are summarised in Table 8.2

Table 8.2 Quality Control

	N
Variant *detected* in index with DD that was *not detected* in their unaffected relative	12
Variant *not detected* in index or unaffected relative	5
Variant detected in index and an affected relative	7
Variant detected in index but not detected in affected relative	1
Variant detected in affected but not index	1
Variant detected in an index and affected but not in an unaffected relative	4

8.1.3 Identification of Pathogenic Mutations

For 66 index individuals a DNA variant that could be causing DD was detected. Five of these variants were identified in more than one individual. In total, 59 possible DD causing variants were detected in 66 individuals, reported in Table 8.3. In the case of six individuals, more than one variant was identified. In all cases, one variant was selected as the variant most likely to be causing DD in that individual. The variants not selected are listed at the end of the table. An intronic variant was identified in an individual who also had a large 86 base pair deletion in exon 10 (Mutation ID 23), the latter was selected as the variant most likely to be causing DD. A missense mutation in exon 12 was identified in an individual with an in-frame deletion of one amino acid in exon 13 (Mutation ID 28). The latter mutation was also detected in an affected relative from the same family and was therefore selected as the mutation most likely to be causing DD in that family. Two mutations detected in the 3' untranslated region were identified

in 4 individuals in whom a DNA variation was also detected in the coding region of the gene. In all cases, the mutations detected in the coding region were selected as the mutations most likely to be causing DD (Mutation IDs 13, 44, 50, 52).

Additional mutation information was known for seven index individuals, also summarised in Table 8.3. In two cases, no DNA could be collected from the index individuals, but another family member with DD provided a DNA sample. In these cases the mutation information from the affected relative has been used for the index individual (ID 21 and ID 38). A pathogenic mutation was already known for three individuals, although these mutations were not detected in the current screen (ID 59, 62 and 63). In all three cases, the known pathogenic mutations were either in exon 1, for which no PCR product could be generated, (ID 62), or were in a DNA fragment in which failed PCR amplification in those particular individuals (ID 63 and 59). One of these mutations (ID 59) was also identified in another unrelated index individual. A further mutation was known for an individual from whom a DNA sample was not collected (ID 65). A frameshift variant in exon 13 was detected in the affected relative of an index individual, but not in the index individual (Mutation ID 64). However, PCR amplification of the fragment of DNA containing the variant did not fail in the index individual. One explanation for this could be that there was a further pathogenic variant not detected in either the index individual or affected relative causing DD in this family. However, the variant detected in the affected relative was a frameshift variant in a functional location (N domain) of the SERCA2 protein and therefore likely to be a pathogenic variant. There are no previous reports of more than one pathogenic variant in *ATP2A2* being detected in individuals with DD. A further explanation could be that the frameshift mutation was not detected in the index individual in the current mutation detection process. As this explanation is more likely the variant was included in the following analyses.

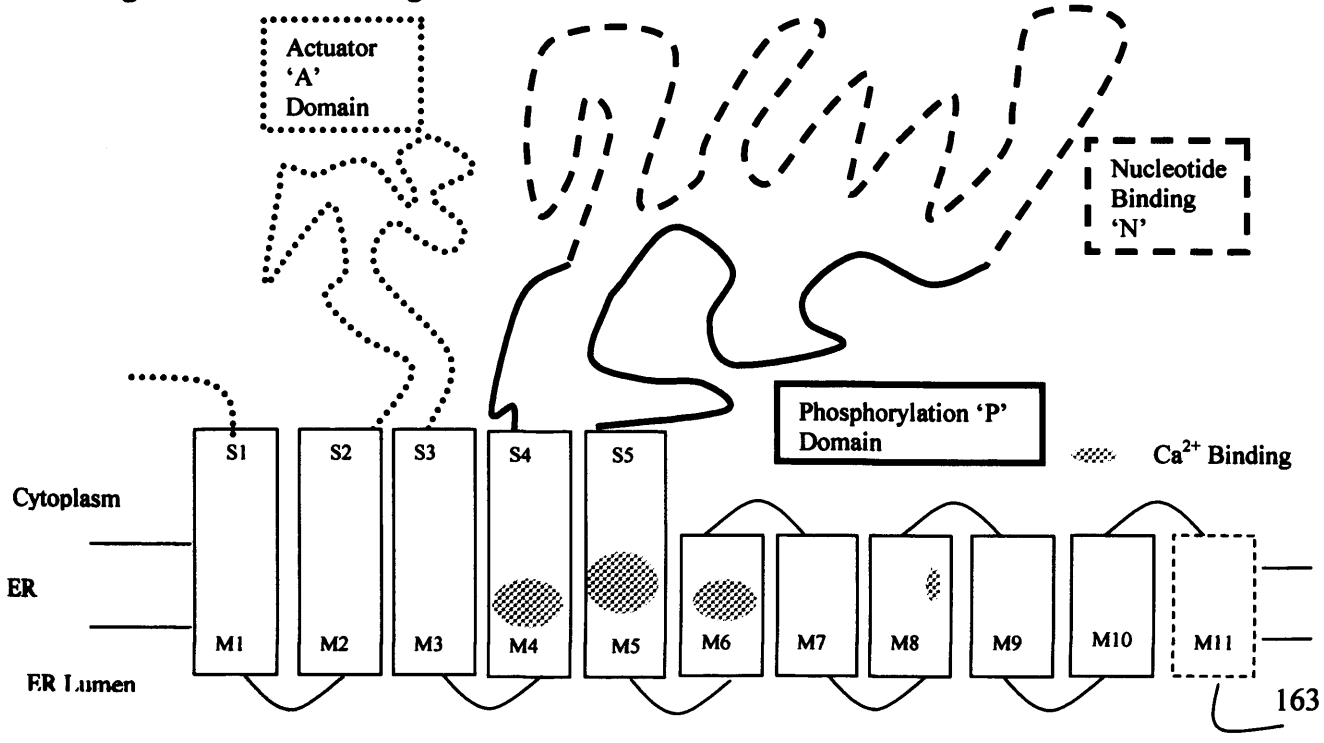
Table 8.3 Possible DD Causing DNA Variants Detected in *ATP2A2*

ID	Nucleotide Change	Exon/Intron	Amino Acid Change	Type	SERCA2b Protein Domain (See Figure 8.1)	Number of individuals
1	137-3 C>G	Intron 2		Splice site	S1	1
2	142 ins 18bp	Exon 3		Large Insertion	S1	1
3	167 A>G	Exon 3	Q56R	Missense	M1	1
4	194 T>C	Exon 3	L 65 S	Missense	M1	1
5	219+5 insert AA	Intron 3		Intronic	M1-M2 lumenal	1
6	325-2 A>G	Intron 4		Splice site	M2	1
7	464-2 A>C	Intron 5		Splice site	A	1
8	490 A>G	Exon 6	R164G	Missense	A	1
9	543delA	Exon 6		Frameshift Deletion	A	1
10	698 G>T	Exon 8	G233V	Missense	A	1
11	826 15bp del	Exon 8	IGHFN del	In-frame Deletion	M3-M4 lumenal	1
12	923 C>A	Exon 8	P308H	Missense	M4	1
13	925 G>A	Exon 8	E309K	Missense	M4	1
14	929 G>T	Exon 8	G310V	Missense	M4	1
15	948 del C	Exon 8		Frameshift Deletion	S4	1
16	949 7bp del	Exon 8		Frameshift Deletion	S4	1
17	958 G>C	Exon 8	A320P	Missense	S4	1
18	1000C>T	Exon 8	R334X	Nonsense	P	2
19	1043 T>C	Exon 8	I348T	Missense	P	1
20	1070 C>G	Exon 8	T357R	Missense	P	1
21	1070C>A	Exon 8	T 357 K	Missense	P	1*
22	1095+1 G>C	Exon 8		Splice site	N	1
23	1228 del 86 bp	Exon 10		Large Deletion	N	1
24	1321A>C	Exon 11	T 441 P	Missense	N	1
25	1413 C>A	Exon 11	C471X	Nonsense	N	1
26	1484 C>T	Exon 12	S495L	Missense	N	2
27	1508 delC	Exon 12		Frameshift Deletion	N	1
28	1628 del AGA	Exon 13	Del K543	In-frame Deletion	N	1
29	1713 del AA	Exon 13		Frameshift Deletion	N	1
30	1762-1 G>C	Intron 13		Splice site	N	1
31	1919 Ins T	Exon 14		Frameshift Insertion	P	1
32	2017 del C	Exon 14		Frameshift Deletion	P	1
33	2046 Ins C	Exon 14		Frameshift Insertion	P	1
34	2048 A>T	Exon 14	K683M	Missense	P	1
35	2098-2 A>C	Intron 14		Splice site	P	1
36	2104G>A	Exon 15	D 702 N	Missense	P	1
37	2116 G>A	Exon 15	D706N	Missense	P	2
38	2123C>A	Exon 15	P 708 H	Missense	P	1*
39	2249 G>A	Exon 15	R750Q	Missense	S5	1
40	2287 C>G	Exon 15	L763V	Missense	M5	1
41	2294 C>T	Exon 15	S765L	Missense	M5	2
42	2300 A>G	Exon 15	N767S	Missense	M5	4
43	2317 T>C	Exon 15	C773R	Missense	M5	1
44	2319-1 G>A	Intron 15		Splice site	M5	1
45	2384 A>G	Exon 16	N795S	Missense	M6	1
46	2405 C>G	Exon 16	P802R	Missense	M6	1
47	2406C>T	Exon 16	P 802 P	Silent	M6	1
48	2417 T>G	Exon 16	L806R	Missense	M6	1

Table 8.3 Continued.						
ID	**Nucleotide Change**	**Exon/Intron**	**Amino Acid Change**	**Type**	**SERCA2b Protein Domain (See Figure 8.1)**	**Number of individuals**
49	2527 G>T	Exon 17	V843F	Missense	M7	1
50	2584 Ins G	Exon 17		Frameshift Insertion	M7-M8	1
51	2620 C>T	Exon 18	Q874X	Nonsense	M7-M8	1
52	2678dupC	Exon 18	2678dupC	Frameshift Insertion	M7-M8	1
53	2684 C>T	Exon 18	P895L	Missense	M7-M8	1
54	2709 del 6bp	Exon 18	Del V904 and T905	In-frame Deletion	M8	1
55	2730 Ins C	Exon 18		Frameshift Insertion	M8	1
56	2741+1G>T	Intron 18		Splice site	M8	1
57	2741+5 G>C	Intron 18		Intronic	M8	1
58	2741+74 G>T	Intron 18		Intronic	M8	1
59	2759 C>A	Exon 19	S920Y	Missense	M8-M9	1 + 1*
60	2759 C>T	Exon 19	S920F	Missense	M8-M9	1
61	2777C>G	Exon 19	P926R	Missense	M8-M9	1
62	1A>G	Exon 1	M1V	Missense	N terminus	1*
63	1418delCA	Exon 11		In-frame exon (11) skipping	N domain	1*
64	1697dupA	Exon 13		Frameshift Insertion	N domain	1*
65	2258del3bp	Exon 15	Del N755	In-frame Deletion	S5	1*
Variants identified in individuals with more than mutation – selected as the variant least likely to be causing DD in that individual						
	219+179 G>T	Intron 3		Intronic	M1-M2 lumenal	1
	1484 C>T	Exon 12	S495L	Missense	N	1
	3126+238 A>G	3'UTR (20B)		transl 3'UTR		1
	3126+306 T>C	3'UTR (20B		transl 3'UTR		3

Protein Domains: M*n* = transmembrane domains, S*n* =stalk domains, A= Actuator Domain, P= Phosphorylation Domain, N= Nucleotide Binding Domain. * Mutation not detected in index individual in current mutation detection.

Figure 8.1 Schematic Diagram of Domains of SERCA2b Protein



Including the mutation information known for the additional seven individuals, 65 possible pathogenic DD causing variants were detected/known in 73 index individuals. In order to decide which of these variants were likely to be a pathogenic mutation, the variants were ranked in categories ranging from the mutation types most likely to be causing DD to variants less likely to be causing DD, shown in Table 8.4. Sixty of the 65 variants were ranked as likely to be pathogenic and the remaining five variants, including a non-synonymous amino acid change, a synonymous amino acid change and intronic variants were ranked as being less likely to be pathogenic.

In summary, 60 variants detected and/or known in 68 unrelated individuals were ranked as likely to be pathogenic. Genotype-phenotype correlations were carried out between the type and location of the pathogenic mutations detected and the neuropsychiatric phenotypes observed in these 68 individuals.

Table 8.4 Ranking of Possible DD Causing DNA Variants Detected in *ATP2A2*

Pathogenic Mutation Rank	**Mutation ID**	**Total**
1. Reported in literature as pathogenic	4 14 19 21 26 32 36 39 41 42 45 49 52 53 59 62 63 65	18
2. Large insertion/deletions	2 23	2
3. Nonsense or Frameshift	9 15 16 18 25 27 29 31 33 50 51 55 64	13
4. Non-synonymous a.a change: Non conservative (predicted as not tolerated with SIFT)	3 8 10 12 13 17 20 24 34 37 38 40 46 48 60 61	16
5. In-frame insertion/deletion	11 28 54	3
6. Splice site	1 6 7 22 30 35 44 56	8
Mutations ranked as likely to be pathogenic =60		
7. Non-synonymous a.a change: Conservative (predicted as tolerated with SIFT)	43	1
8. Synonymous a.a change (Silent)	47	1
9. Intronic	5 57 58	3
Mutations ranked as less likely to be pathogenic=5		

SIFT; Sorting Intolerant From Tolerant, a.a; amino acid.

8.2 Comparison of Type and Location of Mutations Detected Compared to Previously Reported Mutations in ATP2A2

This section compares the type and location of the 60 mutations detected/known in 68 unrelated individuals in the present sample to the distribution of 143 *ATP2A2* mutations previously reported in 175 unrelated individuals in the literature. Eighteen of the mutations detected/known in individuals in the present study had previously been reported in the literature. Therefore 42 novel pathogenic mutations in *ATP2A2* were detected in the present study. The frequencies of mutations detected in the present sample were combined with mutations previously reported in the literature. Ten individuals in the present study whose mutations have previously been reported in the literature were counted only once. A total of 185 mutations in *ATP2A2* have now been detected in 233 unrelated individuals with DD.

The frequencies and percentages of pathogenic mutations and individuals in the present study according to *mutation type, ATP2A2 exon/intron mutation location* and *SERCA2 domain mutation location* are summarised in Appendix D.i. pg 316 (Table D-1 to D-6) where they are compared to the distribution of mutations and unrelated individuals previously reported in the literature. In all cases, the distribution of mutation types and locations were very similar to the distributions previously reported in the literature. The largest percentage difference in *ATP2A2 exon/intron mutation* distribution was found in exon 8, 20% of mutations in the present study were found in exon 8 compared to 10.5% previously reported in the literature and 19.1% of individuals in the present study had mutations in exon 8 compared to 10.9% of individuals in the literature (see Tables D-3 pg. 317 and D-4 pg. 318). Differences were also found in exon 1 (1.7% mutations in present study vs. 5% in literature and 1.5% of individuals vs. 7.4%). This was expected since no PCR product for this exon could be generated in the present study. One of the seven individuals in the present study with an already known pathogenic mutation had a mutation

in exon 1. The largest percentage difference in *SERCA2 domain mutation location* was in the A domain (6.7% mutations vs. 21% and 5.9% individuals vs. 22.3%) (see Tables D-5 pg. 319 and D-6 pg. pg. 320). However, this difference would be expected as exon 1 is translated into part of the A domain.

8.3 Comparison of the Clinical Features of DD and Neuropsychiatric Phenotype in Individuals in whom a Pathogenic Mutation was and was not Detected/Known

This section compares the clinical features of DD and the neuropsychiatric phenotype in the 68 individuals in whom a pathogenic mutation was detected /known to the 28 individuals where a pathogenic mutation was not detected/known. Four individuals from whom no DNA was collected and no mutation was known were not included in the following analyses. As highlighted in the previous section, 7.4% of individuals previously reported in the literature were found to have a mutation in Exon 1 for which no PCR product could be generated in the present study. One individual in the present sample was already known to have a pathogenic mutation in Exon 1 (Table 8.3 pg. 162). Therefore it would be expected that approximately six further individuals in the present sample would have a mutation in Exon 1. In addition, in 17 of the 28 individuals in whom a pathogenic mutation was not detected, there were between one and three exon fragments for which no PCR product could be generated. Therefore one reason for a pathogenic mutation not being detected in individuals is that they had a pathogenic mutation in a fragment of DNA for which no PCR product could be generated. A further possibility is that for a subset of individuals, the clinical features similar to DD may be caused by factors other than a pathogenic mutation in *ATP2A2* (i.e. individuals might be phenocopies of DD).

8.3.1 Comparison of Clinical Features of DD

Comparisons of reported family history of DD, DD severity and Dermatology Life Quality Index (DLQI) worst week scores amongst individuals in whom a pathogenic mutation *was* and *was not* detected are shown in Appendix D.ii Table D-7 and D-8 pg. 321.The DLQI has been previously described in Chapter 3, section 3.2.2.5 pg. 38. Significantly more individuals in whom a pathogenic mutation *was* detected reported a definite or probable family history of DD (79% vs. 57%; χ^2=4.465, df=1, p=0.035). Within individuals in whom a pathogenic mutation *was* detected, 18%, 72% and 10% were classified as having mild, moderate and severe DD respectively compared to 36%, 50% and 14% individuals in whom a pathogenic mutation *was not* detected. There was no significant relationship between a pathogenic mutation being identified and DD severity (χ^2 =4.571, df=2, p= 0.102). Individuals in whom a pathogenic mutation *was* and *was not* detected did not differ significantly in their median scores on the DLQI- worst week (15 vs. 15.5; U=849, n=95, p=0.467).

8.3.2 Comparison of Neuropsychiatric Features

Comparisons of positive lifetime DSM-IV diagnosis, lifetime history of suicide attempts, history of contact with psychiatric services, history of learning difficulties and history of investigations for blackouts, loss of consciousness or fainting episodes respectively amongst individuals in whom a pathogenic mutation *was* and *was not* detected are shown Appendix D.ii Table D-9 pg. 322 and summarised graphically in Figure 8.2 pg. 169. No significant relationships were found, although in all cases, there was a trend for the percentages of individuals to be higher amongst individuals in whom a pathogenic mutation *was* detected.

Comparisons of scores on the Bipolar Affective Disorder Dimensional Scale (BADDS) mania and depression dimensions and age of onset of psychiatric illness among individuals in

whom a pathogenic mutation *was* and *was not* detected are shown in Table D-10 pg. 322. The BADDS has previously been described in Chapter 3, section 3.2.5 pg. 42. Individuals in whom a pathogenic mutation *was* detected had significantly higher median scores on the BADDS Mania Dimension (8 vs. 4; U=344.5, n=70, p=0.014) and non-significantly higher median scores on the BADDS depression dimension (54 vs. 45; U=148, n=45, p=0.132). The two groups did not significantly differ in their age of onset of psychiatric illness. The box plots in Figure 8.3 and Figure 8.4 represent the median, interquartile range and minimum and maximum scores on the BADDS mania and depression dimensions amongst individuals in whom a pathogenic mutation *was* and *was not* detected.

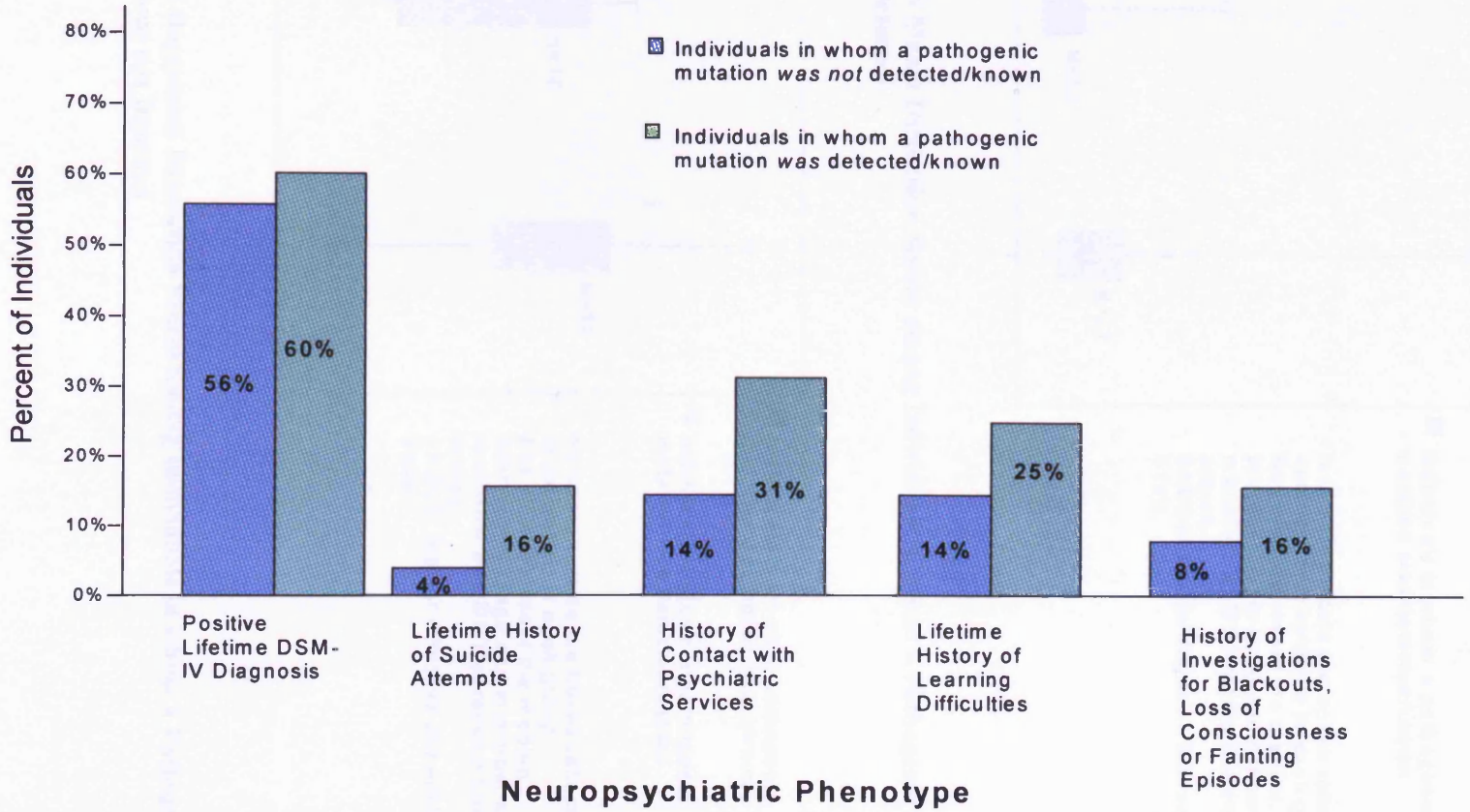


Figure 8.2 Summary of the Neuropsychiatric Phenotype in Individuals in whom a Pathogenic Mutation *was* and *was not* Detected/Known

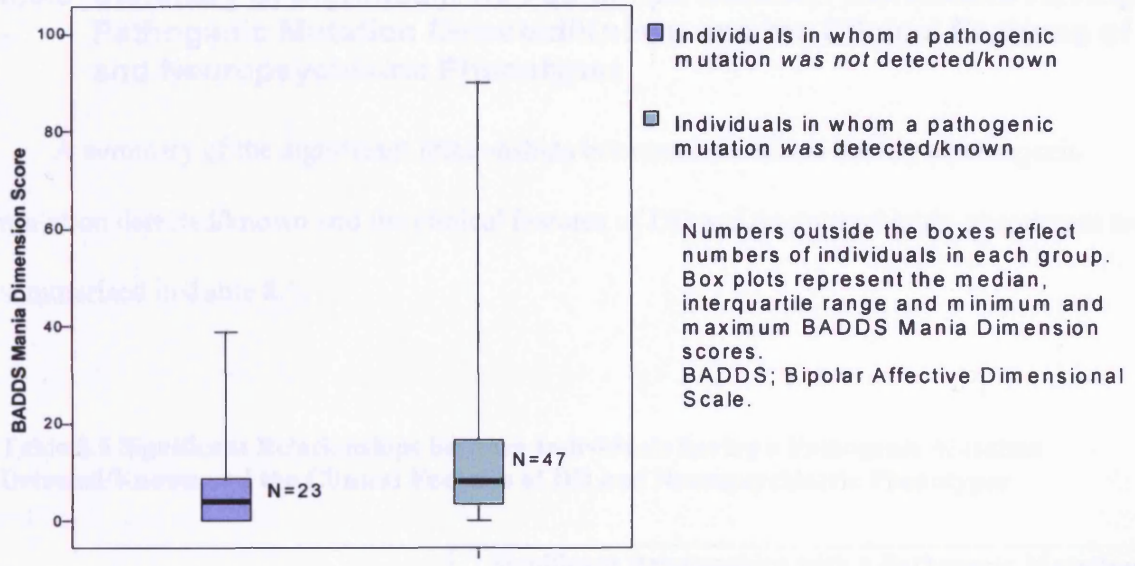


Figure 8.3 BADDS Mania Dimension Scores among Individuals in whom a Pathogenic Mutation *was* and *was* not Detected

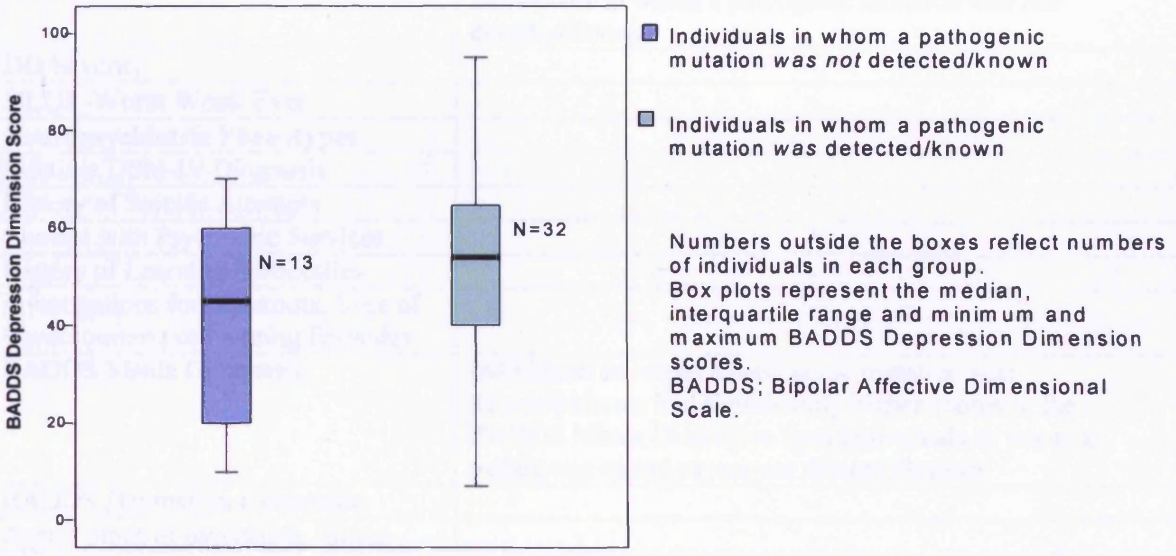


Figure 8.4 BADDS Depression Dimension Scores among Individuals in whom a Pathogenic Mutation *was* and *was* not Detected

8.3.3 Summary of Significant Relationships Between Individuals Having a Pathogenic Mutation Detected/Known and the Clinical Features of DD and Neuropsychiatric Phenotypes

A summary of the significant relationships between individuals having a pathogenic mutation detected/known and the clinical features of DD and neuropsychiatric phenotypes are summarised in Table 8.5.

Table 8.5 Significant Relationships between Individuals having a Pathogenic Mutation Detected/Known and the Clinical Features of DD and Neuropsychiatric Phenotypes

	Significant Relationships with a Pathogenic Mutation Being Detected/Known
Clinical Features of DD	
Family History of DD	A significantly higher proportion of individuals in whom a pathogenic mutation *was* detected/known had a definite/probable family history of DD compared to individuals in whom a pathogenic mutation *was not* detected/known
DD Severity	-
DLQI -Worst Week Ever	-
Neuropsychiatric Phenotypes	
Lifetime DSM-IV Diagnosis	-
History of Suicide Attempts	-
Contact with Psychiatric Services	*
History of Learning Difficulties	-
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes	-
BADDS Mania Dimension	Individuals in whom a pathogenic mutation *was* detected/known had significantly higher scores in the BADDS Mania Dimension than individuals in whom a pathogenic mutation *was not* detected/known.
BADDS Depression Dimension	-
Age of onset of psychiatric illness	-

DLQI; Dermatology Life Quality, BADDS; Bipolar Affective Disorder Dimensional Scale.
* Relationship almost reached significance; a higher proportion of individuals in whom a pathogenic mutation *was* detected/known had a history of contact with psychiatric services compared to individuals in whom a pathogenic mutation *was not* detected/known (31% vs. 14%; p=0.085).

8.4 Investigations of Neuropsychiatric Genotype-Phenotype Correlations- Schematic Diagram of SERCA2b

The neuropsychiatric phenotypes observed in the 68 individuals in whom a pathogenic mutation was detected/known are summarised in Table 8.6 pg. 173. Known neuropsychiatric features in individuals' first-degree relatives with DD are also displayed in the table. The pathogenic mutations detected/known in the 68 individuals were mapped onto schematic diagrams of the SERCA2b protein and highlighted according to the presence of a specific neuropsychiatric phenotype. Two of these diagrams, highlighting the location of pathogenic mutations in individuals with a history of contact with psychiatric services and individuals with a personal history of bipolar disorder or first degree relative with DD and a history of bipolar disorder, are shown below in Figure 8.5 pg. 177 and Figure 8.6 pg. 178 and the remainder are shown in Appendix D.iii pg 323 (Figures D-1 to D-6). The numbering of the mutations corresponds to the ID numbers previously reported in Table 8.3 pg. 162. Mutations with the ID numbers 5,43,47,57 and 58 were not included in the diagrams as they were considered unlikely to be pathogenic (as discussed in section 8.1.3 pg. 160).

A summary of mutations detected within SERCA2b according to specific neuropsychiatric phenotypes is shown in Table 8.7 pg. 179 and observations of possible clustering of mutations are reported in Table 8.8 pg. 181.

Table 8.6 Neuropsychiatric Features in 68 Individuals in whom a Pathogenic Mutation was Detected/Known and their First Degree Relatives with DD

Study ID	ID**	Neuropsychiatric Features (including DSM-IV Lifetime Main Diagnosis, History of Suicide Attempts, Contact with Psychiatric Services, Learning Difficulties and Investigations for Blackouts, Loss of Consciousness or Fainting Episodes)	BADDS M Score	BADDS D Score	Neuropsychiatric Features in first-degree relatives with DD (n)
DAR36-1	1	Tonic clonic epilepsy, learning difficulties.	-	-	
DAR91-1	2	Bipolar I Disorder*. Fit aged 10 months, CT and EEG investigations for periods of unconsciousness in late 20s.	81	50	Psychiatric treatment for depression and anxiety, possibly a bipolar illness (1)
DAR14-1	3	MDDR*, mild high mood after birth of children followed by episodes of depression.	30	64	ECT treatment for depression (1)
DAR95-1	4	MDDR, learning difficulties.	-	51	Mild form of epilepsy (1)
DAR75-1	6		-	-	
DAR81-2	7	Bipolar I Disorder*.	65	70	Depression and epilepsy (2), puerperal psychosis (1), depression (1)
DAR11-1	8	MDDS*, learning difficulties.	8	60	
DAR97-1	9	MDDR*	-	53	
DAR7-1	10	Depression NOS*, suicide attempt, diagnosis of dyslexia.	-	39	
DAR38-1	11		3	-	
DAR6-1	12	Depression NOS.	11	-	
DAR65-1	13		5	-	Bipolar Disorder (1) Seen by educational psychologist (1)
DAR3-1	14		17	-	***
DAR70-1	15	Unknown DSM-IV*, suicide attempt, cyclothymic personality, idiopathic epilepsy.	-	-	
DAR55-1	16	MDDR*, suicide attempt, extensive psychiatric history, diagnosis of somatoform disorder. Brain scan to investigate a blackout.	16	74	Treated by psychiatrist for depression (1)
DAR43-1	17		1		Psychiatric hospital treatment for depression (1)
DAR1-1	18a		1		
DAR21-1	18b	Unknown DSM-IV.	17		
DAR77-1	19		-		
DAR10-1	20		4		
DAR76-1	21	MDDR, learning difficulties.	-	51	

Table 8.6 Continued					
Study ID	**ID****	**Neuropsychiatric Features (including DSM-IV Lifetime Main Diagnosis, History of Suicide Attempts, Contact with Psychiatric Services, Learning Difficulties and Investigations for Blackouts, Loss of Consciousness or Fainting Episodes)**	**BADDS M Score**	**BADDS D Score**	**Neuropsychiatric Features in first-degree relatives with DD (n)**
DAR27-1	22	MDDR, possible symptoms of mania after birth of first child, query of epileptic fit in GP notes, learning difficulties.	17	41	Treated by psychiatrist for depression (1)
DAR30-1	23	MDDR*, suicide attempt, extensive psychiatric history, learning difficulties.	-	69	Schizophrenia (1)
DAR99-1	24	EEG and CT scans for fainting episodes.	5		
DAR4-1	25	MDDR*, multiple suicide attempts, extensive psychiatric history, brain scan investigations for blackouts, learning difficulties.	17	72	
DAR39-1	26a	MDDR, suicide attempt.	-	65	
DAR62-1	26b	MDDS, chronic fatigue symptoms for 16 years.	9	50	
DAR84-1	27	Panic Disorder Without Agoraphobia.	12	7	
DAR51-2	28		5		Treated by psychiatrist for depression (1) psychiatric hospital treatment for depression (1)
DAR29-1	29	MDDR*.	0	61	
DAR56-1	30	Unknown DSM-IV*.	6	-	Treated by psychiatrist for depression (1)
DAR60-1	31		3	-	
DAR28-1	32	MDDR, CT scan for syncopal attacks.	15	62	Treated by psychiatrist for depression and multiple suicide attempts (2)
DAR61-1	33	Anxiety Disorder NOS, learning difficulties - born with hole in the heart.	-	-	
DAR92-1	34	Two-five blackouts, investigated with CT scan.	17	39	
DAR88-1	35		-	-	
DAR103-1	36	Dysthymic Disorder*, learning difficulties.	17	39	Seen by educational psychologist and mental health services (1)
DAR85-1	37a	MDDS*, suicide attempt, learning difficulties.	7	60	
DAR44-1	37b		3	-	No details-adopted
DAR86-1	38		2	-	

Table 8.6 Continued					
Study ID	**ID****	**Neuropsychiatric Features (including DSM-IV Lifetime Main Diagnosis, History of Suicide Attempts, Contact with Psychiatric Services, Learning Difficulties and Investigations for Blackouts, Loss of Consciousness or Fainting Episodes)**	**BADDS M Score**	**BADDS D Score**	**Neuropsychiatric Features in first-degree relatives with DD (n)**
DAR40-1	39	MDDS, fainting episodes for over 30 years.	8	60	
DAR79-1	40	Depression NOS.	16	39	Suicide attempt (1)
DAR8-1	41a		1		
DAR26-1	41b		7		
DAR72-1	42a	MDDR*, brain scan to investigate blackouts.	15	63	
DAR41-1	42b	Depression NOS.	-	41	
DAR74-1	42c	MDDR, paranoia not reaching delusional intensity.	-	70	Diagnosis of schizophrenia, depression and suicide attempt (1)
DAR50-1	42d	Unknown DSM-IV, learning difficulties.	14	-	
DAR24-1	44	Depression NOS, CT for headache and fainting episodes.	10	30	Treated by mental health services (1)
DAR89-1	45	Anxiety Disorder NOS.	0		
DAR90-1	46	Bipolar I Disorder*, puerperal psychosis following the birth of only child.	70	10	
DAR47-1	48	MDDR	-	51	
DAR52-1	49	MDDR*, suicide attempt, two week period of blackouts investigated by a neurologist and brain scans.	5	66	
DAR78-1	50		-	-	
DAR16-1	51	MDDR*, described in medical notes as having "a curious affect", learning difficulties, labile mood at interview.	-	55	
DAR98-1	52	MDDR.	-	-	
DAR19-1	53	Depression NOS, suicide attempt.	5	19	
DAR9-1	54		16	-	
DAR94-1	55	Depression NOS.	2	-	
DAR83-1	56		-	-	
DAR68-1	59a	Learning difficulties.	13		Learning disability and epilepsy (1)
DAR5-1	59b	Unknown DSM-IV*, learning difficulties.	-		Depression and suicide attempt (1)
DAR53-1	60	Depression NOS.	7	30	
DAR64-1	61	Cyclothymic Disorder*, learning difficulties	17		
DAR54-1	62	Learning difficulties.	-		
DAR15-1	63	GP investigations for fainting episodes since childhood.	1	-	Learning disabilities (1).

Table 8.6 Continued					
Study ID	**ID****	**Neuropsychiatric Features (including DSM-IV Lifetime Main Diagnosis, History of Suicide Attempts, Contact with Psychiatric Services, Learning Difficulties and Investigations for Blackouts, Loss of Consciousness or Fainting Episodes)**	**BADDS M Score**	**BADDS D Score**	**Neuropsychiatric Features in first-degree relatives with DD (n)**
DAR18-1	64	Bipolar I Disorder*, suicide attempt, learning difficulties. EEG and CT investigations following an episode of loss of consciousness.	90	95	
DAR69-1	65	Learning Difficulties.	2	-	

****ID numbers correspond to those previously reported in Table 8.4 pg. 162.**

* Contact with psychiatric services.

***Mother was admitted to psychiatric hospital with 'high mood' although neither parent was reported to have DD.

BADDS; Bipolar Affective Disorder Dimensional Scale Mania (M) and Depression (D) Dimensions, MDDR; Major Depressive Disorder Recurrent Episodes, MDDS; Major Depressive Disorder Single Episode, NOS; Not Otherwise Specified

Figure 8.5 Genotype-Phenotype Correlation: History of Contact with Psychiatric Services

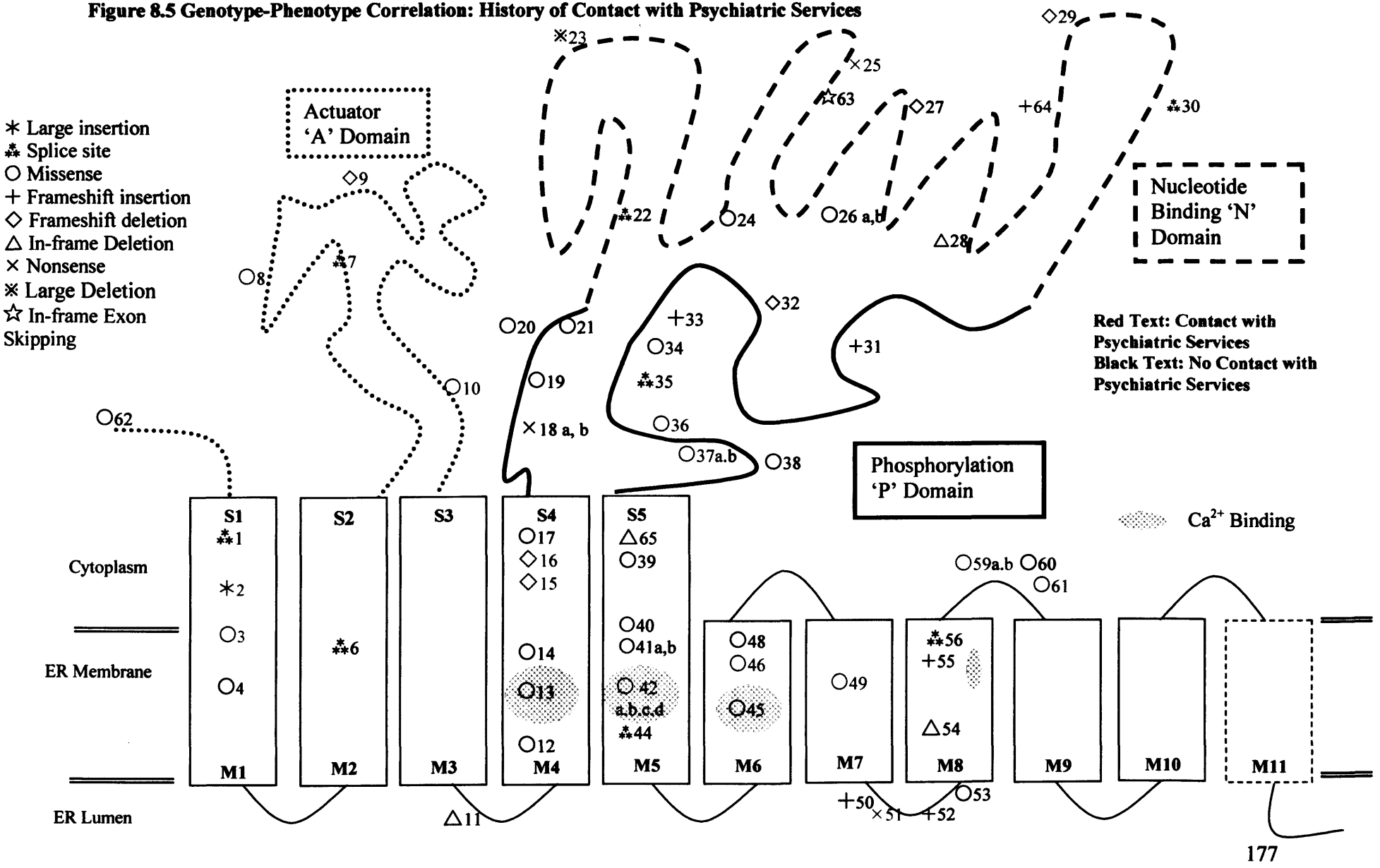


Figure 8.6 Genotype-Phenotype Correlation: Personal History of Bipolar Disorder or First Degree Relative with DD and a History of Bipolar Disorder

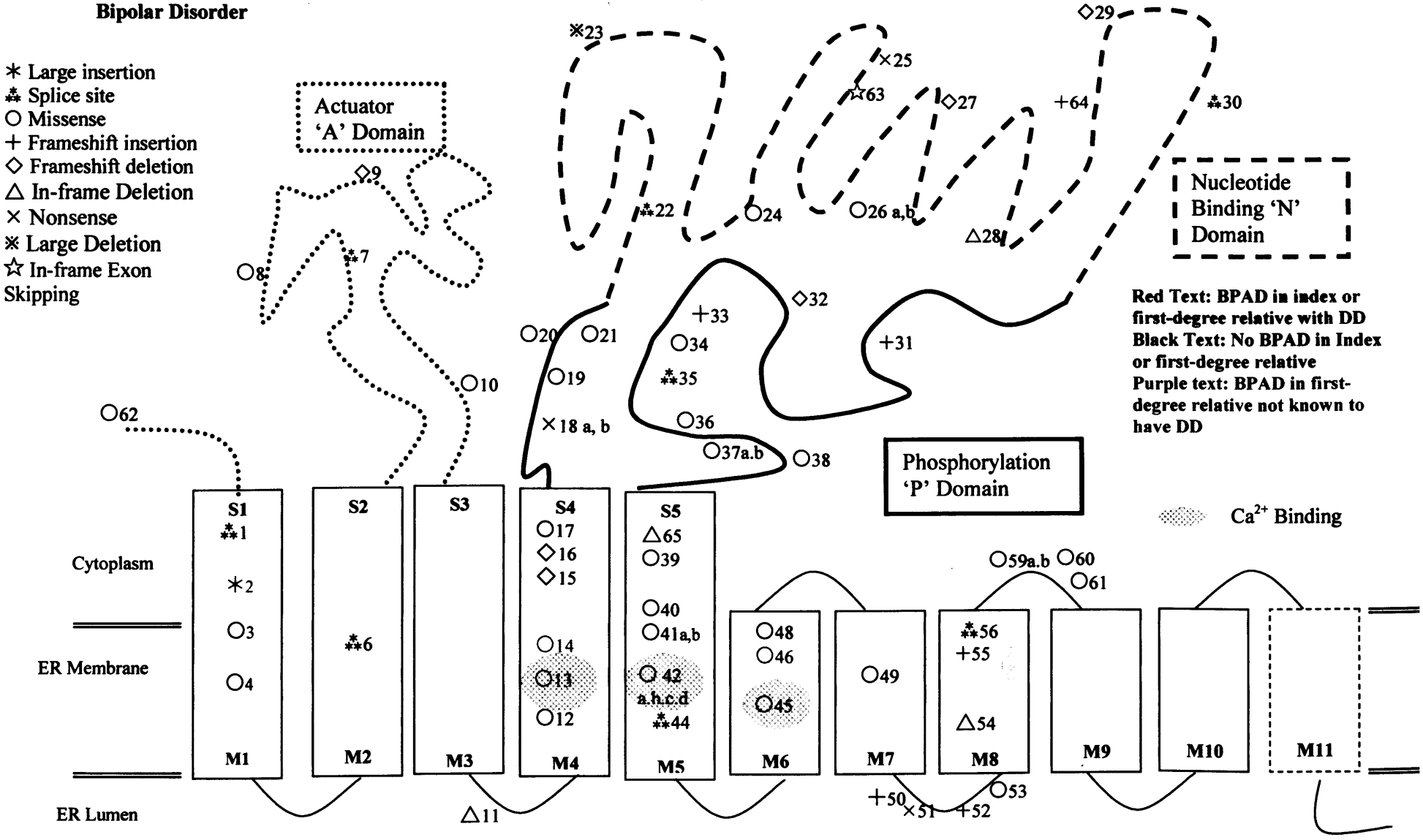


Table 8.7 Summary of Mutations within the SERCA2b Protein according to Neuropsychiatric Phenotypes

ID**	Mutation Type	SERCA2b Protein Domain	History of Contact with Psychiatric Services	History of Bipolar Disorder or First Degree Relative with DD and a History of Bipolar	BADDS Mania Scores ≥10	History of Suicide Attempts	DSM-IV Lifetime Diagnosis	Learning Difficulties	Investigations for Blackouts, Loss of Consciousness or Fainting Episodes
62	○	N terminus							
1	⁂	S1							
2	✳	S1							
3	○	M1							
4	○	M1							
6	⁂	M2							
7	⁂	A							
8	○	A							
9	◇	A							
10	○	A							
11	△	M3-M4 lumenal							
12	○	M4							
13	○	M4							
14	○	M4							
15	◇	S4							
16	◇	S4							
17	○	S4							
18a	×	P							
18b	×	P							
19	○	P							
20	○	P							
21	○	P							
22	⁂	N							
23	※	N							
24	○	N							
25	×	N							
63	☆	N							
26a	○	N							
26b	○	N							
27	◇	N							
28	△	N							
64	+	N							
29	◇	N							
30	⁂	N							
31	+	P							
32	◇	P							
33	+	P							
34	○	P							
35	⁂	P							

Table 8.7 Continued.

ID**	Mutation Type	SERCA2b Protein Domain	History of Contact with Psychiatric Services	History of Bipolar Disorder or First Degree Relative with DD and a History of Bipolar	BADDS Mania Scores ≥10	History of Suicide Attempts	DSM-IV Lifetime Diagnosis	Learning Difficulties	Investigations for Blackouts, Loss of Consciousness or Fainting Episodes
36	○	P							
37a	○	P							
37b	○	P							
38	○	P							
65	◇	S5							
39	○	S5							
40	○	M5							
41a	○	M5							
41b	○	M5							
42a	○	M5							
42b	○	M5							
42c	○	M5							
42d	○	M5							
44	⁂	M5							
45	○	M6							
46	○	M6							
48	○	M6							
49	○	M7							
50	+	M7-M8							
51	×	M7-M8							
52	+	M7-M8							
53	○	M7-M8							
54	△	M8							
55	+	M8							
56	⁂	M8							
59a	○	M8-M9							
59b	○	M8-M9							
60	○	M8-M9							
61	○	M8-M9							

****ID numbers correspond to those previously reported in** Table 8.4 **pg 164.**

Protein Domains: M*n* = transmembrane domains, S*n* =stalk domains, A= Actuator Domain, P= Phosphorylation Domain, N= Nucleotide Binding Domain.

Coloured cells indicate the presence of a specific neuropsychiatric phenotype.

∗ Large insertion, ⁂ Splice site, ○ Missense , + Frameshift insertion, ◇ Frameshift deletion, △ In-frame Deletion, × Nonsense, ※ Large Deletion, ☆ In-frame Exon Skipping.

Table 8.8 Observations of Possible Clustering of Mutations within the SERCA2b Protein according to Specific Neuropsychiatric Phenotypes

Figure; Page number	Neuropsychiatric Phenotype	Observations
Figure 8.5;177	Contact with Psychiatric Services	All four individuals with mutations in the 'A' domain had a history of contact with psychiatric services (ID 7,8, 9, 10). Other observed clusters of history of contact with psychiatric services: • Two individuals with missense mutations four a.a apart in the 'P' domain (ID 36 and 37a). • Two individuals with frameshift mutations only one base pair apart in the S4 (Stalk 4) domain (ID 15 and 16). • Two individuals with missense mutations 6 a.a. apart in the M8-M9 cytosolic loop (ID 59b and 61). • Three individuals with mutations located close together in the ' N' domain (ID 64, ID 29 and ID 30).
Figure 8.6;178	Personal History of BPAD or 1st Degree Relative with DD with a History of BPAD	• An individual with a missense mutation at one of the seven Ca^{2+} binding sites within the M4 domain (ID 13) had a 1st degree relative with DD and Bipolar Disorder. An individual with a missense mutation (ID 14) at an adjacent amino acid, with a BADDS mania score of 17, also had a parent who had been treated in a psychiatric hospital for 'high mood' on two occasions although there was no reported history of DD in the family. • A further individual with bipolar disorder had a missense mutation in the M5 domain close another Ca^{2+} binding site.
Figure D-1; 323	BADDS Mania Score	• Two individuals with BADDS mania scores of 81 and 30 (ID 2 and 3) respectively both had mutations in the M1 domain. • Four individuals with mutations in the M5 domain had a BADDS Mania Score equal to or greater than 10 (ID 40, 42a, 42d and 44). Two of these individuals had the same missense mutation. • Two individuals with missense mutations in the M8-M9 cytosolic loop (ID 59a and 61) had BADDS mania scores of 13 and 17 respectively.
Figure D-2; 324	History of Suicide Attempts	• Two individuals with frameshift mutations only one base pair apart in the S4 domain had a history of suicide attempts (ID 15 and 16).
Figure D-3; 325	Lifetime DSM-IV Diagnosis	• Three individuals with the same missense mutation at one of the seven Ca^{2+} binding sites in the M5 domain (ID 42a, b and c) all had a DSM-IV diagnosis of a mood disorder. An individual with the same mutation (ID 42d) and an unknown DSM-IV diagnosis. • Two individuals with the same missense mutation in the N domain (ID 26 a and b) had a lifetime DSM-IV diagnosis of major depression.
Figure D-4; 326	Learning Difficulties	• Three individuals with missense mutations in the M8-M9 cytosolic loop were classified as having learning difficulties (ID 59a, 59b, 61). Two of these individuals had the same mutation (ID 59).
Figure D-6; 328	Diagnosis of Epilepsy or 1st Degree Relative with DD and a Diagnosis of Epilepsy	• Two individuals with mutations in the S1 and M1 domains had a personal diagnosis of epilepsy (ID 1) or first degree relative with a diagnosis of epilepsy (ID 4).

a.a; amino acid, BADDS; Bipolar Affective Disorder Dimension Scale.

8.5 Investigations of Genotype-Phenotype Correlations- Mutation Type

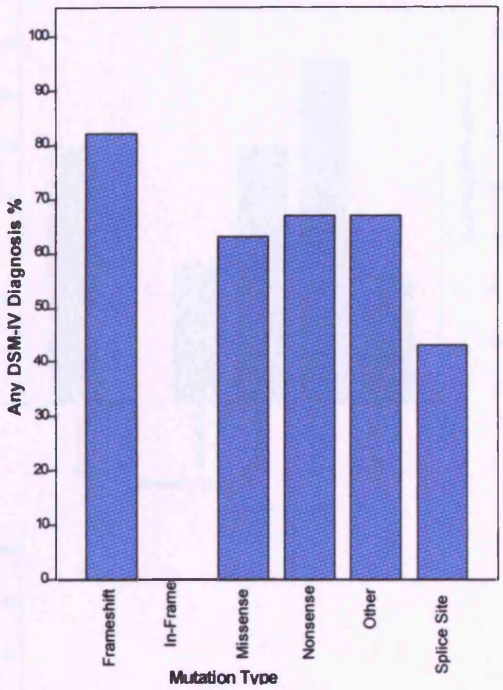
This section investigates possible relationships between the types of pathogenic mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed in the 68 individuals in whom a pathogenic mutation was detected. Comparisons of the percentages of neuropsychiatric phenotypes, BADDS mania and depression scores and age of onset of psychiatric illness among individuals with different mutation types are shown in Appendix D.iv Table D-11 pg. 329 to D-13 pg. 330 and summarised graphically in Figure 8.7 to Figure 8.9 pg. 183 to 185. Table 8.9 summarises the relationships investigated between the types of mutation detected in *ATP2A2* and specific neuropsychiatric phenotypes, no significant relationships were found.

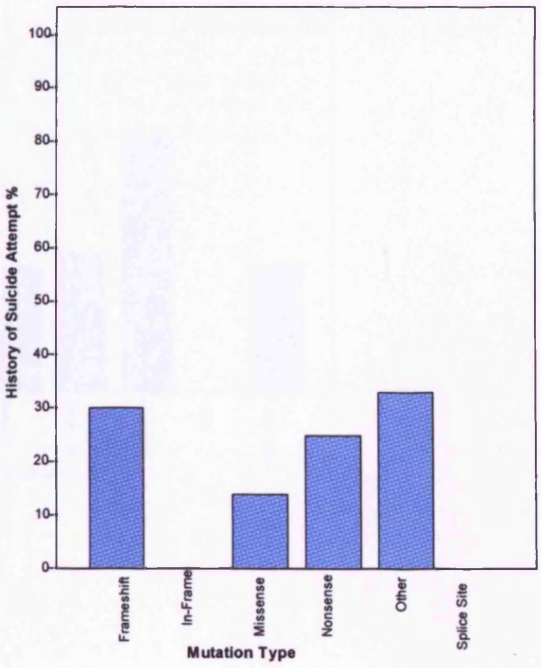
Table 8.9 Summary of Relationships between Type of Pathogenic Mutation and Neuropsychiatric Phenotypes

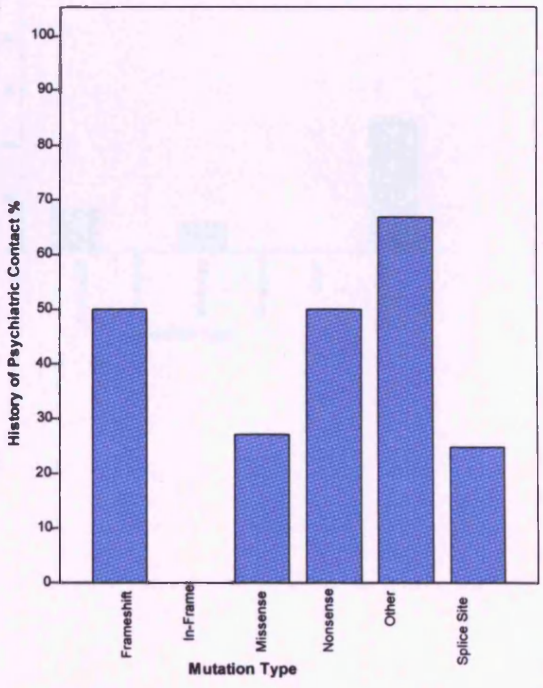
Neuropsychiatric Phenotype	Relationship with Type of Pathogenic Mutation	Table/ Figure
Any Lifetime DSM-IV Diagnosis	*χ^2=9.291, df=5, p= 0.090	D-11 pg. 329 Figure 8.7
History of Suicide Attempts	*χ^2=4.817, df=5, p= 0.441	
History of Psychiatric Contact	*χ^2=6.242, df=5, p= 0.284	
Learning Difficulties	*χ^2=2.859, df=5, p= 0.760	
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes	*χ^2=3.866, df=5, p= 0.527	
Personal History of Bipolar Disorder and/or 1st Degree Relatives with DD and Bipolar Disorder	*χ^2=3.570, df=5, p= 0.657	D-12 pg. 329 Figure 8.7
Diagnosis of Epilepsy and/or 1st Degree Relatives with DD and a Diagnosis of Epilepsy	*χ^2=4.753, df=5, p= 0.413	
BADDS Mania Dimension	**H=2.620, df=5, p=0.758	D-13 pg. 330 Figure 8.8 &Figure 8.9
BADDS Depression Dimension	**H=3.369, df=4, p=0.498	
Age of onset of psychiatric Illness	**H=0.424, df=4,p=0.981	

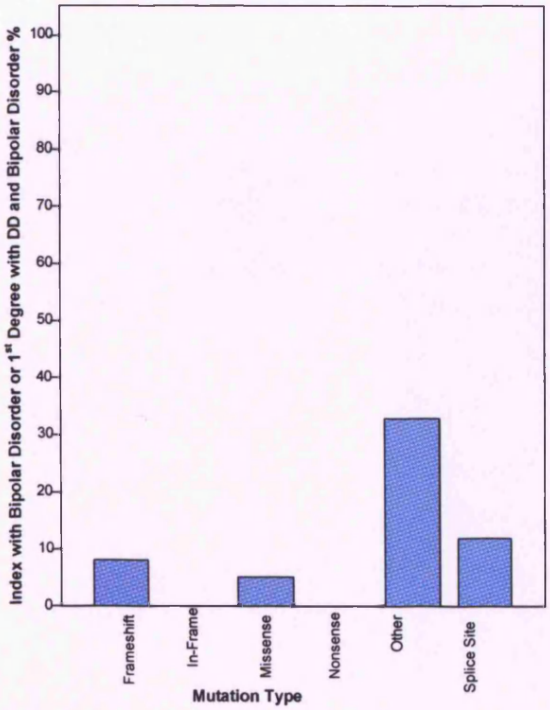
* Exact significance tests for Pearson's chi-square, ** Kruskal-Wallis H tests.

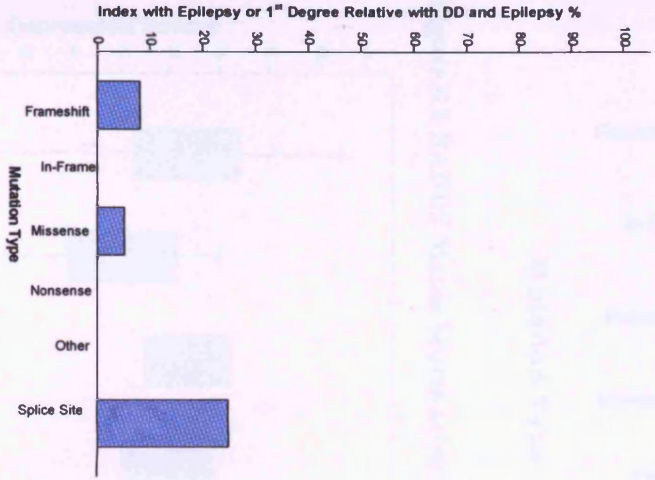
Figure 8.7 Percentages of Individuals with Specific Neuropsychiatric Phenotypes according to Mutation Types

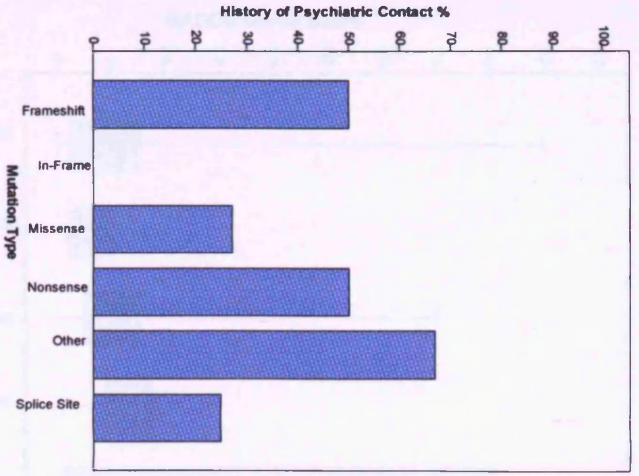


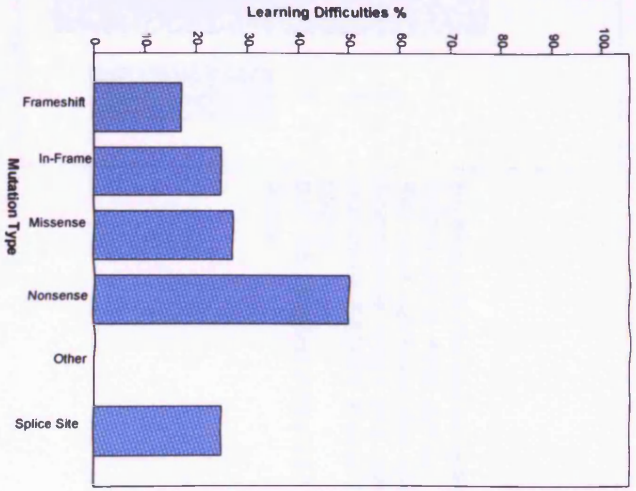


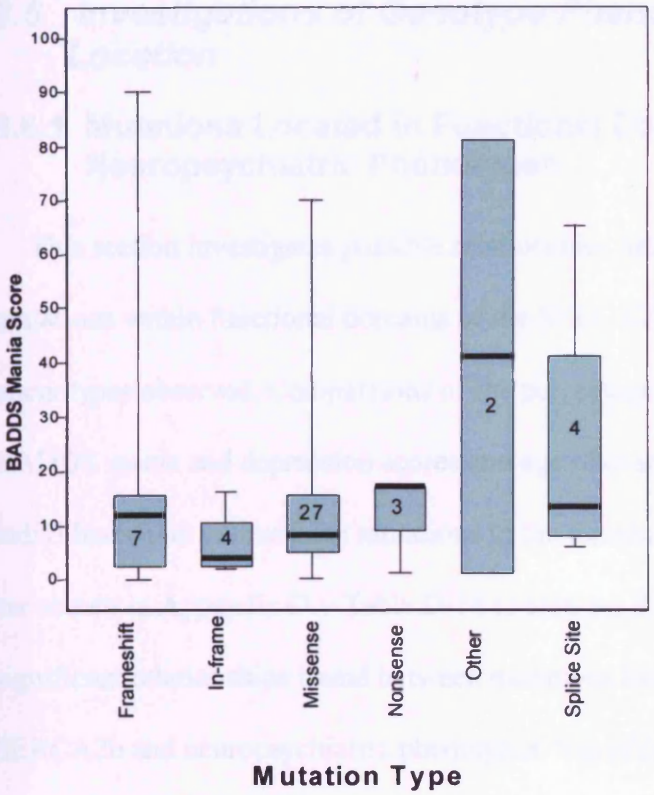





Index with Epilepsy or 1st Degree Relative with DD and Epilepsy %
0
10
20
30
40
50
60
70
80
90
100
Mutation Type
Frameshift
In-Frame
Missense
Nonsense
Other
Splice Site

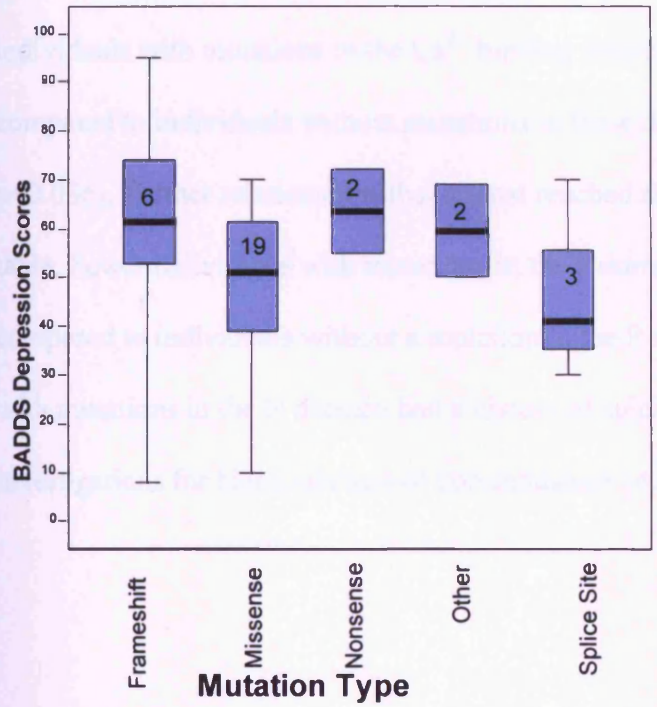

History of Psychiatric Contact %
0
10
20
30
40
50
60
70
80
90
100
Mutation Type
Frameshift
In-Frame
Missense
Nonsense
Other
Splice Site


Learning Difficulties %
0
10
20
30
40
50
60
70
80
90
100
Mutation Type
Frameshift
In-Frame
Missense
Nonsense
Other
Splice Site



Numbers in the boxes reflect numbers of individuals in each group.
Box plots represent the median, interquartile range and minimum and maximum BADDS Mania Dimension scores.
BADDS; Bipolar Affective Dimensional Scale.

Figure 8.8 BADDS Mania Scores according to Mutation Types



Numbers in the boxes reflect numbers of individuals in each group.
Box plots represent the median, interquartile range and minimum and maximum BADDS Depression Dimension scores.
BADDS; Bipolar Affective Dimensional Scale.

Figure 8.9 BADDS Depression Scores according to Mutation Type

8.6 *Investigations of Genotype-Phenotype Correlations- Mutation Location*

8.6.1 Mutations Located in Functional Domains of SERCA2b and Neuropsychiatric Phenotypes

This section investigates possible relationships between the location of pathogenic mutations within functional domains of the SERCA2b protein and the neuropsychiatric phenotypes observed. Comparisons of the percentages of neuropsychiatric phenotypes, BADDS mania and depression scores and age of onset of psychiatric illness among individuals with and without mutations in the functional A, N, P and Ca^{2+} binding domains are shown in Appendix D.v Table D-14 to D16 pg. 331-333. Table 8.10 summarises the two significant relationships found between mutations located in the functional domains of SERCA2b and neuropsychiatric phenotypes. Significantly more individuals with mutations in the A domain had a history of psychiatric contact compared to individuals without mutations in the A domain (100% vs. 27%; Fisher's exact test, p= 0.008) and significantly fewer individuals with mutations in the Ca^{2+} binding domain had a history of learning difficulties compared to individuals without mutations in these domain (12% vs. 39 %; χ^2 =4.390, df=1, p=0.036). Further relationships that almost reached significance are also summarised in the table. Fewer individuals with mutations in the P domain had a lifetime DSM-IV diagnosis compared to individuals without a mutation in the P domain (38% vs. 66%). More individuals with mutations in the N domain had a history of suicide attempts (33% vs. 12%) and investigations for blackouts/loss of consciousness (40% vs. 15%).

Table 8.10 Summary of Relationships between Mutations Located in Functional Domains of SERCA2b and Neuropsychiatric Phenotypes

	A Domain	N Domain	P Domain	Ca^{2+} Binding Domains
Lifetime DSM-IV Diagnosis			Lower % of individuals: 38 % vs. 66% (χ^2 =3.3269, df=1, p= 0.071).	
History of Suicide Attempts		Higher % of individuals: 33% vs. 12% (Fisher's exact test, p= 0.082)		
History of Psychiatric Contact	**Significantly higher % of individuals: 100% vs. 27% (Fisher's exact test, p=0.008)**			
Learning Difficulties				**Significantly lower % of individuals: 12% vs. 39 % (χ^2 =4.390, df=1, p=0.036)**
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes		Higher % of individuals 40% vs. 15% (Fisher's exact test, p=0.082)		
Personal History of Bipolar Disorder and/or 1st Degree Relatives with DD and Bipolar Disorder				
Diagnosis of Epilepsy and/or 1st Degree Relatives with DD and a Diagnosis of Epilepsy				
BADDS M				
BADDS D				
Age of onset of psychiatric illness				

Significant relationships are displayed in **bold.**

8.6.2 Mutation Location within *ATP2A2* and Neuropsychiatric Phenotypes

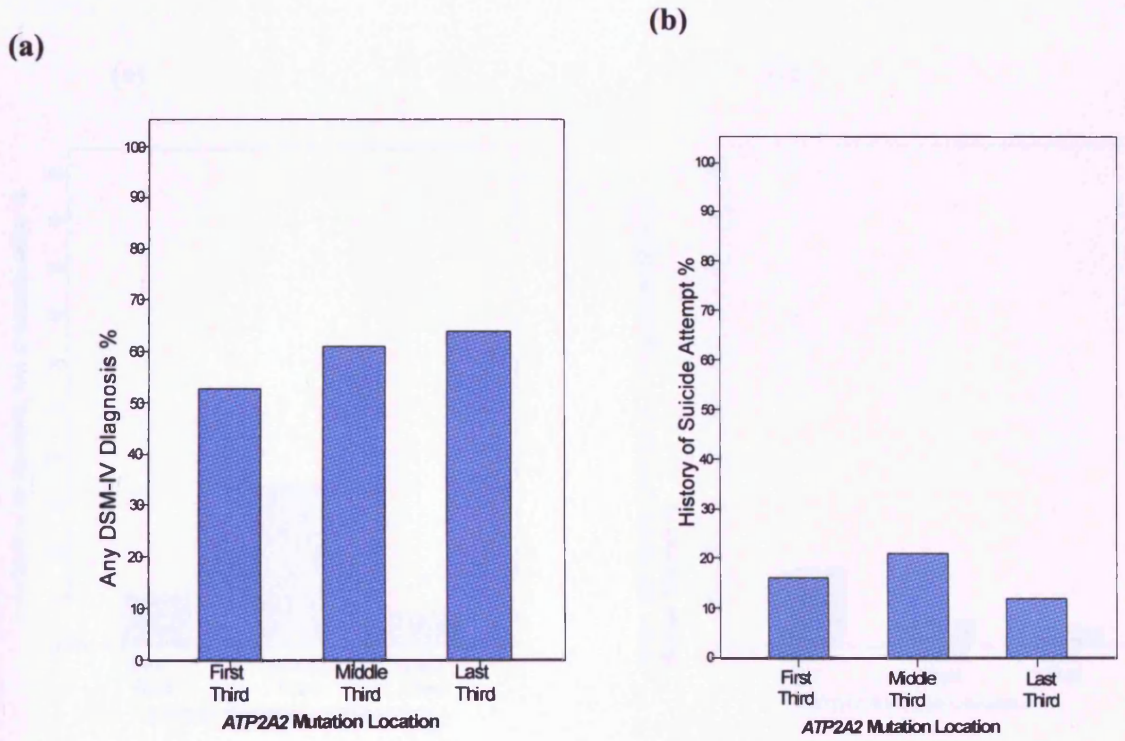
This section investigates possible relationships between the location of pathogenic mutations within the *ATP2A2* gene and the neuropsychiatric phenotypes observed. Comparisons of the percentages of neuropsychiatric phenotypes, BADDS mania and depression scores and age of onset of psychiatric illness among individuals with mutations in the first, middle and last third of *ATP2A2* are shown in Appendix D.vi Table D-17-19 pg.334 and summarised graphically in Figure 8.10 and Figure 8.11 pg. 189 to 191. Table 8.11 summaries the relationships between mutation location within *ATP2A2* and neuropsychiatric phenotypes. A significantly higher proportion of individuals with mutations in the middle third of *ATP2A2* had a history of investigations for blackouts, loss of consciousness or fainting episodes compared to individuals with mutations in the last third of the gene (34% vs. 7%; Fisher's exact test, p= 0.045) (Figure 8.10e) There was also a significant relationship between mutation location and diagnosis of epilepsy in index and/or 1st degree relatives with DD and a diagnosis of epilepsy (χ^2 =7.454, df=2, p=0.039) (Figure 8.10g).

Table 8.11 Summary of Relationships between Mutations Located in the First, Middle and Last Third of *ATP2A2* and Neuropsychiatric Phenotypes

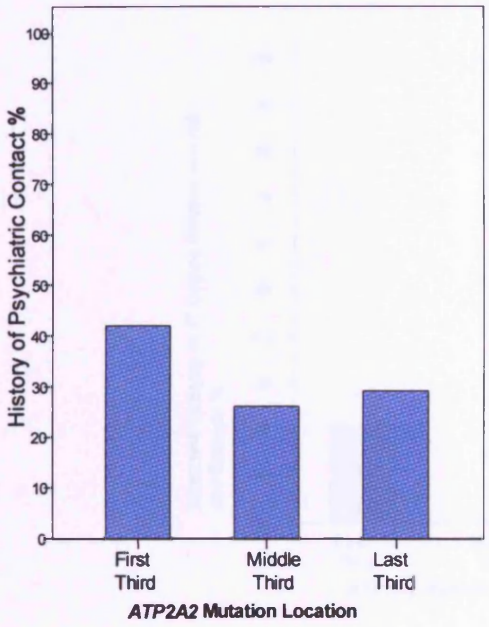
Neuropsychiatric Phenotype	Relationship with Location of Pathogenic Mutation	Table/ Figure
Any Lifetime DSM-IV Diagnosis	χ^2 =0.575, df=2, p= 0.750	D-17 pg .334
History of Suicide Attempts	*χ^2 =0.754, df=2, p= 0.763	
History of Psychiatric Contact	χ^2 =1.328, df=2, p= 0.515	
Learning Difficulties	*χ^2 =0.220, df=2, p= 0.939	Figure 8.10
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes	***χ^2 =6.039, df=2, p=0.048**	
Personal History of Bipolar Disorder and/or 1st Degree Relatives with DD and Bipolar Disorder	*χ^2 =2.818, df=2, p= 0.371	D-18 pg .334
Diagnosis of Epilepsy and/or 1st Degree Relatives with DD and a Diagnosis of Epilepsy	***χ^2 =7.454, df=2, p=0.039**	Figure 8.10
BADDS Mania Dimension	** H=1.209, df=2, p=0.546	D-19 pg .335
BADDS Depression Dimension	** H=3.206, df=2, p=0.201	Figure 8.11
Age of onset of psychiatric Illness	** H=0.267, df=2, p=0.875	

* Exact significance tests for Pearson's chi-square, ** Kruskal-Wallis H tests.

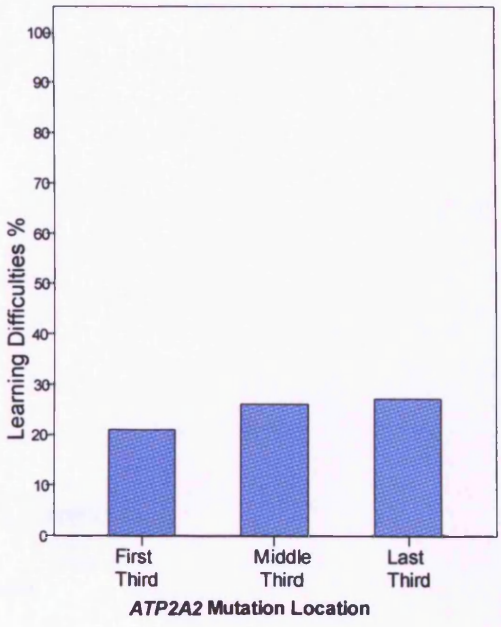
Figure 8.10 Percentages of Individuals with Specific Phenotypes according to Mutation Location within *ATP2A2*



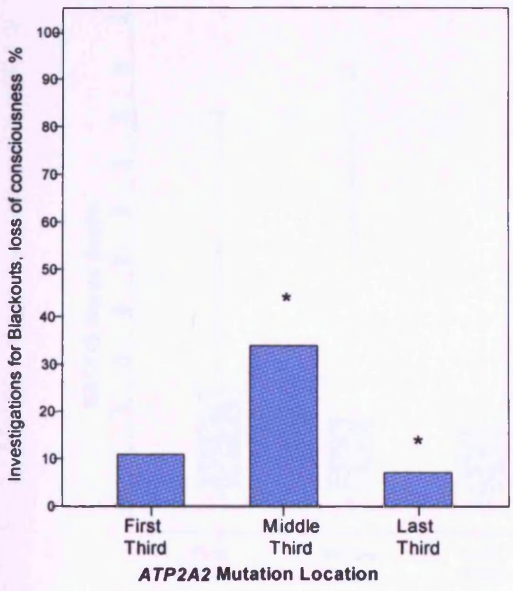
(c)



(d)

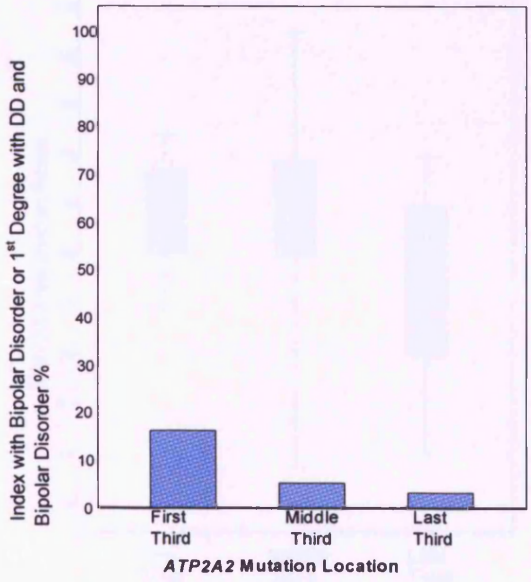


(e)

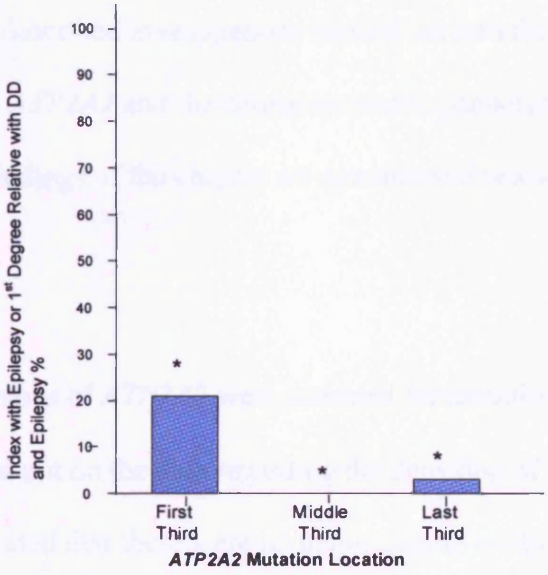


*significant difference p=0.045

(f)

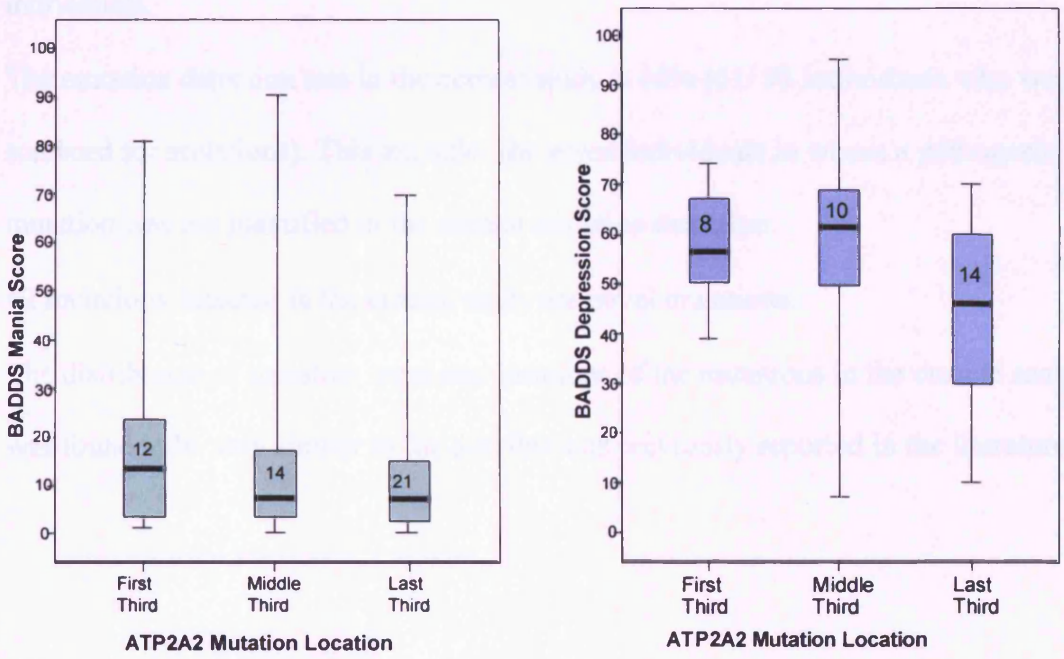


(g)



* significant difference; p= 0.039

Figure 8.11 BADDS Mania and Depression Scores according to *ATP2A2* Mutation Location



BADDS; Bipolar Affective Disorder Dimensional Scale. Numbers in boxes reflect numbers of individuals with each mutation type. Box plots represent the median, interquartile range and minimum and maximum BADDS Mania and Depression Dimension Scores.

8.7 Summary of Chapter 8

This chapter has described investigations carried out into the relationship between mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD. The main findings of the chapter are summarised below.

Mutation Detection

- 20 of the 21 exons of *ATP2A2* were screened for mutations. Exon 1 was not screened.
- Checks carried out on the data regarding the detection of DNA variants within *ATP2A2* indicated that there were no major quality control issues. There were no cases in which an individual with an already known pathogenic mutation was found to have a different pathogenic mutation in the current study.
- In total, 60 pathogenic mutations were detected and/or known in 68 unrelated individuals.
- The mutation detection rate in the current study is 66% (61/ 93 individuals who were screened for mutations). This excludes the seven individuals in whom a pathogenic mutation was not identified in the current mutation detection.
- 42 mutations detected in the current study are novel mutations.
- The distribution of mutation types and locations of the mutations in the current sample was found to be very similar to the distributions previously reported in the literature.

Comparison of the Clinical Features of DD and Neuropsychiatric Phenotype In Individuals in whom a Pathogenic Mutation Was and Was Not Detected/Known

- Significantly more individuals in whom a pathogenic mutation *was* detected reported a definite or probable family history of DD compared to individuals in whom a mutation *was not* detected.
- There was a consistent trend for the prevalence of lifetime neuropsychiatric phenotypes to be higher among individuals in whom a pathogenic mutation *was* detected.

Neuropsychiatric Genotype-Phenotype Correlations

1. Observations of possible clustering of mutations among individuals with certain neuropsychiatric phenotypes included:

- All four individuals with mutations in the functional 'A' domain of the protein had a history of contact with psychiatric services.
- Two individuals with frameshift mutations only one base pair apart in the S4 (Stalk 4) domain of the protein both had a history of suicide attempts in addition to having a cyclothymic personality and idiopathic epilepsy (ID 15) and an extensive psychiatric history including major depressive disorder, somatoform disorder and investigations for a blackout (ID16).
- Three individuals with the same missense mutation at one of the seven Ca^{2+} binding sites in the M5 domain all had a DSM-IV diagnosis of a mood disorder.

2. The main findings of genotype-phenotype investigations carried out between the type and location of pathogenic mutations detected in *ATP2A2* and the prevalence and/or severity of the neuropsychiatric phenotypes are summarised in Table 8.12. For six of the seven neuropsychiatric phenotypes investigated, the highest or second highest prevalence of these phenotypes was found among individuals with frameshift mutations or other types of mutations (large deletions or insertions).

Table 8.12 Summary of Relationships between the Types and Locations of Mutation within *ATP2A2* and Neuropsychiatric Phenotypes Observed in Individuals with DD

	Mutation Types With Highest Prevalence	**Mutations Located in Functional Domains of SERCA2b**	**Mutations Located in the First, Middle and Third of *ATP2A2***
Lifetime DSM-IV Diagnosis	1. Frameshift 2. Other/Nonsense	*Least frequent among individuals with mutations in the P domain*	
History of Suicide Attempts	1. Other 2. Frameshift	*Most frequent among individuals with mutations in the N domain*	
History of Psychiatric Contact	1. Other 2. Frameshift	**Most frequent among individuals with mutations in the A domain**	
Learning Difficulties	1. Nonsense 2. Missense	**Least frequent among individuals with mutations in the Ca^{2+} Binding domain**	
Investigations for Blackouts, Loss of Consciousness or Fainting Episodes	1. Other/Nonsense 2. Frameshift	*Most frequent among individuals with mutations in the N domain*	**Most frequent among individuals with mutations in the middle third**
Personal History of Bipolar Disorder and/or 1st Degree Relatives with DD and Bipolar Disorder	1. Other 2. Splice Site		
Diagnosis of Epilepsy and/or 1st Degree Relatives with DD and a Diagnosis of Epilepsy	1. Splice Site 2. Frameshift		**Most frequent among individuals with mutations in the first third**

Significant relationships are reported in **bold**, relationships almost reaching significance are reported in *italics*.

The following chapter (Chapter 9) discusses these findings.

9 GENOTYPE-PHENOTYPE CORRELATIONS BETWEEN MUTATIONS DETECTED IN ATP2A2 AND NEUROPSYCHIATRIC PHENOTYPES: DISCUSSION

This chapter will discuss the results presented in Chapter 8. These results and discussions relate to the second aim of the thesis, which was to investigate possible genotype-phenotype correlations between the type and/or locations of pathogenic mutations detected in the *ATP2A2* gene and the neuropsychiatric features observed in the current sample. Evidence for such correlations would support the hypothesis that mutations in the *ATP2A2* gene may have pleiotropic effects in the skin and brain and therefore confer susceptibility to neuropsychiatric features in individuals with DD.

First, general issues concerning the detection of mutations in *ATP2A2* in the current sample are discussed. This is followed by a discussion of the possible reasons for the consistent trend for the prevalence of lifetime neuropsychiatric features to be higher among individuals in whom a pathogenic mutation *was* detected compared to individuals in whom a pathogenic mutation *was not* detected. The findings of the investigations into genotype-phenotype correlations are then discussed and compared to the findings of the small number of previous genotype-phenotype correlation studies conducted in individuals with DD. A key finding reported in Chapter 8 was the observation of the clustering of mutations within the SERCA2b protein among individuals with similar neuropsychiatric phenotypes. These observations will be combined with the previous descriptions of individuals with known mutations in these regions of the protein to investigate whether this provides further support for the suggestion that certain mutations in *ATP2A2* may confer susceptibility to certain types of neuropsychiatric illness in individuals with DD.

9.1 Detection of Mutations In ATP2A2

In the current study, 93 index individuals with DD and a smaller number of their affected and unaffected family members were screened for mutations in *ATP2A2*. The findings of the mutation detection screen are reported in section 8.1 pg. 158.Unfortunately, one of the exons (Exon 1) could not be screened for technical reasons. In addition, certain fragments failed PCR amplification in some samples. These technical difficulties are likely to explain why three previously known mutations in *ATP2A2* were not detected during the current mutation screen. In total 60, pathogenic mutations were detected in 68 apparently unrelated individuals. The decision to classify a mutation as being pathogenic was based on ranking the mutations according to how likely they were to be pathogenic and deciding a cutoff above which mutations were thought to be highly likely to be pathogenic (see Table 8.4 pg. 164). It is possible that this method incorrectly classified mutations as non-pathogenic and vice versa. However, as a fairly conservative approach was used, it is more likely that pathogenic mutations may have been misclassified as non-pathogenic rather than non-pathogenic mutations being misclassified as pathogenic. Functional studies investigating the effects of specific mutations on the functioning of the SERCA2b protein would be needed to confirm whether a mutation was actually pathogenic. The criteria used to decide whether or not a mutation was likely to be pathogenic are very similar to those used in previous studies. Since the mutation types classified as being pathogenic in the current sample had all been reported as pathogenic mutations previously in the literature, this suggests that they were responsible for causing DD. This is also supported by the fact that the distributions of mutation types and locations of mutations in the current sample were very similar to the distributions previously reported in the literature.

Eighteen of the pathogenic mutations detected in the current study were among the 143 mutations previously reported in the literature, whereas 42 were novel. This study therefore greatly adds to the number of known mutations in DD and takes the worldwide known total from 143 to 185. In the current study, only six of the 60 mutations (10%) were found in more than one unrelated individual and the majority of individuals were found to have a unique DD causing mutation. This finding is consistent with the previous literature in which 17% of the mutations reported have been found in more than one unrelated individual. Four individuals were found to have the same missense mutation (N767S) in the M5 (fifth transmembrane) domain of the SERCA2b protein (see Table 8.3 pg. 162 Mutation ID 42). This mutation is one of only four missense mutations that have been previously reported in three or more apparently unrelated families in the literature. The remaining five mutations found more than once were each found in two unrelated individuals. It is possible that these individuals may have shared a common ancestor, although this could only be known if extensive family history research and/or further genetic analysis were carried out.

9.2 Comparison of the Clinical Features of DD and Neuropsychiatric Phenotype In Individuals in whom a Pathogenic Mutation was and was not Detected/Known

The mutation detection rate in the current study is 66% with mutations not being detected in 28 index individuals. This rate is consistent with those reported in larger scale mutation screens of the *ATP2A2* gene, which have ranged from 50% (Ikeda *et al.*, 2003) to 70% (Onozuka *et al.*, 2004). In the current study, pathogenic mutations located in exon 1 would not have been detected (although this is only likely to account for six individuals, see section 8.3 pg. 166). Another possible explanation for mutations not being detected is poor DNA quality and/or technical

reasons, for example, some individuals may have had a pathogenic mutation in a DNA fragment that failed PCR amplification. However since only a very small percentage of fragments failed amplification (3.6%), this explanation is not likely to account for many cases. It is also possible that undetected mutations may have been located in an unscreened region of the *ATP2A2* gene such as the promoter region, although pathogenic mutations in DD have not previously been reported in this region. A final possibility could be that a proportion of individuals in the current sample had clinical features similar to DD caused by factors other than a pathogenic mutation in *ATP2A2* i.e. they could be a phenocopy of DD. These individuals would be unlikely to report a family history of DD. Therefore, the suggestion that the sample may contain phenocopies of DD is supported by the finding that a significantly higher proportion of individuals in whom a pathogenic mutation *was* detected reported a family history of DD compared to individuals in whom a pathogenic mutation *was not* detected (see section 8.3.1 pg. 167).

The prevalence and severity of lifetime neuropsychiatric phenotypes, including suicide attempts, history of psychiatric contact, learning difficulties and investigations for periods of loss of consciousness, was higher among the individuals in whom a pathogenic mutation *was* detected compared to those in whom a pathogenic mutation *was not* detected (see Figure 8.2 to Figure 8.4 pg. 169 to 170). Although only one significant relationship was found, for scores on the Bipolar Affective Disorder Dimensional Scale (BADDS) mania dimension, the small group sizes mean that power to detect significant differences between the two groups was limited.

A number of explanations could account for the increased prevalence of neuropsychiatric features among individuals in whom a pathogenic mutation *was* detected. This observation could be due to chance, although this seems unlikely due to the consistent trend seen across all of the neuropsychiatric phenotypes. However, there is a degree of overlap in the phenotypes measured,

for example individuals with a history of suicide attempts are likely to have also had contact with psychiatric services. Another explanation could be that individuals within this group had a more severe form of DD and/or had experienced a greater impact of DD on their quality of life. The increased prevalence of psychiatric features seen in this group would therefore reflect a psychosocial reaction to the symptoms of DD. There was a trend for a higher percentage of individuals in whom a pathogenic mutation *was not* detected to have been classified as having mild DD. This difference did not reach statistical significance although it is possible that this could be due to limited power. The two groups however, did have very similar scores on the worst week version of the Dermatology Life Quality Index. This indicates that differences in the subjective impact DD had caused on quality of life could not account for the group differences in the prevalence and severity of psychiatric phenotypes.

A further explanation is that a proportion of the individuals in whom a pathogenic mutation *was not* detected are phenocopies of DD, i.e. they do not have a pathogenic mutation in *ATP2A2.* Based on the hypothesis that mutations in *ATP2A2* have pleiotropic effects in the skin and brain, it would be expected that these individuals would have a lower prevalence of neuropsychiatric features than individuals with a confirmed mutation in *ATP2A2.* Comparison of the prevalence of reported family history of DD between the two groups described above, suggests that there is a possibility that individuals in whom a pathogenic mutation *was not* detected may be phenocopies of DD. However, this cannot be confirmed in the current study and it remains possible that all of the individuals in whom a pathogenic mutation *was not* detected did have a mutation in *ATP2A2* that was not detected in the current mutation screen.

In addition to potentially providing support for mutations in *ATP2A2* having pleiotropic effects in the skin and brain, the increased prevalence of neuropsychiatric illness among

individuals in whom a pathogenic mutation *was* detected has potential treatment implications for individuals with DD, particularly if this finding is replicated in future studies. DD is currently clinically diagnosed by a skin biopsy, however, it is possible that in the future, DNA samples will be routinely taken from individuals with biopsy proven clinical DD to be screened for a pathogenic mutation within *ATP2A2.* The findings of the present study highlight the need for the assessment and recognition of neuropsychiatric symptoms in DD particularly among individuals found to have pathogenic mutation in *ATP2A2.*

9.3 Investigations of Genotype-Phenotype Correlations in DD

The main findings of the genotype-phenotype investigations carried out between the type and location of pathogenic mutations detected in *ATP2A2* and the prevalence and/or severity of the neuropsychiatric phenotypes measured in the current sample are summarised in Table 8.12 pg. 194. A small number of significant findings were observed, although due to the number of investigations carried out these would not remain significant after corrections for multiple testing. Therefore these investigations, by looking at mutation types and locations, did not demonstrate statistically significant genotype-phenotype correlations. However, such correlations would not be expected given the very limited power due to the small group sizes.

The possible clustering of mutations within the SERCA2b protein among individuals with similar neuropsychiatric phenotypes was also examined in this study. This is a more useful way of looking for possible genotype-phenotype correlations as these associations are likely to be more complex than being related to just the type and/or location of mutations. The observation of the clustering of mutations among individuals with similar neuropsychiatric phenotypes,

described in the following section, found evidence supporting the existence of genotype-phenotype correlations in DD.

9.3.1 Clustering of Mutations within SERCA2b and Neuropsychiatric Phenotypes

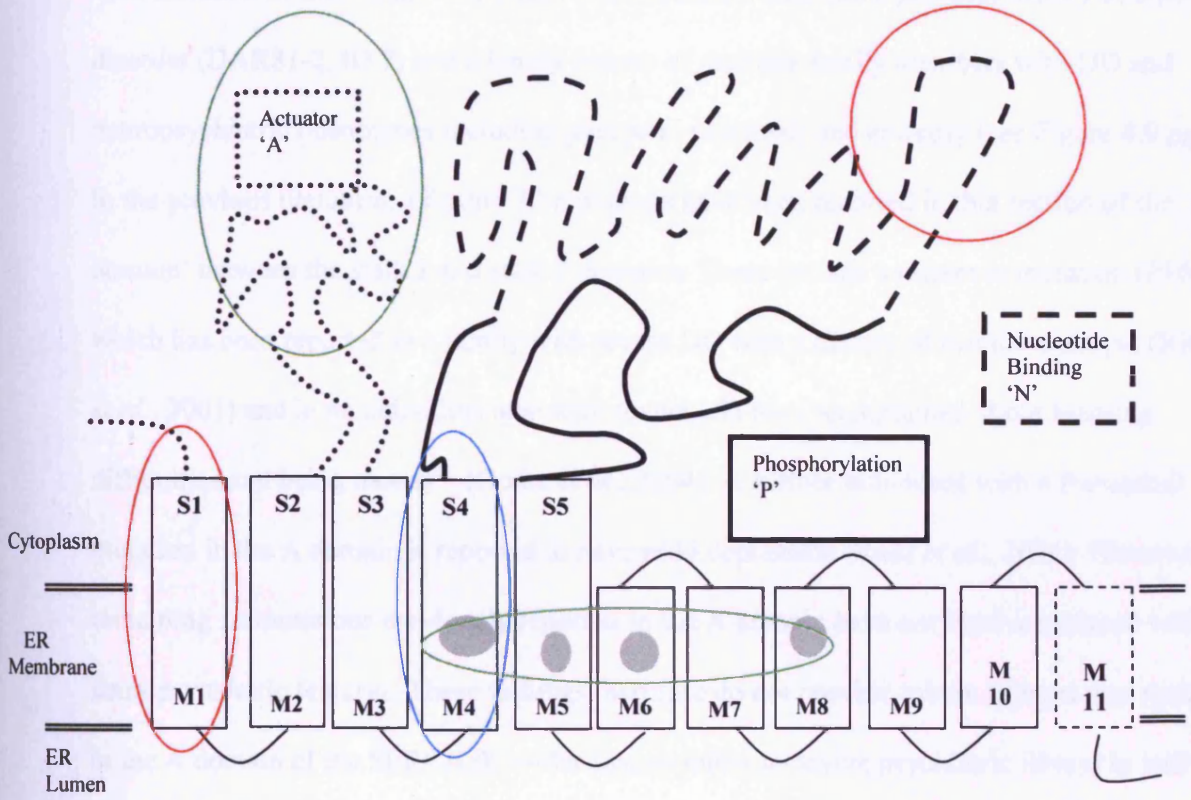
In the current sample, mutations found among individuals with similar neuropsychiatric phenotypes appeared to cluster in certain locations within the SERCA2b protein (see Table 8.8 pg. 181 for a summary of these observations). The key observations are illustrated in Figure 9.1. Most evidence was found for a clustering of mutations among individuals with similar severe psychiatric phenotypes including suicide attempts and a lifetime history of contact with psychiatric services.

In the following sections the neuropsychiatric phenotypes of individuals with mutations in these highlighted domains of the SERCA2b protein will be combined with the previous descriptions of individuals with mutations in these regions. Dermatologists have carried out the majority of the previous mutation detection studies in DD. Therefore, a limitation of combining the findings of the current study with these previous studies is that they will not all have recorded and/or reported neuropsychiatric features. Similarly, where neuropsychiatric features have been reported previously in the literature, in many cases only brief descriptions are provided.

The neuropsychiatric features observed among individuals in the current sample and their first-degree relatives with DD are summarised in Table 8.6 pg. 173. Where individuals reported in this table are described in the following sections, their study ID (first column e.g. DAR36-1) and/or mutation ID (second column e.g. ID 1) will be provided for reference.

A domain
All four individuals with mutations in the 'A' domain had a history of contact with psychiatric services.

N domain
Three individuals with mutations located close together in the ' N' domain had a history of psychiatric contact.



ER; endoplasmic reticulum, S*n;* stalk domains, M*n;* transmembrane domains

S1-M1 Domain
Two individuals with mutations in the S1 and M1 domains had a personal diagnosis of epilepsy or first degree relative with a diagnosis of epilepsy.

S4-M4 Domain
Two individuals with frameshift mutations only one base pair apart in the S4 domain had a history of contact with psychiatric services and suicide attempts.

Ca^{2+} Binding Sites
An individual with a missense mutation at one of the seven Ca^{2+} binding sites within the M4 domain had a 1st degree relative with DD and bipolar disorder.
Three individuals with a missense mutation at another of the seven Ca^{2+} binding sites in the M5 domain all had a DSM-IV diagnosis of a mood disorder.

Figure 9.1 Key Observations of Evidence for Possible Clustering of Mutations within the SERCA2b Protein among Individuals In the Current Sample with Similar Neuropsychiatric Phenotypes

9.3.1.1 A Domain

All four individuals with mutations in the functional 'A' domain of the SERCA2b protein had a history of contact with psychiatric services (mutation IDs 7-10) (see Figure 8.5 pg. 177). This included an individual with a splice-site mutation who had a personal history of bipolar disorder (DAR81-2, ID 7) and a family history of multiple family members with DD and neuropsychiatric phenotypes including puerperal psychosis and epilepsy (see Figure 4.9 pg. 80). In the previous literature, a further 22 mutations have been reported in this section of the 'A domain' between the stalk 2 and stalk 3 domains. These include a missense mutation (P160L) which has been reported in a family with severe DD with a history of suicide attempts (Ringpfeil *et al.*, 2001) and in an individual also with severe DD who "complained about learning difficulties and being moody" (Godic *et al.*, 2004). A further individual with a frameshift mutation in the A domain is reported to have mild depression (Racz *et al.*, 2004). However, the remaining 20 mutations previously reported in the A domain have not been associated with any neuropsychiatric features. These findings therefore do not provide robust support that mutations in the A domain of the SERCA2b confer susceptibility to severe psychiatric illness in individuals with DD

9.3.1.2 N Domain

Three individuals with mutations located close together in the ' N' domain had a history of psychiatric contact (IDs 64, 29 and 30). These mutations along with the mutations previously reported in the literature in this section of the N domain are shown in Figure 9.2. Mutations identified in families where any family member with DD also had a known neuropsychiatric phenotype are highlighted in red. One of the individuals in the current study with a frameshift

mutation in this region had a diagnosis of bipolar disorder (DAR18-1, ID 64). Interestingly, a further missense mutation (C560R) in this region was previously reported in two unrelated individuals with mood disorders (Jacobsen *et al.*, 1999). One of these individuals had bipolar disorder and another had a diagnosis of schizoid personality disorder not otherwise specified. Of the seven mutations that have been identified in this region of the N domain of the SERCA2b protein, six have been found in individuals with additional neuropsychiatric phenotypes. This includes two individuals with bipolar disorder and a further two individuals who have received treatment from secondary psychiatric services for depression. These findings therefore provide further strong support for the suggestion that mutations in this section of the N domain may confer susceptibility to mood disorders in individuals with DD.

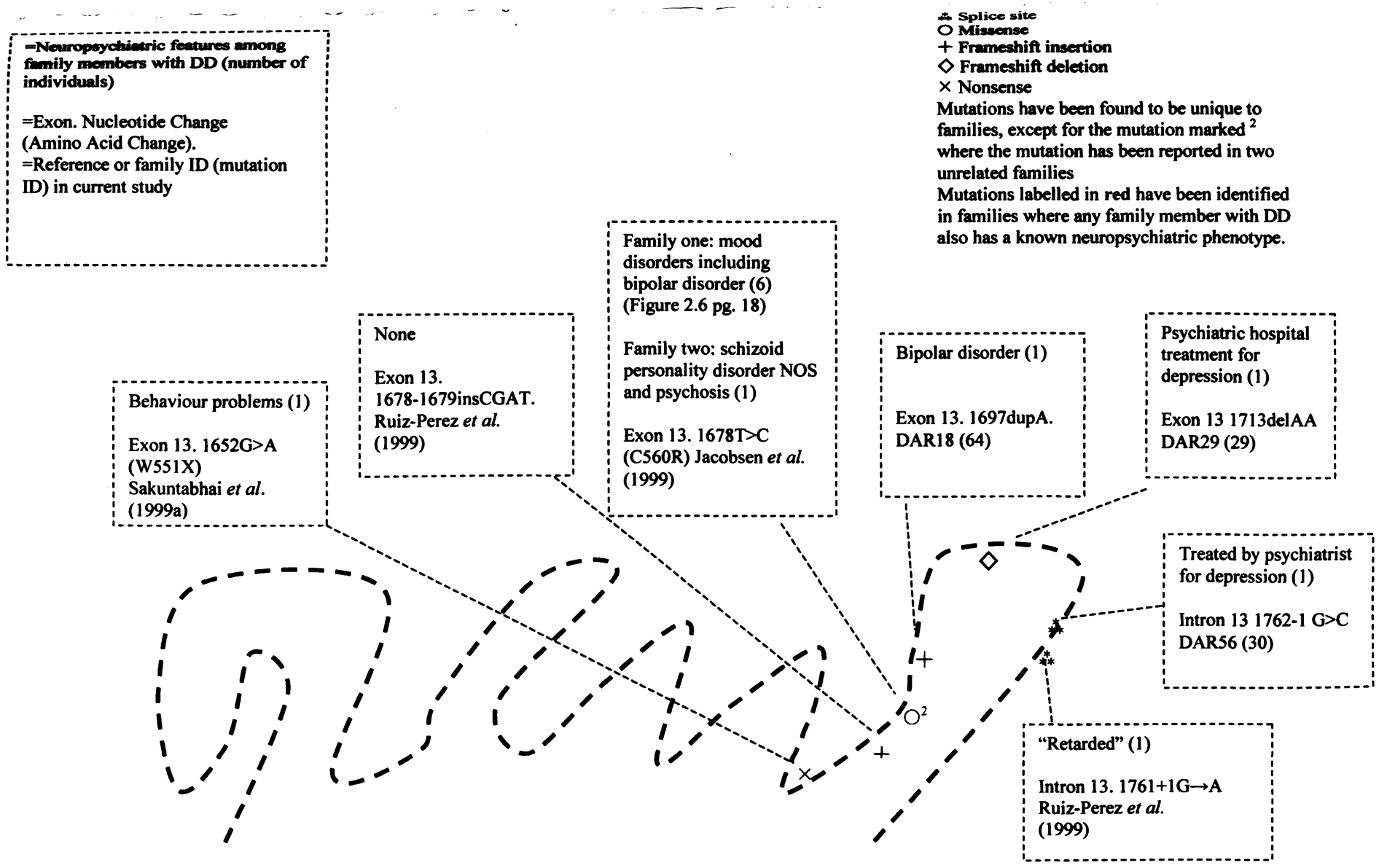
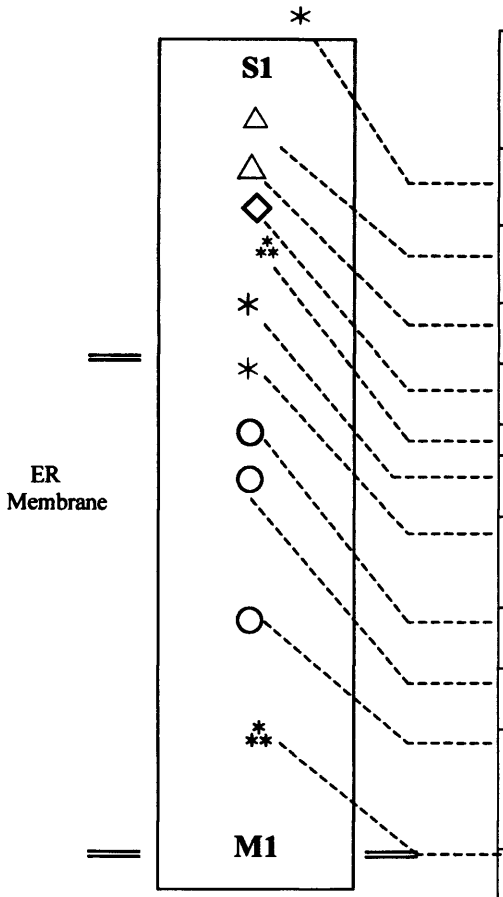


Figure 9.2 Neuropsychiatric Phenotypes of all Known Individuals with DD Causing Mutations in the Last Segment of the N Domain

9.3.1.3 S1-M1 Domain

Two individuals with mutations in the S1 and M1 domains of the SERCA2b protein had a personal diagnosis of epilepsy (DAR36-1, ID 1) or first degree relative with a diagnosis of epilepsy (DAR95-1, ID 4). Both of these individuals were also classified as having learning difficulties. These mutations, along with those reported in the literature, are shown in Figure 9.3. There were no additional reported cases of epilepsy. However, of the 10 mutations that have been reported in this domain, six have been found in individuals with neuropsychiatric illness including bipolar disorder. A large insertion mutation was identified in the current study in an individual with a diagnosis of bipolar disorder (DAR91-1, ID 2). The mother of this individual with DD is also reported to possibly have a bipolar illness. A further individual in the current study with a missense mutation in this domain had a diagnosis of major depression and reported episodes of high mood after the birth of her children (DAR14-1, ID 3). The father of this individual who also had DD had received ECT treatment for depression. A large insertion mutation located just outside the S1-M1 domain has also been reported in a family containing multiple members with affective disorder including bipolar disorder (Jones *et al.*, 2002). These findings provide evidence to suggest that mutations in the S1-M1 region of the SERCA2b protein may confer susceptibility to more severe psychiatric phenotypes in DD, including bipolar disorder.



Neuropsychiatric Features Among Family Members with DD (number of individuals)	Reference/ family ID (mutation ID) in current study	Exon. Nucleotide Change (Amino Acid Change).
Multiple members with affective disorder including bipolar disorder (incomplete co-segregation with DD)	Jones *et al.* (2002)	Intron 1/Exon 2.
Suicide in family, "emotional problems" (?n)	Ringpfeil *et al.* (2001)	Exon 2. 121 del 3bp (L41del)
Depression (?n)	Ringpfeil *et al* (2001)	Exon 2. 124 del 3bp (P42del)
Not reported	Sakuntabhai *et al.* (1999a)	Exon 2. 130delG
Tonic clonic epilepsy, learning difficulties (1)	DAR36-1 (1)	Intron 2. 137-3 C>G
Not reported	Sakuntabhai *et al.* (1999a)	Exon 3. 137-12ins 18bp
Bipolar disorder, fit aged 10 months, CT and EEG investigations for periods of unconsciousness in late 20s (1). Psychiatric treatment for depression and anxiety possibly a bipolar illness (1).	DAR91-1 (2)	Exon 3. 142 ins 18bp.
None	Wada *et al.* (2003)	Exon 3. 160A>G (I54V)
Mild high mood after birth of children followed by episodes of depression (1) ECT treatment for depression (1).	DAR14-1 (3)	Exon 3. 167 A>G (Q56R)
Depression and learning difficulties learning difficulties (1). Mild form of epilepsy (1).	DAR95-1 (4) also reported in Ruiz-Perez *et al.* (1999)	Exon 3. 194 T>C (L 65 S)
None	Sakuntabhai *et al.* (1999a)	Intron 3. 219+1G>A Splice-site

Mutation Type
✳ Large insertion
⁂ Splice site
○ Missense
◇ Frameshift deletion
△ In-frame Deletion

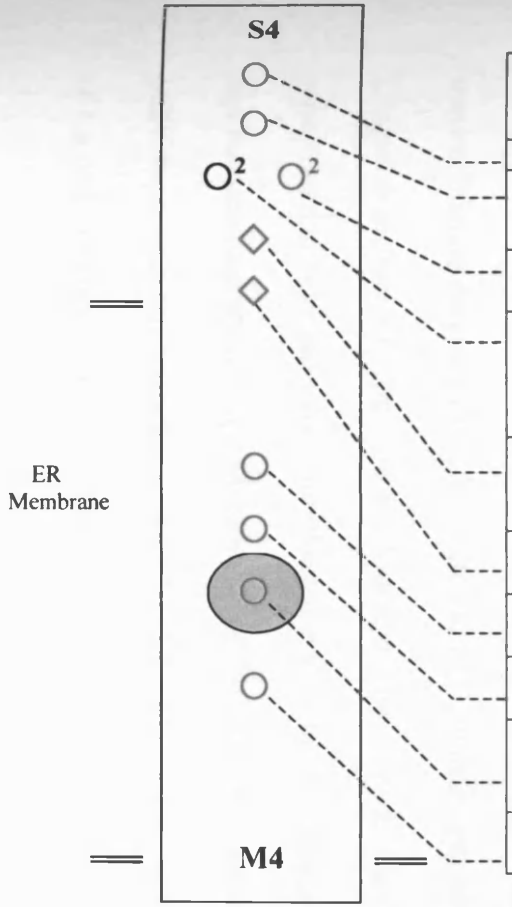
ER; Endoplasmic reticulum, S; stalk domain, M; transmembrane domain. Mutations labelled in red have been identified in families where any family member with DD also has a known neuropsychiatric phenotype.

Figure 9.3 Neuropsychiatric Phenotypes of all Known Individuals with DD Causing Mutations in the S1-M1 Domain of SERCA2b

9.3.1.4 S4-M4 Domain

Two individuals with frameshift mutations only one base pair apart in the S4 (Stalk 4) domain of the protein both had a history of suicide attempts in addition to having a cyclothymic personality and idiopathic epilepsy (DAR70-1, ID 15) and an extensive psychiatric history including major depressive disorder, somatoform disorder and investigations for a blackout (DAR55-1, ID 16). These mutations along with those previously reported are shown in Figure 9.4.

Of the 10 mutations that have been reported in the M4-S4 domain, seven have been found in families with members with severe psychiatric phenotypes including bipolar disorder.
An individual in the current study with a missense mutation (E309K), at one of the Ca^{2+} binding sites (DAR65-1, ID 13) had a 1st degree relative with DD and bipolar disorder, although they themselves did not have a history of mood disorder.



Neuropsychiatric Features Among Family Members with DD (number of individuals)	Reference/ Study ID (mutation ID) in current study	***Nucleotide Change Amino Acid Change).
Neuropsychiatric disorder and behaviour problems (2)	Sato *et al.* (2004)	(L321F)
Psychiatric hospital treatment for depression (1)	DAR43 (ID 17)	958 G>C (A320P)
Mild mental retardation and psychiatric abnormality (1) (Wang et al., 2006).	Ren *et al.* (2006) ;Wang *et al.*(2006)	953G>T (C318F)
None	Onozuka *et al.* (2004); Sakuntabhai *et al.* (1999a)	952T>C (C318R)
Major depression, multiple suicide attempts, brain scan to investigate blackouts, somatoform disorder, BADDS mania score of 16 (1). Treated by psychiatrist for depression (1).	DAR55 (ID 16)	949 7bp del
Cyclothymic personality described in medical notes, suicide attempt, idiopathic epilepsy (1)	DAR70 (ID 15)	948 del C
Bipolar disorder (1). Treated by psychiatrist for depression (1)	(*)	935 C>G (P312R)
Below average IQ, BADDS Mania Score of 17 (1) (**)	DAR3 (ID 14)	929 G>T (G310V)
Bipolar Disorder (1) Seen by educational psychologist for poor concentration (1) Seen by child psychiatrist and treated by psychiatrist for depression and heroin addiction as an adult, bipolar disorder not otherwise specified (1)	DAR65 (ID13)	925 G>A (E309K)
Depression NOS, BADDS Mania Score of 11 (1)	DAR6 (ID12)	923 C>A (P308H)

ER; Endoplasmic reticulum, S; stalk domain, M; transmembrane domain. Mutations have been found to be unique to families, except for mutations marked [2] where mutations have been reported in two unrelated families. Mutations labelled in red have been identified in families where any family member with DD also has a known neuropsychiatric phenotype. BADDS; Bipolar Affective Disorder Dimension Scale.

*Unpublished mutation identified in an individual seen by our research group who was not included in the current study.** Mother was admitted to psychiatric hospital with 'high mood' although neither parent was reported to have DD. *** All mutations are located in Exon 8.

Figure 9.4 Neuropsychiatric Phenotypes of all Known Individuals with DD Causing Mutations in the S4-M4 Domain of SERCA2

A further missense mutation three amino acids away (P312R) was identified in an individual with bipolar disorder who had previously been interviewed by our research team, although was not included in the current study. Interestingly, a missense mutation identified in between these two mutations (G310V) was identified in an individual with severe DD with a notable score of 17 on the mania dimension of the Bipolar Affective Disorder Dimensional Scale. The mother of this individual had been admitted to a psychiatric hospital with high mood, although she was not known to have DD. Sato *et al* (2004) found a unique missense mutation (L321F) in the S4 domain which led to a SERCA2b protein with markedly reduced sensitivity to feedback inhibition by ER Ca^{2+}. This mutation was reported in a female and her daughter both with severe DD and severe neuropsychiatric disorders and behaviour problems.

These observations suggest that mutations within the S4-M4 domain may confer increased susceptibility to more severe neuropsychiatric phenotypes, in particular psychiatric symptoms and bipolar disorder, in individuals with DD. As this domain contains a Ca^{2+} binding site, mutations in this domain may be expected to have a more dramatic effect on the functioning of the protein. The neuropsychiatric phenotypes of individuals with mutations at the other Ca^{2+} binding sites of the SERCA2b protein are discussed in the following section.

9.3.1.5 Ca^{2+} Binding Sites

The SERCA2 protein has seven Ca^{2+} binding amino acids within four of the transmembrane domains (M4, M5, M6 and M8), which form two Ca^{2+} binding sites (see Chapter 6 section 6.2.1 pg. 138 and Figure 6.1 pg. 137). In the current study, six individuals were found to have missense mutations in a Ca^{2+} binding amino acid. Four of these individuals had the same mutation in the M5 domain of the SERCA2b protein (N767S, ID 42). Three individuals with this mutation (DAR72-1, DAR41-1 and DAR74-1) all had DSM-IV diagnosis of a mood disorder including major depression and depression not otherwise specified. An

individual with the same mutation (DAR50-1) had experienced possible symptoms of anxiety that did not reach diagnostic criteria. As described in the previous section, an individual with a missense mutation (E309K) at another of the Ca^{2+} binding sites within the M4 domain (DAR65-1, ID 13) had a 1st degree relative with DD and bipolar disorder. Two other members of this family with DD had been seen by a psychiatrist and an educational psychologist. A further missense mutation (N795S) at another Ca^{2+} binding site was found in an individual in the current study who had a diagnosis of anxiety not otherwise specified (DAR89-1, ID 45). These findings suggest mutations in the Ca^{2+} binding sites may confer susceptibility to mood or anxiety symptoms. However, this is not supported by the previous literature. Of six further individuals/families reported to have mutations within one of the seven Ca^{2+} binding sites, only one had a diagnosis of depression (Jacobsen *et al.*, 1999). However, as discussed previously, the neuropsychiatric features of the remaining individuals may not have been recorded and/or reported.

9.3.2 Functional Genotype-Phenotype Studies in DD

The findings of the present study combined with the previous literature provide strong evidence to suggest genotype-phenotype correlations in DD. This is particularly evident in the S4-M4 region where a number of unrelated individuals have been found/reported to have severe psychiatric symptoms including multiple cases of bipolar disorder. In addition, unrelated individuals with the same DD causing mutation have been found to have similar neuropsychiatric phenotypes. However, replication of these findings is difficult since the majority of DD causing mutations are unique to families.

Site-directed mutagenesis functional studies are a further way of investigating genotype-phenotype correlations in DD. To date only a small number of these studies have been conducted, although the findings suggest that the variable neuropsychiatric phenotypes observed among individuals with DD may be accounted for by certain mutations having

specific effects on the functioning of the SERCA2b protein. Ahn *et al.*(2003) indicated that the effects of DD mutations on Ca^{2+} transport activity could not account for the variable phenotypes observed in DD since the majority of mutations they examined (11 out of 12) had no Ca^{2+} pumping activity. Interestingly, they found that five of these mutations led to 'mutant' SERCA2b pumps that interact with, and subsequently reduce, the activity of the co-expressed wild-type normal SERCA2b pumps. Further functional studies have identified a small number of unique mutations leading to proteins with an enhanced Ca^{2+} transport activity relative to the wild type SERCA2b and a reduced sensitivity to inhibition by lumenal Ca^{2+} (L321F and S920Y) ((Dode *et al.*, 2003; Sato *et al.*, 2004). It has been suggested that these unique types of mutations could account for the variable clinical features observed in individuals with DD including the presence of more severe neuropsychiatric features (Ahn *et al.*, 2003; Sato *et al.*, 2004).

Of the 60 mutations in the current study, 11 had been previously examined in site-directed mutagenesis functional studies in DD. These mutations have been found to have varying effects on the levels of SERCA2b protein expression, Ca^{2+}-ATPase activity and Ca^{2+}-transport activity. The functional effects of specific mutations vary across the different studies making it difficult to summarise these findings. One of these 11 mutations (S920Y), has previously been shown to result in a 'mutant' pump that reduces activity of the wild-type normal pumps (Ahn *et al.*, 2003). A separate study found that this mutation leads to a protein that has a unique reduced sensitivity to inhibition by lumenal Ca^{2+} (Dode *et al.*, 2003). This missense mutation (ID 59) is located in the cytoplasmic loop between M8 and M9 and was detected in two individuals in the current study (DAR68-1 and DAR5-1). Both of these individuals had severe DD and a personal and family history of neuropsychiatric features. The first of these individuals (DAR68-1) had a personal history of learning difficulties, according to the definition used in the present study, and had a sibling with DD who suffered from epilepsy and learning disabilities severe enough

to require residential treatment. A further family member had committed suicide, which was reported as being a direct reaction to the severity of their DD. The neuropsychiatric features among members of this family had been reported previously in the literature (Jacobsen *et al.*, 1999; Sakuntabhai *et al.*, 1999a). The second individual with this mutation in the present study (DAR5-1) also had learning difficulties and had received psychiatric treatment following a severe episode of septicaemia related to their DD. They had an adult child with DD with a diagnosis of major depression who had made a suicide attempt following relationship problems. Another family member with DD had been admitted to a psychiatric hospital.

These findings highlight the need for further site-directed mutagenesis functional studies in DD. These studies would enable further genotype-phenotype investigations into the association between the functional effects of certain *ATP2A2* mutations and the presence of severe neuropsychiatric features in individuals with DD. Varied neuropsychiatric phenotypes have been observed among members of the same family with DD. Since these individuals will all have the same mutation in *ATP2A2*, this suggests that additional genetic and/or environmental susceptibility factors are likely to be involved in the development of neuropsychiatric features in individuals with DD.

9.3.3 Implications of Evidence for Genotype-Phenotype Correlations in DD

As discussed in the previous section, the current study has found evidence for possible genotype-phenotype correlations in DD. This provides support for the hypothesis that mutations in the *ATP2A2* gene have pleiotropic effects in the skin and brain and therefore confer susceptibility to neuropsychiatric features, in particular psychiatric illness, in individuals with DD. This hypothesis is also supported by the small number of functional studies that have provided evidence of how certain mutations in *ATP2A2* may have high penetrance with respect to neuropsychiatric phenotypes by their specific effects on the functioning of the SERCA2b protein.

The suggestion of mutations in *ATP2A2* being involved in conferring susceptibility to neuropsychiatric illness is highly plausible since the gene is widely expressed in the brain (Baba-Aissa *et al.*, 1998). The dual role of the SERCA2b protein in intracellular Ca^{2+} signalling and in the synthesis and post-translational modification of proteins within the endoplasmic reticulum (ER) also provides support for this suggestion. Intracellular Ca^{2+} signalling has been shown to play a role in a range of neuronal functions including neuronal excitability, neurotransmitter release, gene expression, neuronal growth and synaptic plasticity (Berridge, 2002; Berridge *et al.*, 1998; Verkhratsky, 2005). Furthermore, there is recent evidence suggesting the role of ER function in the pathophysiology and treatment of mood disorders (Shao *et al.*, 2006; So *et al.*, 2007).

It is possible that the function of SERCA2b pumps may be more critical in the skin and the brain than in other tissues. Both tissues may have a particular susceptibility to a reduction in SERCA2b activity possibly relating to changes in ER Ca^{2+} concentration and ER functioning such as the post-translational processing of proteins. For example, trafficking of desmoplakin, a plasma membrane protein involved in cell adhesion, has been found to be impaired in DD skin cells (Dhitavat *et al.*, 2003a).

These findings have potential treatment implications for individuals with DD, particularly if future site-directed functional studies identify further mutations that appear to have high penetrance with respect to neuropsychiatric phenotypes. The findings also have implications for the identification of genetic factors involved in conferring susceptibility to psychiatric illness in individuals without DD. Other genes encoding proteins in the same biological system as and/or encoding proteins with a similar function to SERCA2b would be good candidates for involvement in predisposing individuals to developing psychiatric illness, in particular mood disorders. Further studies are needed to look for supportive evidence of the clustering of mutations among DD individuals with similar neuropsychiatric phenotypes. In addition further

functional studies are needed to determine the likely biological plausibility that variation within the cluster domains can affect a neuropsychiatric phenotype.

The following chapters (Chapters 10-12) are concerned with addressing the third aim of the thesis, which was to compare the presence of neuropsychiatric features in individuals with DD and their first-degree relatives, unaffected by DD. These investigations were designed to allow the detection of milder neuropsychiatric phenotypes in DD and allow control for additional genetic and non-genetic factors that could influence the presence of these features.

10 COMPARISON OF DEMOGRAPHICS, LIFETIME NEUROPSYCHIATRIC FEATURES AND QUESTIONNAIRE SCORES IN A SAMPLE OF INDIVIDUALS WITH DD AND THEIR UNAFFECTED RELATIVES: METHODS

Chapters 3 to 5 were concerned with investigating the neuropsychiatric phenotype in 100 unrelated individuals with DD, looking at relationships between the clinical features of DD and the neuropsychiatric features observed and then comparing the prevalence of neuropsychiatric features observed in individuals with DD to normative data and literature regarding the prevalence of psychiatric illness in other dermatological conditions. This was then followed by investigations carried out into the relationship between DD causing mutations detected in *ATP2A2* and the neuropsychiatric phenotypes observed in individuals with DD in Chapters 6 to 9.

It was also intended that the neuropsychiatric test battery previously described in Chapter 3 would be administered to available and willing unaffected relatives without DD. Comparison of the presence of neuropsychiatric features in a sample of individuals with DD and their unaffected relatives allows control for additional genetic and non-genetic factors that could influence the presence of neuropsychiatric features in individuals with DD.

This chapter describes the recruitment and clinical assessment of 24 unaffected first-degree relatives with no history of DD. This is followed by a description of the measures and design used to compare the presence of neuropsychiatric features in individuals with DD and their unaffected relatives. Additional information relating to this chapter can be found in section E of the Appendix starting at pg. 336, and will be referenced throughout the chapter.

10.1 Recruitment and Assessment of Unaffected Relatives

10.1.1 Available Unaffected Relatives

The 100 index individuals with DD described and discussed in Chapters 3 to 5 were asked whether they had an available unaffected first-degree relative, preferably a sibling, over the age of 18 whom they would ask to take part in the research. Thirty-one individuals did not have any available unaffected relatives who could be contacted to be asked to take part in the research, reasons are summarised in Table 10.1.

Table 10.1 Unavailable unaffected relatives

	N
No unaffected 1st degree relatives	10
Not in contact with any 1st degree relatives	8
1st degree unaffected relatives all under 18 or over 80	2
Did not want to ask	10
1st degree relatives not in the UK	1
Total	**31**

10.1.2 Approaches to Recruiting Available Unaffected Relatives

Sixty-nine index individuals with DD had a first-degree unaffected relative suitable to be asked to participate in the study. During the initial stages of interviewing the 100 index individuals with DD, it was also attempted to interview unaffected relatives, described below in section 10.1.2.1. However, due to the low number of unaffected relatives agreeing to be interviewed, a second approach was carried out in which index individuals were asked if they could pass on a questionnaire version of the interview to their unaffected relatives, described below in section 10.1.2.2.

10.1.2.1 Approach 1-Interviewing Unaffected Relatives

I initially attempted to interview available unaffected relatives of the 100 index individuals with DD. Where possible, the unaffected relative was a sibling of the index individual, if an unaffected sibling was not available the unaffected relative was a parent or child of the index case over the age of 18. Individuals with available unaffected relatives were asked if they would pass on or post (envelopes and stamps provided) an invitation letter and information sheet to their unaffected relative (Appendix E.i pg. 336). Unaffected relatives willing to take part in the study or wishing to discuss taking part over the telephone were asked to complete an attached contact form, which could be returned in the SAE enclosed with the letter. For the first 40 index participants with DD, this approach was used to attempt to interview their unaffected relatives. Nine unaffected relatives agreed to participate in the study and were interviewed in their homes.

The clinical assessment of unaffected relatives was identical to the assessment of individuals with DD, described in Chapter 3 section 3.2 pg. 35 and summarised in Figure 3.2 pg. 36 with the exception of the section relating to the clinical features of DD. Unaffected relatives were asked if they had ever been treated by a dermatologist or had ever experienced any skin problems in case they might have had a mild case of DD that had not been diagnosed.

Reasons I thought could contribute to the low number of unaffected relatives willing to participate in the study included individuals being unable find the time to take part in the study due to work, family and other commitments, and individuals not feeling comfortable/willing to discuss personal details with a researcher. I also thought that the relationship individuals with DD had with their unaffected relative would influence the willingness of their unaffected relative to take part in the study.

10.1.2.2 Approach 2 -Questionnaire Pack for Unaffected Relatives

Due to the low number of unaffected relatives agreeing to be interviewed a second approach, which involved asking unaffected relatives to complete a shortened questionnaire pack, was attempted (shown in Appendix E.ii pg. 340). The pack consisted of a covering letter, information sheet about the study, two copies of a consent form, a questionnaire version of the neuropsychiatric assessment and a set of personality, temperament and current mood state questionnaires (described later in section 10.1.3.3). Index individuals with DD with available unaffected relatives were asked if they would be willing to pass on or post (envelopes and stamps provided) the questionnaire pack to their unaffected relatives.

Where possible the unaffected relative was a sibling of the index individual, if an unaffected sibling was not available the unaffected relative was a parent or child over the age of 18. Willing unaffected relatives were asked to return the completed questionnaires in a SAE provided along with a signed consent form. Sixty index individuals with DD were given or sent a questionnaire pack to pass on to an available unaffected relative. Fifteen unaffected relatives returned a completed questionnaire pack. Therefore in total 24 unaffected relatives (35%) were recruited (9 interviewed plus 15 completing a questionnaire pack).

10.1.2.3 Confirmation that Unaffected Relatives did not have a Pathogenic Mutation in ATP2A2

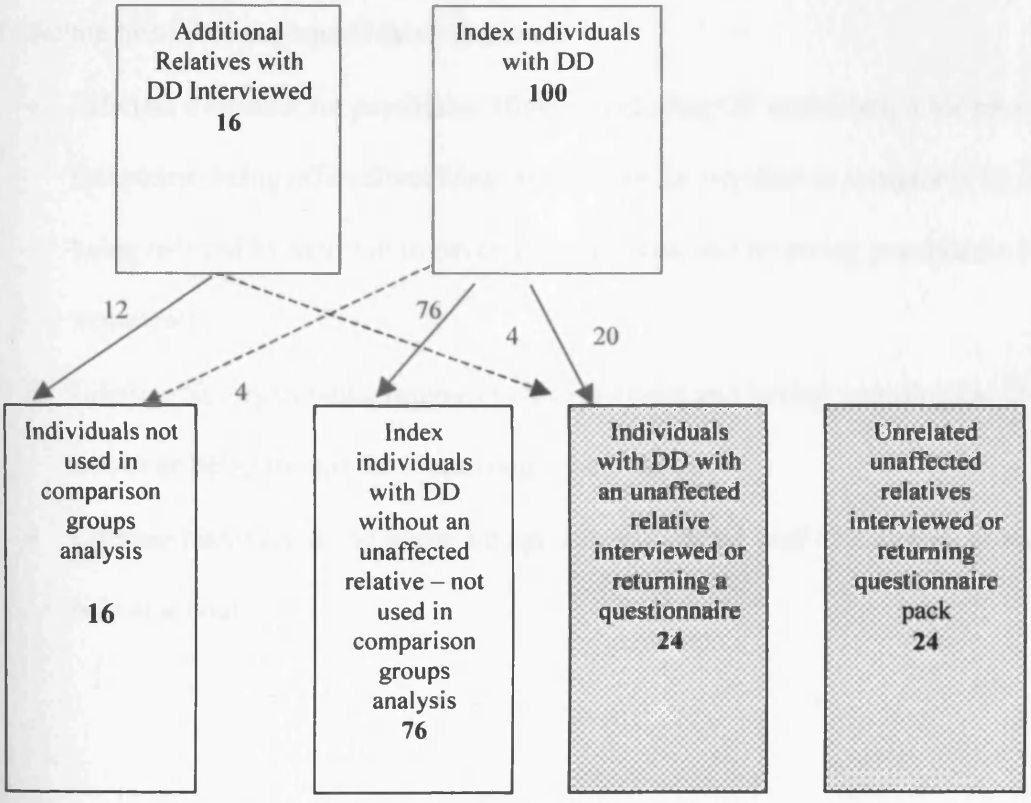
Blood or Oragene™ saliva samples were collected from 17 of the 24 unaffected relatives. The DNA extraction techniques, carried out by two members of the psychiatric genetics laboratories at Birmingham and Cardiff Universities, and the detection of variants in the *ATP2A2* gene, carried out by the Wales Gene Park, are previously described in Chapter 7 Section 7.1 and

7.2 respectively pg. 145. As reported in Chapter 8, section 8.1.1 pg. 158, no other possible DD causing variants in *ATP2A2* were detected in any of these unaffected relatives.

10.1.2.4 Attempts to Match Individuals with DD and their Unaffected Relatives For Age and Gender

In addition to the 100 index individuals with DD and the 24 unaffected relatives, 16 additional first-degree relatives with DD were also recruited as part of the study. This was usually because they had been present at the house when the index individual was interviewed and were also willing to participate in the study. In the case of four of these additional relatives, they were either closer in age to the unaffected relative than the index and/or were the same gender as the unaffected relative whereas the index was not. These four additional affected relatives were included in the DD comparison group instead of their four index relatives. These figures are summarised in Figure 10.1.

Figure 10.1 Comparison Groups- 24 Individuals with DD and their Unaffected Relatives



10.1.3 Comparison of Demographics, Lifetime Neuropsychiatric Features and Scores on Personality, Temperament and Current Mood State Questionnaires

The demographic characteristics, prevalence of history of neuropsychiatric features and scores on a number of personality, temperament and current mood state questionnaires were compared in a group of individuals with DD and their unaffected first-degree relatives. These are described in the following sections.

10.1.3.1 Demographics

Demographic information was collected from all study participants including current age, gender, marital status, current social and employment circumstances, main lifetime occupation and highest level of educational qualifications.

10.1.3.2 Lifetime History of Neuropsychiatric Features

Individuals with DD and their unaffected relatives were compared on the following measures of lifetime history of neuropsychiatric features:

- Lifetime treatment for psychiatric illness (including GP consultation for psychiatric symptoms, being offered/receiving medication for psychiatric symptoms by their GP, being referred by their GP to psychiatric services, and receiving psychiatric inpatient treatment).
- Lifetime history of being referred to a neurologist and having neurological investigations and/or or being treated for a neurological condition.
- Lifetime history of being given a diagnosis of dyslexia and/ or a history of receiving extra help at school.

10.1.3.3 Personality, Temperament and Current Mood State Questionnaires

As previously described in Chapter 3 section 3.2.9 pg 45, at the end of the clinical and neuropsychiatric assessment, individuals with DD were asked if they would be willing to have a pack of personality, temperament and current mood state questionnaires to be completed in their own time and returned in a stamped addressed envelope. Unaffected relatives were also asked to complete the same set of questionnaires. A brief description of each questionnaire is given below and their scoring is displayed in pg. 226. All of the questionnaires are self-rated and validated measures. Copies of all of the questionnaires are displayed in Appendix Eiii pg. 348.

Beck Depression Inventory (BDI) (Beck & Steer, 1987)

The Beck Depression Inventory (BDI) is a widely used measure of the presence and/or severity of *current* depressive symptoms. The questionnaire was included to investigate whether there were any significant differences in the current depression scores of individuals with DD and their unaffected relatives. As current depressive symptoms are known to affect scores on a number of personality and temperament questionnaires, the questionnaire was also included to enable differences between the groups on these measures to be investigated whilst controlling for individuals' current level of depression.

Altman Self-Rating Scale for Mania (ASRM) *(Altman et al., 1997)*

The Altman Self-Rating Scale for Mania (ASRM) is a measure of the presence and/or severity of *current* manic symptoms. The questionnaire was included for the same reasons outlined above for the BDI.

Rosenberg Self-Esteem Scale (RSE) *(Rosenberg, 1965)*

The Rosenberg Self-Esteem Scale (RSE) is a widely used measure of trait self-esteem. The scale has a total score and gives two subscale scores, a *negative subscale* relating to five statements including "At times I think I am no good at all" and a *positive subscale* relating to the remaining five statements including "On the whole I am satisfied with myself". Studies have found lower self-esteem using the RSE among euthymic individuals with unipolar and bipolar disorder compared to controls (Jones *et al.*, 2005; Scott *et al.*, 2000). These studies, in addition to a number of others, suggest that low self-esteem reflects a vulnerability to affective illness. The RSE was included in the present study as significant differences in self-esteem scores may reflect differences between the groups in the prevalence of individuals with vulnerability to mood disorders.

Eysenck Personality Questionnaire (EPQ) *(Eysenck & Eysenck, 1975)*

The Eysenck Personality Questionnaire (EPQ) is a widely used measure of personality. The questionnaire gives scores for three dimensions of personality: Extraversion, Neuroticism and Psychoticism. Similar to low self-esteem, numerous studies have shown that high neuroticism scores reflect an individual's vulnerability to major depression (Duggan *et al.*, 1995; Kendler *et al.*, 2006). One study has suggested, that in women, EPQ neuroticism is a better reflection of vulnerability to depression than self-esteem measured using the RSE (Roberts & Kendler, 1999). Although neuroticism is most commonly reported as being associated with risk of developing unipolar depression, high neuroticism has also been associated with bipolar disorder (Solomon *et al.*, 1996) and anxiety disorders including social phobia, agoraphobia and panic disorder (Bienvenu *et al.*, 2001). The EPQ was included in the present study to compare scores on the personality dimensions between individuals with DD and their unaffected relatives. Significant differences in scores on the neuroticism dimension in particular may reflect differences between the groups in the prevalence of individuals with increased vulnerability to major depression and other mood and anxiety disorders.

Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version (TEMPS-A)(Akiskal et al., 2005)

The Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version (TEMPS-A) is designed to measure variations in individuals' temperament. The questionnaire measures five temperaments/subscales: Cyclothymic, Dysthymic, Irritable, Hyperthymic and Anxious. It has been suggested by the author of the questionnaire that dysregulation of these temperaments may represent sub-clinical presentations of mood and anxiety disorders and reflect increased vulnerability to these disorders. This is supported by a study using the scale to compare the temperament scores of euthymic individuals with bipolar disorder, their healthy unaffected relatives (a sample of individuals at risk of developing mood disorders) and normal controls (Mendlowicz *et al.*, 2005). Euthymic individuals with bipolar disorder and their unaffected relatives were found to have significantly higher scores on the cyclothymic and anxious temperament subscales than controls. The cyclothymic subscale includes the items/statements *"my moods and energy are either high or low, rarely in between"* and *"I constantly switch between being lively and sluggish"* For each statement individuals are asked to indicate if this applies to them for much of their life.

The TEMPS-A questionnaire was included in the present study as significant differences between the groups on one or more of the subscales may reflect differences in the proportion of individuals with features of mood and anxiety disorders including sub-clinical features. Such differences may not be apparent when comparing the groups on objective measures such as lifetime history of treatment for psychiatric symptoms. An advantage of the TEMPS-A is that high scores on each of the temperaments/subscales may indicate vulnerability to specific types of mood and/or anxiety disorders.

Kings Schizotypy Questionnaire (KSQ) (Jones et al., 2000; Williams, 1993)

The Kings Schizotypy Questionnaire (KSQ) is a measure of schizotypy, a term used to describe a set of characteristics observed in the unaffected relatives of individuals with schizophrenia and suggested as reflecting liability to schizophrenia (Claridge, 1987; Meehl, 1962). These characteristics include difficulty in close relationships, odd speech and behaviours and perceptual disturbances. The KSQ measures seven subscales: Social Isolation, Social Anxiety, Recurrent Illusions I, Recurrent Illusions II, Magical Thinking, Paranoid Ideation and Ideas of Reference. Scores on the KSQ have been found to be significantly higher in individuals with schizophrenia and bipolar disorder than controls (Heron et al., 2003). In addition the presence of schizotypal personality traits have been found to be similar in relatives of individuals with schizophrenia and affective psychosis (McGilvarry et al., 2001). These finding suggest that high schizotypy scores may reflect vulnerability to functional psychosis rather than just schizophrenia. The KSQ was included in the present study as significant differences in schizotypy may reflect difference between the groups in the prevalence of individuals with vulnerability to functional psychosis.

Table 10.2 Current Mood State, Personality and Temperament Questionnaires

Questionnaire (Reference)	Description	Subscales	Scoring
Beck Depression Inventory (BDI) (Beck & Steer, 1987)	Widely used measure of the presence and/or severity of current depressive symptoms 21 items each rated on a scale of 0 (absent) to 3 (present and severe)	-	*Total scores range from 0 (no depressive symptoms) to 63 (severe depression)
Altman Self-Rating Scale for Mania (ASRM) (Altman *et al.*, 1997)	Measure of the presence and/or severity of current manic symptoms 5 items each rated on a scale of 0 (absent) to 4 (present and severe)	-	Total scores range from 0 (no manic symptoms) to 20 (significant manic symptoms)
Rosenberg Self-Esteem Scale (RSE) (Rosenberg, 1965)	Widely used measure of global self esteem 10 items/statements (5 positive, 5 negative) rated on a scale of 1-4 *strongly disagree-strongly agree* on the positive items/statements and *strongly agree -strongly disagree* on the negative items/statements	Positive Subscale Negative Subscale	Total score range from 10-40. Positive and negative subscale scores range from 5-20. Higher scores reflect higher self-esteem.
Eysenck Personality Questionnaire (EPQ) (Eysenck & Eysenck, 1975)	Widely used measure of personality. 90 yes-no items	3 personality dimensions: Extraversion, Neuroticism, Psychoticism + Lie Scale	Subscale Scores Extraversion 0-21 Neuroticism 0-23 Psychoticism 0-25 Lie 0-21
Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version (TEMPS-A) (Akiskal *et al.*, 2005)	Measure of temperament 39 true-false items/statements – true items are added up to give a score for each of the 5 temperaments	5 temperaments: Depressive, Cyclothymic, Hyperthymic, Irritable and Anxious	Cyclothymic 0-12 Depressive 0-8 Irritable 0-8 Hyperthymic 0-8 Anxious 0-3
Kings Schizotypy Questionnaire (Jones *et al.*, 2000; Williams, 1993)	Measure of schizotypal features 63 yes-no items	7 subscales: Social Isolation, Social Anxiety, Recurrent Illusions I, Recurrent Illusions II, Magical Thinking, Paranoid Thinking, Ideas of Reference	Total Score 0-63 All 7 subscales 0-9

*BDI Cut off scores: <10; none or minimal depression, 10-18; mild to moderate depression, 19-29; moderate to severe depression, 30-63; severe depression

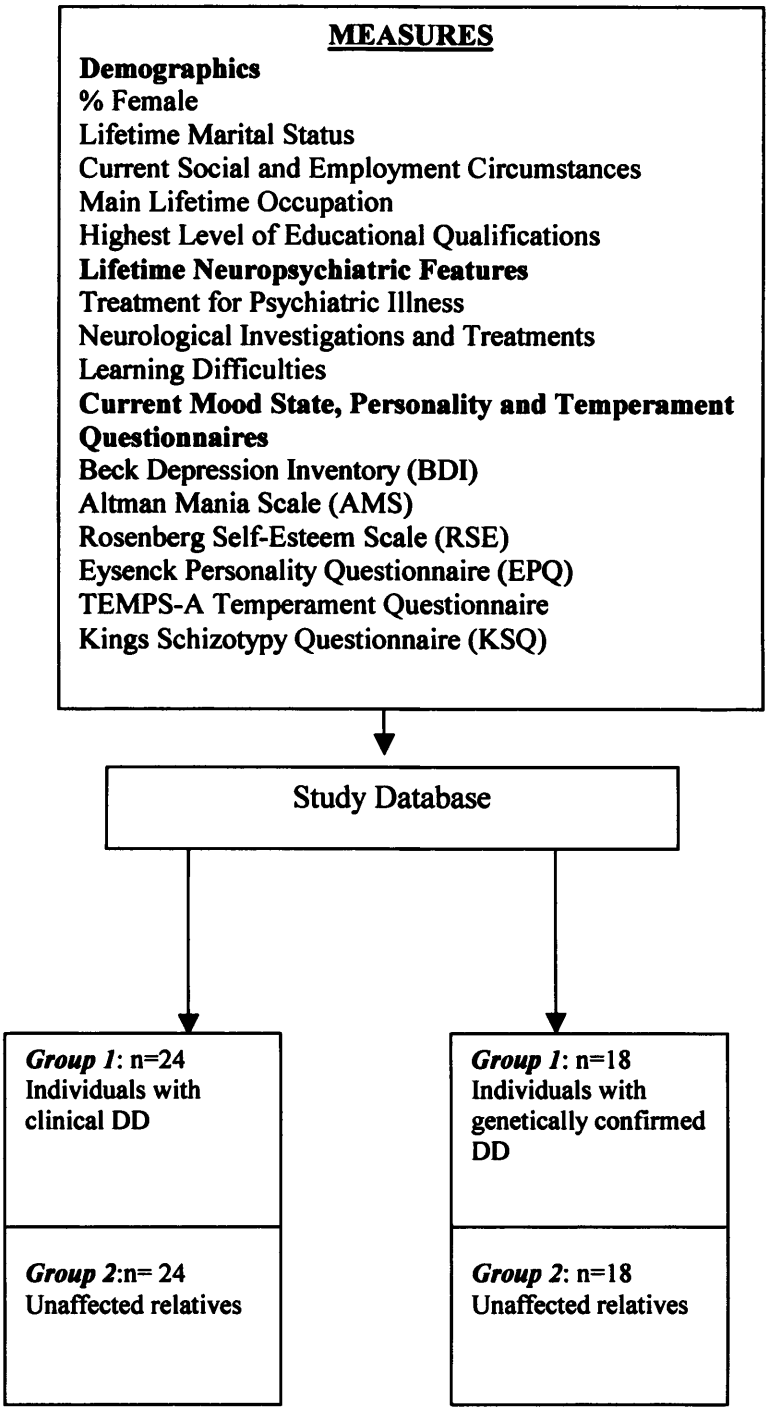
10.1.4 Pathogenic Mutation Detection in 24 Individuals with DD

In six of the 24 individuals with DD a pathogenic mutation was not detected in *ATP2A2*. As previously described in Chapter 9 section 9.2 pg. 197, it is possible that one or more of these individuals may have clinical features similar to DD that may be caused by factors other than a pathogenic mutation in *ATP2A2* in other words a be a phenocopy of DD. As a result, two sets of analyses were carried out, one comparing all 24 individuals with a diagnosis of DD to their unaffected relatives and another excluding the six individuals in whom a pathogenic mutation was not detected in *ATP2A2* and their unaffected relatives i.e. N=18 in each group.

10.1.5 Summary of the Comparison of a Sample of Individuals with DD and Their Unaffected Relatives

A summary of the measures and design used to compare a sample of individuals with DD and their unaffected relatives is shown in Figure 10.2.

Figure 10.2 Comparison of a Sample of Individuals with DD and their Unaffected Relatives- Measures and Design



10.2 Statistical Approaches

Data were extracted from the study Access database into the statistical package SPSS version 12.0.1 (SPSS Inc., 2003). The statistical tests used to examine differences between the DD and unaffected relatives groups in both sets of analyses are outlined below. Statistical tests were considered significant at the $p<0.05$ level (two tailed).

Continuous data: Paired-samples t tests (normality of the differences between the two groups was assessed using the Kolmogorov-Smirnov Test and were found to approximate normality on all measures)

Categorical data: Fisher's exact tests (2x2 tables) and exact significance tests for Pearson's chi-square (2x3 tables and greater).

Within-subject associations between scores on the BDI and the personality and temperament measures were assessed using Spearman's rho correlations.

Binary logistic regression (forward stepwise likelihood ratio) was carried out to determine which combination of temperament and personality questionnaire measures best-predicted group membership (i.e. DD or unaffected relatives).

Due to the modest sample size of the groups and limited power, corrections were not routinely made for multiple testing. Therefore, any significant differences between the groups were treated with caution and an emphasis was placed on looking for trends in the differences between individuals with DD and their unaffected on the neuropsychiatric measures and scores on the personality and temperament questionnaires.

The following chapter (Chapter 11) compares the presence of neuropsychiatric features in a sample of individuals with DD and their unaffected first-degree relatives.

11 COMPARISON OF DEMOGRAPHICS, LIFETIME NEUROPSYCHIATRIC FEATURES AND QUESTIONNAIRE SCORES IN A SAMPLE OF INDIVIDUALS WITH DD AND THEIR UNAFFECTED RELATIVES: RESULTS

This chapter describes and compares the demographic characteristics, prevalence of lifetime neuropsychiatric features and scores on a number of personality, temperament and current mood state questionnaires in a group of individuals with DD and a group of their unaffected relatives. Two sets of analyses were carried out, one comparing 24 individuals with a clinical diagnosis of DD to their unaffected relatives and another only comparing 18 of the original 24 individuals with genetically-confirmed DD to their unaffected relatives. Additional information relating to this chapter can be found in section F of the Appendix starting at pg. 363, and will be referenced throughout the chapter.

11.1 Comparison of Demographics

The demographic characteristics of all the groups are summarised in Table 11.1. In both sets of analyses, the DD group and unaffected relatives groups did not differ significantly on any of the demographic variables, which included current age, the proportion of females, lifetime marital status, current social circumstances, current employment status, main-lifetime occupation and highest level of educational qualifications (see Appendix F Table F-1 pg. 363).

Table 11.1 Comparison of Demographic Characteristics of Individuals with DD and their Unaffected Relatives - Descriptives and Frequencies

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD (N=24)	Unaffected (N=24)	DD (N=18)	Unaffected (N=18)
Age (years)				
Mean (95% CI)	45.04 (39.34-50.74)	43.50 (37.72-49.28)	43.89 (37.73-50.05)	44.22 (37.74-50.71)
Standard Deviation	13.502	13.680	12.395	13.036
Range	20-67	19-74	21-61	19-74
	%	%	%	%
Female	67	71	67	72
Lifetime Marital Status				
Has married/lived as married	75	79	78	83
Has never married/lived as married	25	21	22	17
Current Social Circumstances				
Lives in own home with spouse and/or children	54.2	54.2	55.6	61.1
Lives alone	12.5	8.3	16.7	5.6
Lives in home of parents or children	25	29.2	22.2	27.8
Lives with partner at least one year but not married	8.3	8.3	5.6	5.6
Current Employment Status				
Employed full time	41.7	37.5	50	38.9
Employed part time	20.8	33.3	22.2	33.3
Not working-receiving benefits	12.5	8.3	16.7	11.1
Full-time student	8.3	4.2	0	0
Homemaker	4.2	8.3	5.6	5.6
Retired	12.5	8.3	5.6	11.1
Main Lifetime Occupation				
Legislator/senior officials, managers and professionals	25	41.7	16.7	33.4
Technicians & associate professionals (& civil servants)	8.3	12.5	11.1	16.7
Clerks	25	20.8	27.8	22.2
Service workers & shop & market workers	20.8	8.3	22.2	11.1
Craft & related trade workers	12.5	4.2	16.7	5.6
Elementary occupations	4.2	8.3	5.6	11.1
Full-time student	4.2	4.2	0	0
Highest Level of Educational Qualifications				
No qualifications	20.8	20.8	27.8	22.2
Other qualifications*	20.8	4.2	16.7	5.6
GCSE A-C or equivalent	33.3	33.3	44.4	38.9
A Level or equivalent	12.5	12.5	5.6	11.1
Teaching, HND, Nursing, Degree	12.5	29.2	5.6	22.2

* Other qualifications include CSEs and GCSE grades D-G

11.2 Comparison of Lifetime Neuropsychiatric Features

11.2.1 Comparison of Lifetime Treatment for Psychiatric Illness

Table 11.2 pg. 234 summarises the frequencies and percentages of individuals with DD and their unaffected relatives receiving treatment for psychiatric illness in their lifetime. Individuals who had received more than one type of treatment, for example had consulted their GP for symptoms of depression, received antidepressants and been referred to secondary psychiatric services, are included in more than one treatment category. In the first set of analyses, a significantly higher proportion of individuals with DD compared to their unaffected relatives had consulted their GP for psychiatric symptoms (62.5% vs. 25%; Fisher's, p=0.019) and been referred by their GP to secondary psychiatric services (33.3% vs. 4.2%; Fisher's, p=0.023). In the second set of analyses, which only included individuals with genetically confirmed DD, there were greater differences in the proportions of individuals with DD and their unaffected relatives having a lifetime history of treatment for psychiatric illness. A significantly higher proportion of individuals with DD compared to their unaffected relatives had consulted their GP for psychiatric symptoms (66.7% vs. 16.7%; Fisher's, p=0.006) and been referred by their GP to secondary psychiatric services (44% vs. 0%; Fisher's, p=0.003).

11.2.2 Comparison of Lifetime History Neurological Investigations and Treatments

Table 11.3 pg. 234 summarises the frequencies and percentages of individuals with DD and their unaffected relatives with a lifetime history of being referred to a neurologist and having neurological investigations or being treated for a neurological condition. In both sets of analyses there were identical proportions of individuals with DD and their unaffected relatives having a lifetime history of neurological investigations and treatments.

Table 11.2 Comparison of Lifetime Treatment for Psychiatric Illness amongst Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD N=24	Unaffected N=24	DD N=18	Unaffected N=18
	N (%)	N(%)	N (%)	N (%)
GP consultation for psychiatric symptoms	15 (62.5)	6 (25) (p=0.019)	12 (66.7)	3 (16.7) (p=0.006)
*Offered/received medication for psychiatric symptoms by GP	10 (41.7)	6 (25) (p= 0.359)	9 (50)	3 (16.7) (p=0.075)
Referred by GP to psychiatric services	8 (33.3)	1 (4.2) (p= 0.023)	8 (44.4)	0 (0) (p= 0.003)
Psychiatric inpatient treatment	1 (4.2)	0 (0) (p=1.0)	1(5.6)	0 (0) (p=1.0)

Fisher's Exact Tests. * Includes antidepressants, anxiolytics or hypnotics.

Table 11.3 Comparison of Lifetime History of Neurological Investigations and Treatments amongst Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD N=24	Unaffected N=24	DD N=18	Unaffected N=18
	N (%)	N (%)	N (%)	N (%)
Neurological Treatments or Investigations	3* (12.5)	3** (12.5) (p= 1.0)	3 (16.7)	3 (16.7) (p=1.0)

Fisher's Exact Tests. *Viral Encephalitis; syncope attacks; recurrent headaches. ** Multiple Sclerosis; recurrent headaches x2.

11.2.3 Comparison of Learning Difficulties

Table 11.4 summarises the frequencies and percentages of individuals with DD and their unaffected relatives with a lifetime history of being given a diagnosis of dyslexia and/or a history of receiving extra help at school. Individuals with a history of being given a diagnosis of dyslexia and receiving extra help at school are included in both categories. In the first set of analyses, a non-significantly higher proportion of individuals with DD compared to their unaffected relatives had previously been given a diagnosis of dyslexia (13% vs. 4.3%; Fisher's, p=0.608) and a history of receiving extra help at school (21.7% vs. 4.3%; Fisher's,

p=0.187). In the second set of analyses, there were greater differences in the proportions of individuals with genetically confirmed DD and their unaffected relatives having a lifetime history of a diagnosis of dyslexia (11.8% vs. 0%; Fisher's, p=0.485) and a history of receiving extra help at school (24% vs. 0%; Fisher's, p=0.103). These differences were not significant.

Table 11.4 Comparison of Learning Difficulties amongst Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD N=23*	Unaffected N=23*	DD N=17*	Unaffected N=17*
	N (%)	N (%)	N(%)	N(%)
Diagnosis of Dyslexia	3(13)	1(4.3) (p=0.608)	2(11.8)	0(0) (p=0.485)
**Received extra help at school	5 (21.7)	1 (4.3) (p=0.187)	4 (24)	0(0) (p=0.103)

Fisher's Exact Tests. *Missing cases are one DD-Unaffected pair where information on either one individual or both was unknown. ** Includes extra help with reading and/or writing and/or spelling and/or maths.

11.3 Comparison of Current Mood State, Personality and Temperament Questionnaires

The following sections compare the DD and unaffected relatives groups on a number of personality, temperament and current mood state questionnaires. A brief description of each questionnaire and their scoring is displayed in Chapter 10 Table 10.2 pg. 227.

11.3.1 Current Mood State Questionnaires

Descriptives for scores on the Beck Depression Inventory (BDI) and Altman Self-Rating Scale for Mania (ASRM) among individuals with DD and their unaffected relatives are displayed in Table 11.5. In the first set of analyses, there was a very small difference in the mean BDI scores of individuals with DD and their unaffected relatives (6.95 vs. 6.86; t=0.042, df=20, p=0.967). In the second set of analyses, only including individuals with genetically confirmed DD, there was a greater difference in the mean BDI scores, which almost reached significance (7.93 vs. 4.20; t=1.813, df=14, p=0.091). In both sets of analyses,

there were very small non-significant differences in the mean ASRM scores (2.90 vs. 3.62; t=0.821, df=20, p=0.422) and (3.27 vs. 3.73; t=0.450, df=14, p=0.660).

Table 11.5 Comparison of BDI and ASRM Scores in Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD *N=21	Unaffected *N=21	DD *N=15	Unaffected *N=15
BDI Mean/ Median	6.95/5.00	6.86/3.00	7.93/5.00	4.20/1.00
Standard Deviation	7.018	9.074	7.411	6.700
Range (IqR)	0-24 (10.00)	0-31 (10.50)	0-24 (9)	0-25 (6)
	(t=0.042, df=20, p=0.967)		(t=1.813, df=14, p=0.091)	
ASRM Mean/ Median	2.90/2.00	3.62/3.00	3.27/2.00	3.73/3.00
Standard Deviation	2.528	4.080	2.658	4.511
Range (IqR)	0-9 (3)	0-15 (7)	0-9 (2)	0-15 (8)
	(t=0.821, df=20, p=0.422)		(t=0.450, df=14, p=0.660)	

Paired Samples T Tests. BDI; Beck Depression Inventory, ASRM; Altman Self-Rating Scale for Mania. IqR; Interquartile Range. *Missing cases are three DD-Unaffected pairs where either one individual or both did not complete the questionnaires.

11.3.2 Personality and Temperament Questionnaires

11.3.2.1 Rosenberg Self-Esteem Scale (RSE)

Descriptives for total and subscale scores on the Rosenberg Self-Esteem Scale (RSE) among individuals with DD and their unaffected relatives are displayed in Table 11.6. A similar pattern of results was observed in both sets of analyses. Both groups of individuals with DD had lower mean *total scores* on the RSE (indicating lower self esteem) than their unaffected relatives. In the second set of analyses, this difference was statistically significant (26.44 vs. 31.50; t=2.219, df=15, p=0.042). Mean scores on the *positive subscale* of the RSE were not significantly different in both sets of analyses. Individuals with DD had significantly lower mean scores on the *negative scale* of the RSE (indicating lower self-esteem) than their unaffected relatives in both sets of analyses (13.38 vs. 15.81; t=2.112, df=20, p=0.047) and (12.38 vs. 15.69; t=2.374, df=15, p=0.031). In the three sets of analyses where individuals with DD had significantly lower self-esteem scores than their unaffected relatives, correlations between self-esteem scores and individuals' current level of depression were

investigated (see Table F-2 pg. 363). In all cases there was a non significant negative correlation between self-esteem and current depressive symptoms, individuals with lower self-esteem reported more current depression. To investigate whether the significant differences in self-esteem between individuals with DD and their unaffected relatives still existed once current level of depression was controlled for, further analyses were carried out only including individuals with DD and their unaffected relatives where both individuals had a score of less than 10 on the BDI (the cut-off for none or minimal depression) (see Table F-3 pg 364). The trend for individuals with DD to have lower self-esteem scores than their unaffected relatives remained but these differences did not reach statistical significance. This trend was also found when a linear regression model was used to adjust RSE scores for variation in BDI scores and the corrected total RSE scores of individuals with DD and their unaffected relatives were compared using a paired sample t- test (mean RSE scores; 24.46 vs. 27.93; t=2.085, df=14, p=0.056).

Table 11.6 Comparison of Rosenberg Self-Esteem Scale Total and Subscale Scores in Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD *N=21	Unaffected *N=21	DD **N=16	Unaffected **N=16
Total				
Mean/Median	28.10/28.00	31.62/32.00	26.44/27.00	31.50/31.50
Standard Deviation	6.123	6.407	5.656	6.303
Range	17-39	16-40	17-39	16-40
Interquartile Range	10	9	8	10
	(t=1.874, df=20, p=0.076)		**(t=2.219, df=15, p=0.042)**	
Positive subscale				
Mean/Median	14.71/15.00	15.81/15.00	14.06/15.00	15.81/15.00
Standard Deviation	3.068	2.462	3.043	2.287
Range	7-19	11-20	7-19	11-20
Interquartile Range	5	3	5	3
	(t=1.263, df=20,p=0.221)		(t=1.635, n=15, p= 0.123)	
Negative subscale				
Median/ Mean	13.38/13.00	15.81/17.00	12.38/12.00	15.69/16.00
Standard Deviation	3.653	4.167	3.364	4.254
Range	9-20	5-20	9-20	5-20
Interquartile Range	6	6	6	6
	(t=2.112, df=20, p=0.047)		**(t=2.374, df=15, p=0.031)**	

Paired Samples T Tests. Missing cases are three* and two** DD-Unaffected pairs where either one individual or both did not complete the questionnaire.

11.3.2.2 Eysenck Personality Questionnaire (EPQ)

Descriptives for subscale scores on the Eysenck Personality Questionnaire (EPQ) among individuals with DD and their unaffected relatives are displayed in Table 11.7. In both sets of analyses there were no significant differences in the mean scores of individuals with DD and their unaffected relatives on the *extroversion*, *psychoticism* and *lie* subscales. The groups also did not significantly differ in their mean scores on the neuroticism subscale in the first set of analyses. However, in the second set of analyses, the mean *neuroticism* score of the genetically confirmed DD group was significantly higher than the mean score of the unaffected relatives group (12.80 vs. 8.13; t=3.883, df=14, p=0.002). In these two groups there was a positive correlation between *neuroticism* scores and individuals' current level of depression (see Table F-2 pg. 363). Individuals with DD still had significantly higher mean EPQ *neuroticism* scores than their unaffected relatives when current level of depressive symptoms was controlled for by only including individuals with DD and their unaffected relatives where both individuals had a score of less than 10 on the Beck Depression Inventory, (12.25 vs. 7; t=3.656, df=7, p=0.008) (see Table F-3 pg. 364).

The frequencies and percentages of individuals responding yes or no to EPQ item 68 "*Have you ever wished you were dead?*" are shown in Table 11.8. In the first set of analyses, a non-significantly higher proportion of individuals with DD responded *yes* to the item than their unaffected relatives (48% vs. 38%; Fisher's, p=0.687). In the second set of analyses the difference was greater with nearly twice as many individuals with genetically confirmed DD reporting they had wished they were dead compared to their unaffected relatives. This relationship remained non-significant (60% vs. 33%; Fishers, p= 0.143).

Table 11.7 Comparison of EPQ Subscale Scores in Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
	DD *N=21	Unaffected *N=21	DD *N=15	Unaffected *N=15
Extraversion				
Mean/Median	12.90/13.00	13.00/13.00	13.00/13.00	13.73/15.00
Standard Deviation	4.898	4.461	4.957	4.574
Range (IqR)	5-21(9)	6-20 (8)	5-21(8)	6-20 (9)
	(t=0.091, df=20,p=0.928)		(t=0.615, df=14, p=0.549)	
Neuroticism				
Mean/Median	10.43/11.00	9.52/8.00	12.80/15.00	8.13/7.00
Standard Deviation	7.011	5.988	5.943	5.514
Range (IqR)	0-22 (14)	1-22 (10)	3-22 (11)	1-18 (10)
	(t=0.548, df=20, p= 0.590)		**(t=3.883, df=14, p=0.002)**	
Psychoticism				
Mean/Median	2.00/2.43	2.29/2.00	2.73/3.00	2.73/2.00
Standard Deviation	1.832	1.848	1.907	1.981
Range (IqR)	0-7 (3)	0-8 (2)	0-7 (3)	1-8 (2)
	(t=0.260, df=20, p=0.797)		(t=0.0, df=14, p=1.0)	
Lie				
Mean/Median	10.00/10.24	10.43/9.00	10.33/9.00	10.93/11.00
Standard Deviation	4.242	4.154	4.353	4.131
Range	4-19 (7)	4-18 (7)	6-19 (8)	5-18 (6)
	(t=0.159, df=20, p=0.875)		(t=0.399, df=14, p=0.696)	

Paired Samples T Tests. EPQ; Eysenck Personality Questionnaire. IqR; Interquartile Range.
*Missing cases are three DD-Unaffected pairs where either one individual or both did not complete the questionnaire.

Table 11.8 Comparison of Response to EPQ Item 68 '*Have you ever wished you were dead?*' in Individuals with DD and their Unaffected Relatives

	Individuals with Clinical DD and their unaffected relatives					Individuals with Genetically-confirmed DD and their unaffected relatives				
	No		Yes			No		Yes		
FAMILY MEMBER	**N**	**%**	**N**	**%**	**N**	**N**	**%**	**N**	**%**	**N**
DD	11	52	10	48	21	6	40	9	60	15
Unaffected	13	62	8	38	21	10	67	5	33	15
***Total N**	24		18		42	16		14		30
	(Fisher's, p=0.756)					(Fisher's, p=0.143)				

Fisher's Exact Tests. EPQ; Eysenck Personality Questionnaire.
* Missing cases are six individuals from three DD-Unaffected pairs where either one individual or both did not complete the questionnaire.

11.3.2.3 TEMPS-A Temperament Questionnaire

Descriptives for scores on the temperament dimensions of the TEMPS-A among individuals with DD and their unaffected relatives are displayed in Table 11.9. In both sets of

analyses, individuals with DD had higher mean scores on the *depressive* dimension than their unaffected relatives but these differences were not significant. In the first set of analyses, individuals with DD had non-significantly higher mean scores on the *cyclothymic* dimension than their unaffected relatives (3.32 vs. 2.36; t=1.233, df=21, p=0.231). This difference in mean *cyclothymic* scores became significant when only individuals with genetically confirmed DD were compared to their unaffected relatives (4.06 vs. 2.06; t=2.309, df=15, p=0.036). In both sets of analyses individuals with DD had lower mean scores on the *hyperthymic* temperament dimension than their unaffected relatives. This difference was only significant in the first set of analyses (3.27 vs. 4.55; t=2.096, df=21, p=0.048). There were no significant differences between the groups on the *irritable* and *generalized anxious* temperament dimension in both sets of analyses. In the case of the *cyclothymic* and *hyperthymic* temperament dimensions where significant differences were found between individuals with DD and their unaffected relatives, correlations between the temperament scores and individuals current level of depressive symptoms were investigated (see Table F-2 pg. 363). In both the DD and unaffected relatives groups there was a positive correlation between individuals' *cyclothymic* temperament scores and their current level of depressive symptoms. This relationship was significant in the DD group (rho=0.779, n=15, p=0.001). In the DD group there was also a significant negative correlation between individuals' *hyperthymic* temperament scores and their current level of depressive symptoms (rho=-0.552, n=21, p=0.01) but there was no significant correlation in the unaffected relatives group. When current depression was controlled for there were no significant differences between individuals with DD and their unaffected relatives in their mean scores on the *cyclothymic* and *hyperthymic* temperament dimensions of the TEMPS-A questionnaire (see Table F-3 pg. 364).

Table 11.9 Comparison of TEMPS-A Subscale Scores in Individuals with DD and their Unaffected Relatives

		Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
		DD *N=22	Unaffected *N=22	DD *N=16	Unaffected *N=16
Depressive	Mean/Median	2.00/1.00	1.18/0.00	2.38/1.50	1.00/0.00
	Standard Deviation	2.545	1.680	2.680	1.366
	Range (IqR)	0-8 (3)	0-6 (2)	0-8 (4)	0-4 (2)
		(t=1.328, df=21, p=0.198)		(t=1.752, df=15, p=0.100)	
Cyclothymic	Mean/Median	3.32/2.50	2.36/2.00	4.06/3.00	2.06/1.50
	Standard Deviation	3.272	2.592	3.45	2.46
	Range (IqR)	0-11(5)	0-7 (5)	0-11(5)	0-7 (4)
		(t=1.233, df=21, p=0.231)		(t=2.309, df=15, p=0.036)	
Hyperthymic	Mean/Median	3.27/3.00	4.55/5.00	3.19/3.00	4.50/5.00
	Standard Deviation	2.354	2.041	2.482	2.19
	Range (IqR)	0-8 (4)	1-8 (3)	0-8 (4)	1-8 (4)
		(t=2.096, df=21, p=0.048)		(t=1.774, df=15, p=0.096)	
Irritable	Mean/Median	1.64/1.00	1.68/1.00	2.00/1.50	1.81/1.00
	Standard Deviation	1.620	1.961	1.633	2.228
	Range (IqR)	0-5 (3)	0-8 (2)	0-5 (3)	0-8 (3)
		(t=0.110, df=21,p=0.913)		(t=0.362, df=15, p=0.723)	
Generalized Anxious	Mean/Median	1.05/1.00	0.77/0.00	1.19/1.00	0.88/0.00
	Standard Deviation	1.174	1.193	1.276	1.204
	Range (IqR)	0-3 (2)	0-3 (1)	0-3 (3)	0-3 (2)
		(t=0.810, df=21,p=0.427)		(t=0.847, df=15, p=0.410)	

Paired Samples T Test. TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version. IqR; Interquartile Range. *Missing cases are two DD-Unaffected pairs where either one individual or both did not complete the TEMPS-A questionnaire.

11.3.2.4 Kings Schizotypy Questionnaire (KSQ)

Descriptives for the total and subscale scores on the Kings Schizotypy Questionnaire (KSQ) among individuals with DD and their unaffected relatives are displayed in Table 11.10. In the first set of analyses, individuals with DD and their unaffected relatives did not significantly differ in their total scores on the KSQ or any of the subscales. In the second set of analyses individuals with DD had significantly higher mean *Total* KSQ scores (11.09 vs. 7.64; t=3.121, df=10, p=0.011) and significantly higher mean scores on the *Ideas of Reference* (1.82 vs. 0.91; t=3.194, df=10, p=0.010) and *Recurrent Illusions 1* subscales (1 vs. 0.36; t= 2.609, df=10, p= 0.026). In both groups there was a non-significant positive correlation between individuals' *Total, Ideas of Reference* and *Recurrent Illusions 1* scale scores and their current level of depressive symptoms (see Table F-2 pg. 363). When current level of

depressive symptoms was controlled for, individuals with DD still had significantly higher mean scores on the KSQ *total* scale (10.17 vs. 6.33; t=3.005, df=5, p=0.030) and the *Ideas of Reference* subscale (1.83 vs. 0.50; t=4.00, df=5, p=0.010) (shown in Table F-3 pg. 364).

Table 11.10 Comparison of KSQ Scores in Individuals with DD and their Unaffected Relatives

		Individuals with Clinical DD and their unaffected relatives		Individuals with Genetically-confirmed DD and their unaffected relatives	
		DD *N=16	Unaffected *N=16	DD *N=11	Unaffected *N=11
Total	Mean/Median	10.56/10.00	9.50/7.00	11.09/10.00	7.64/7.00
	Standard Deviation	6.077	9.839	6.172	6.021
	Range	1-21	1-41	3-21	1-23
	Interquartile Range	11	6	12	5
		(t= 0.577, df=15, p=0.572)		**(t=3.121, df=10, p=0.011)**	
Ideas of Reference	Mean /Median	1.63/1.00	1.19/0.00	1.82/1.00	0.91/0.00
	Standard Deviation	1.408	1.797	1.471	1.446
	Range	0-4	0-6	0-4	0-4
	Interquartile Range	3	2	2	2
		(t= 1.385, df=15, p=0.186)		**(t=3.194, df=10, p=0.010)**	
Magical Thinking	Mean/Median	1.31/1.00	1.06/1.00	1.00/1.00	0.82/1.00
	Standard Deviation	1.138	1.389	1.000	0.982
	Range	0-3	0-5	0-3	0-3
	Interquartile Range	2	2	2	1
		(t=0.745, df=15, p=0.468)		(t=0.690, df=10, p=0.506)	
Paranoid Thinking	Mean/Median	1.25/1.00	1.06/1.00	1.45/1.00	1.09/1.00
	Standard Deviation	1.571	0.929	1.809	0.831
	Range	0-5	0-3	0-5	0-2
	Interquartile Range	2	2	3	2
		(t=0.467 , df=15, p=0.654)		(t=0.649, df=10, p=0.531)	
Recurrent Illusions 1	Mean/Median	0.81/0.00	0.94/0.00	1.00/0.00	0.36/0.00
	Standard Deviation	1.424	2.081	1.612	0.924
	Range	0-5	0-8	0-5	0-3
	Interquartile Range	1	1	1	0
		(t=0.212 , df=15, p=0.835)		**(t= 2.609, df=10, p= 0.026)**	
Recurrent Illusions 2	Mean/Median	1.38/1.00	0.94/0.00	1.73/1.00	0.55/0.00
	Standard Deviation	1.857	1.652	2.102	0.820
	Range	0-7	0-6	0-7	0-2
	Interquartile Range	2	2	2	1
		(t=0.835 , df=15, p=0.417)		(t=2.137, df=10, p=0.058)	
Social Anxiety	Mean/Median	2.75/3.00	2.50/2.00	3.00/3.00	2.18/2.00
	Standard Deviation	1.571	1.932	1.673	1.471
	Range	1-6	1-8	1-6	1-6
	Interquartile Range	3	2	3	2
		(t=0.522 , df=15, p=0.609)		(t=1.695, df=10, p=0.121)	
Social Isolation	Mean/Median	1.44/1.00	1.81/1.50	1.09/1.00	1.73/1.00
	Standard Deviation	1.590	1.759	1.044	1.794
	Range	0-6	0-6	0-3	0-6
	Interquartile Range	2	3	2	3
		(t=0.972 , df=15, p=0.347)		(t=1.249, df=10, p=0.240)	

Paired Samples T Tests. KSQ; Kings Schizotypy Questionnaire.
*Missing cases are eight DD-Unaffected pairs where either one individual or both did not complete the questionnaire.

11.3.3 Temperament and Personality Predictors of Individuals with DD vs. Unaffected Relatives

Logistic regression (forward stepwise) was carried out to determine which combination of temperament and personality questionnaire measures best-predicted group membership (i.e. DD or unaffected relative). All total questionnaire scores and/or questionnaire subscales where there were large differences between the scores of individuals with DD and their unaffected relatives were selected as predictor variables in addition to total scores on the two current mood state questionnaires (Beck Depression Inventory and Altman Self-Rating Scale for Mania), these are summarised in Table 11.11.

Table 11.11 Personality and Temperament Measures Selected as Predictor Variables for Logistic Regression

Beck Depression Inventory Total Score (BDI)
Altman Self-Rating Scale for Mania Total Score (ASRM)
Rosenberg Self - Esteem Scale Total Score (RSE)
Eysenck Personality Questionnaire - Neuroticism Subscale (EPQ-N)
TEMPS-A- Depressive Subscale
TEMPS-A- Cyclothymia Subscale
TEMPS-A-Hyperthymia Subscale
Kings Schizotypy Questionnaire Total Score (KSQ)

TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version.

In the first set of analyses, 35 individuals with no missing values for any of the questionnaire variables were included in the analysis. None of the questionnaire variables had a significance value of less than 0.05 and were therefore not significantly predictive of DD vs. unaffected relative status. In the second set of analyses, which only included individuals with genetically confirmed DD, 25 individuals, with no missing values for any of the questionnaire predictor variables, were included in the analysis. The best solution correctly classified 72% of individuals. The significant variable in this solution was EPQ neuroticism (OR=1.202, 95% CI = 1.014-1.424, p= 0.034).

11.4 Summary

This chapter has described and compared the demographic characteristics, prevalence of lifetime neuropsychiatric features and questionnaires scores in a group of individuals with DD and a group of their unaffected relatives. A key finding was that the differences between the two groups were greater in the set of analyses that only included individuals with genetically confirmed DD. Table 11.12 summaries the lifetime neuropsychiatric features and questionnaire scores amongst individuals with genetically confirmed DD and their unaffected relatives.

Table 11.12 Summary of Comparison of Lifetime Neuropsychiatric Features and Questionnaire Scores amongst Individuals with Genetically-Confirmed DD and their Unaffected Relatives

		Individuals with genetically-confirmed DD	Unaffected Relatives	P
		%	%	
GP consultation for psychiatric symptoms		66.7	16.7	**0.006**
Referred by GP to psychiatric services		44.4	0	**0.003**
Psychiatric inpatient treatment		5.6	0	1.0
Neurological Treatments or Investigations		16.7	16.7	1.0
Diagnosis of Dyslexia		11.8	0	0.485
Received extra help at school		24	0	0.103
EPQ Item 68- *Have you ever wished you were dead?*		60	33	0.143
		Mean (Median*)	**Mean (Median*)**	**P (P**)**
BDI		7.93 (5.00)	4.20 (1.00)	0.091
ASRM		3.27 (2.00)	3.73 (3.00)	0.660
RSE	Total	26.44	31.50	**0.042** (0.175)
	Positive subscale	14.06	15.81	0.123
	Negative subscale	12.38	15.69	**0.031** (0.206)
EPQ	Extraversion	13.00	13.73	0.549
	Neuroticism	12.80	8.13	**0.002 (0.008)**
	Psychoticism	2.73	2.73 (2.00)	1.0
	Lie	10.33	10.93	0.696
TEMPS-A	Depressive	2.38 (1.50)	1.00 (0.00)	0.100
	Cyclothymic	4.06	2.06 (1.50)	**0.036** (0.794)
	Hyperthymic	3.19	4.50	0.096
	Irritable	2.00 (1.50)	1.81 (1.00)	0.723
	Generalized Anxious	1.19 (1.00)	0.88 (0.00)	0.410
KSQ	Total	11.09	7.64 (7.00)	**0.011 (0.030)**
	Ideas of Reference	1.82 (1.00)	0.91 (0.00)	**0.010 (0.010)**
	Magical Thinking	1.00	0.82 (1.00)	0.506
	Paranoid Thinking	1.45	1.09	0.531
	Recurrent Illusions 1	1.00 (0.00)	0.36 (0.00)	**0.026** (0.203)
	Recurrent Illusions 2	1.73 (1.00)	0.55 (0.00)	0.058
	Social Anxiety	3.00	2.18/2.00	0.121
	Social Isolation	1.09	1.73	0.240

Fisher's Exact Tests and Paired Samples T Tests. BDI; Beck Depression Inventory, ASRM; Altman Self-Rating Scale for Mania, RSE; Rosenberg Self-Esteem Scale, EPQ; Eysenck Personality Questionnaire, TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire, KSQ; Kings Schizotypy Questionnaire. Median* reported where data are not normally distributed. P** Significance after controlling for current depression. Significant P values are reported in **bold.**

The following chapter (Chapter 12) discusses the finding summarised in Table 11.12

12 COMPARISON OF DEMOGRAPHICS, LIFETIME NEUROPSYCHIATRIC FEATURES AND QUESTIONNAIRE SCORES IN A SAMPLE OF INDIVIDUALS WITH DD AND THEIR UNAFFECTED RELATIVES: DISCUSSION

This chapter will discuss the results presented in Chapter 11. These results and discussions relate to the third aim of the thesis, which was to compare the presence of neuropsychiatric features in a sample of individuals with DD and their unaffected first-degree relatives. First, the possible reasons for the consistent finding that differences between the two groups were greater when the analysis only included individuals with genetically confirmed DD are discussed. This is followed by a discussion of the comparison of the demographic characteristics, lifetime neuropsychiatric features and questionnaire scores among the group of 18 individuals with genetically confirmed DD and their unaffected relatives.

12.1 Comparisons of Individuals with Clinical DD and Genetically Confirmed DD

In the majority of cases there was a trend for the prevalence of neuropsychiatric features/questionnaire scores among individuals with genetically confirmed DD to indicate a greater degree of psychopathology compared to the sample containing all individuals with a clinical diagnosis of DD. This pattern was observed for all of the lifetime treatment for psychiatric illness measures, for example 33% of the "clinical diagnosis of DD" sample had been referred to psychiatric services compared to 44% of the "genetically confirmed DD" sample. This is consistent with the finding, reported and discussed in Chapters 8 and 9, that individuals in whom a pathogenic mutation was detected in *ATP2A2* had a higher lifetime prevalence of neuropsychiatric illness. In 18 of the 21 questionnaire total and subscale scores (excluding the EPQ lie scale) there was a trend for scores indicating more psychopathology to be observed in the "genetically confirmed DD" sample compared to the "clinical DD" sample.

Explanations for the increased prevalence of neuropsychiatric features among individuals with genetically confirmed DD have previously been discussed in Chapter 9 section 9.2 pg. 197). It is possible that a proportion of the individuals in whom a pathogenic mutation *was not* detected are phenocopies of DD, i.e. they do not have a pathogenic mutation in *ATP2A2.* Based on the hypothesis that mutations in *ATP2A2* have pleiotropic effects in the skin and brain, it would be expected that these individuals would have a lower prevalence of neuropsychiatric features. Due to the possibility that individuals in the "clinical DD" sample may not have a pathogenic mutation in *ATP2A2*, the following sections discuss just the comparisons of individuals with genetically confirmed DD and their unaffected relatives.

12.2 Comparison of Individuals With Genetically-Confirmed DD and Their Unaffected Relatives

This is the first case series study of DD to compare the presence of neuropsychiatric features in individuals with DD and their first-degree unaffected relatives. Unaffected family members have only previously been examined in studies reporting the neuropsychiatric features in single families with multiple family members with DD (summarised in Table 2.3 pg. 13). These studies have consistently reported a greater prevalence of neuropsychiatric features in family members with DD compared to family members without DD.

As discussed in Chapter 5, comparison of the prevalence of lifetime neuropsychiatric features in the current sample to the reported rates in the general population suggested a potential population-level association between DD and mood disorders, specifically major depression, suicide attempts and suicidal thoughts. The added advantage of having an unaffected relatives comparison group is that this method allows control for additional genetic and non-genetic factors that could influence the presence of neuropsychiatric features.

12.2.1 Demographics

The two groups were well matched for age (mean age 44 in both groups) and gender (proportion of women; 67% vs. 72%). A very similar proportion of individuals with DD and their unaffected relatives were married and the majority of individuals in both groups were living with a spouse and/or children. A higher proportion of individuals with DD were living on their own compared to their unaffected relatives (16.7% vs. 5.6%). In both groups most individuals were either employed full time or part time (72%).

A very slightly higher proportion of DD individuals reported that they had no formal qualifications compared to their unaffected relatives (27.8% vs. 22.2%). As reported in Chapter 4, 41% of individuals in the current study reported having no qualifications, indicating that the DD group was unrepresentative of the whole sample. A possible explanation for this is that individuals with no qualifications were more likely to be older, and therefore their first-degree relatives may not have been well enough/available to take part in the study.

There was a non-significant trend for individuals with DD to have not reached as high occupational and educational levels as their unaffected relatives. A lower proportion of the DD group reported their main lifetime occupation as being a professional/associate professional (27.8% vs. 50.1%) and a lower proportion had higher education qualifications (5.6% vs. 22%). This finding is consistent with a report in the literature of an individual with DD (as a result of a spontaneous mutation) who left school aged 15 without any qualifications, and had a genetically identical but unaffected twin brother who completed secondary school education and achieved a senior position (Ruiz-Perez *et al.*, 1999). The potential effects of DD on individuals' occupation and educational performance needs to be examined in a larger samples. Individuals with DD may have to take time off from school and/or work due to the severity of their symptoms also, as discussed in Chapter 5, a

proportion of individuals with DD may be impaired in specific aspects of learning. Both of these factors could potentially affect the educational and occupational performance of individuals with DD.

12.2.2 Lifetime Neuropsychiatric Features

Treatment for Psychiatric Illness

As shown in Table 11.2 pg. 234, a significantly higher proportion of individuals with DD had a lifetime history of consulting their GP for psychiatric symptoms and being referred by their GP to psychiatric services compared to their unaffected relatives. This is consistent with the findings in the whole DD sample, reported and discussed in Chapters 4 and 5. The explanations for the increased prevalence of psychiatric features among individuals with DD compared to their unaffected relatives have previously been discussed in Chapter 5, section 5.4 pg. 123 These include psychosocial factors and the pleiotropy hypothesis.

The prevalence of past psychiatric treatment in the DD group differed from that observed in the whole genetically confirmed DD sample. Among DD individuals *with* a participating unaffected relative there was a non-significantly higher prevalence of lifetime history of GP consultation (66.7% vs. 46.0%; Fisher's, p=0.173) and being referred by a GP to psychiatric services (44.4% vs. 29.2%; Fisher's, p=0.257) compared to individuals *without* a participating relative. However this bias does not fully account for the differences between the DD and unaffected groups. It is possible that individuals with a past history of psychiatric illness may have been more likely to encourage their unaffected relatives to participate in the current study. In addition, unaffected relatives may have been more likely to participate if they felt their relatives had experienced psychiatric symptoms as a consequence of DD. In contrast there was a lower prevalence of lifetime history of receiving psychiatric inpatient treatment among individuals *with* a participating unaffected relative compared to individuals *without*

(5.6% vs. 16.3%; Fisher's, p=0.238). This suggests that individuals with a lifetime history of more severe psychiatric symptoms were less likely to have an unaffected relative participating in the study. Finally, it is also possible that the unaffected relatives group may be an unrepresentative sample. Unaffected relatives with a history of psychiatric illness may not have felt comfortable discussing any treatment they had received with a researcher and/or reporting these symptoms/treatments in a questionnaire version of the interview.

Overall the findings of the current study indicate that individuals with DD had a greater prevalence of lifetime treatment for psychiatric illness than their unaffected relatives. Ideally this needs to be examined in larger samples. In the current study every effort was made to recruit unaffected relatives. It is therefore likely that future attempts to collect larger samples would encounter similar difficulties.

Neurological Investigations and Treatments

The DD and unaffected relatives groups had an identical proportion of individuals with a lifetime history of being referred to a neurologist and having neurological investigations (16.7%). However, the prevalence of lifetime treatment for neurological symptoms in the DD group is unrepresentative of the whole DD sample. DD individuals *with* a participating relative had a significantly lower prevalence of treatment for neurological symptoms than individuals *without* a participating relative (16.7% vs. 46.0%; Fisher's; p=0.046). In the current study one of the main reasons for DD individuals being referred to a neurologist was for investigations of periods of loss of consciousness. As discussed in Chapter 5 (section 5.5 pg. 125) in some of these individuals, the episodes of loss of consciousness might have formed part of a psychiatric condition or somatization disorder. These individuals may have been less likely to ask their unaffected relatives to participate and/or to have an unaffected relative willing to participate in the current study. Due to this bias in the DD group it is not

possible to make any conclusions regarding prevalence of neurological features in the two groups

Learning Difficulties

As reported in Chapter 5 (section 5.3.4 pg. 121) difficulties were encountered comparing the prevalence of learning difficulties in the current sample to general population figures. The mean age in the DD and unaffected groups was identical, making comparisons between these two groups more meaningful. A non-significantly higher proportion of the DD group had been given a diagnosis of dyslexia and had a history of receiving extra help at school. The small group sizes are likely to have limited the power to detect a significant difference.

Individuals *with* a participating unaffected relative had a higher prevalence of receiving extra help at school (22% vs. 11%; Fisher's, p= 0.248) and having a diagnosis of dyslexia (11% vs. 4.1%; Fisher's, p= 0.291) compared to individuals *without* a participating relative. This indicates a possible bias in the DD group. It is also possible that the unaffected relatives group may have been unrepresentative. Unaffected relatives with learning difficulties may have been less likely to complete the written questionnaires. The current study suggests an increased prevalence of learning difficulties in individuals with DD. However, group biases are likely to partially explain these differences.

12.2.3 Comparison of Current Mood State, Personality and Temperament Questionnaires

This final section discusses the group differences on the current mood state, personality and temperament questionnaires. A number of these measures have previously been completed by large samples of individuals with bipolar disorder and major recurrent depression participating in our research into mood disorders. These data have been collected by researchers within the Department of Psychiatry at the University of Birmingham and Department of Psychological Medicine at Cardiff University. The scores on the Eysenck Personality Questionnaire (EPQ) and Rosenberg Self-Esteem Scale (RSE) in these samples and a control sample at low risk developing mood disorders have previously been reported (Jones *et al.*, 2005). Particular patterns of scores on the EPQ and RSE have been associated with increased risk of developing mood disorders (Duggan *et al.*, 1995; Jones *et al.*, 2005; Kendler *et al.*, 2006; Scott *et al.*, 2000; Solomon *et al.*, 1996). Similarities in the personality characteristics of individuals with DD and individuals with mood disorders may suggest that individuals with DD have an increased vulnerability to developing these disorders compared to their unaffected relatives.

12.2.3.1 Current Mood State Questionnaires

Individuals with DD had non-significantly higher current mean scores on the Beck Depression Inventory (BDI) than their unaffected relatives although the small group sizes are likely to have limited the power to detect a significant difference. Both groups had scores of less than 10 on the BDI, which is in the range reflecting none or minimal depression. Individuals *with* a participating unaffected relative had lower scores on the BDI than DD individuals *without* a participating relative (median BDI score; 5 vs. 13; U=196, n=50,

p=0.083). This suggests individuals' current level of depressive symptoms may have affected the likelihood of them encouraging an unaffected relative to participate in the research.

There were very small group differences in the mean scores on the Altman Self-Rating Scale for Mania (ASRM). DD individuals *with* and *without* a participating unaffected relative did not differ in their ASRM scores (median ASRM score; 2 vs. 2; U=194.4, n=47, p=0.173). This suggests that the ASRM scores of DD group were representative of the whole DD sample. These findings indicate that the presence of current manic symptoms was similar among individuals with DD and their unaffected relatives.

12.2.3.2 Rosenberg Self-Esteem Scale (RSE)

DD individuals had significantly lower score *total* and *negative subscale* scores on the RSE reflecting lower self-esteem (shown in Table 11.6 pg. 237). The group differences did not remain significant after controlling for current depression, although the trend for DD individuals to have lower self-esteem remained. It is likely that due to the small group sizes there was not enough power to detect a significant difference. DD individuals with a participating unaffected relative had similar RSE scores to individuals without a participating relative (mean *total* RSE scores; 26.44 vs. 26.09; t=0.198, df=46, p=0.844) and (mean *negative scale* scores; 14.06 vs. 13.84; t=0.255, df=46, p=0.800). This indicates that the DD group RSE scores were representative of the scores of individuals in the current study.

The total RSE score among the DD group (26.44) is similar to the mean scores found among our large samples of individuals with bipolar disorder (26.81) and major depression (23.67) (Jones *et al.*, 2005). The mean self-esteem score of the control group in this study (34.48) was higher than the score of the current unaffected group (31.50). This suggests that the unaffected group was not particularly biased towards containing individuals with high

self-esteem. Overall these findings indicate that the lower self-esteem scores in the DD group cannot be explained by group biases.

12.2.3.3 Eysenck Personality Questionnaire (EPQ)

Individuals with DD had significantly higher EPQ *neuroticism (N)* scores, a measure of emotional instability, compared to their unaffected relatives (see Table 11.7 pg. 239). This difference remained significant after controlling for current depression. Logistic regression analysis also indicated that EPQ-N was the only questionnaire variable to be significantly predictive of DD vs. unaffected relative group membership. These findings would not remain significant after corrections for multiple testing although the analyses did have limited power due to the small group sizes. DD individuals *with* a participating unaffected relative had similar EPQ-N scores to individuals *without* a participating relative (mean EPQ-N Score; 12.80 vs. 13.53; t=0.370, df=45, p=0.713). This indicates that the EPQ-N scores of the DD group were representative of the scores of DD individuals in the current study.

The mean EPQ-N score of the DD group (12.80) is lower than the mean EPQ-N scores found in our bipolar sample (14.96) and major depression sample (17.49) (figures not reported in the Jones *et al.* (2005) paper). The mean EPQ-N score of the control sample in this study was 4.81. However, individuals in this sample were selected partially on the basis of having low EPQ-N scores and comparisons with this sample would not be meaningful. No normative data could be obtained for the version of the EPQ used in this study. It is therefore difficult to elicit whether the significant differences between the DD and unaffected groups are due to the unaffected group having particularly low EPQ-N scores. However, the EPQ-N scores of the DD group were closer to the mean scores of our bipolar sample than the unaffected relatives group (8.31). Higher neuroticism scores have been previously been found in euthymic individuals with bipolar disorder compared to controls (Solomon *et al.*, 1996). Therefore overall these finding suggests that the group differences are due to elevated

neuroticism scores in the DD group rather than the unaffected relatives group being biased towards individuals with low levels of neuroticism.

Group Differences in Responses to Item 68

Almost twice as many individuals with DD responded yes to item 68 of the EPQ, indicating that they had wished that they were dead at some point in their life compared to their unaffected relatives (60% vs. 33%) (see Table 11.8 pg. 239). This group difference did not reach statistical significance although the small group sizes are likely to have limited the power to detect a significant difference. The proportion of individuals responding yes to this item was similar among individuals *with* and *without* a participating unaffected relative (60% vs. 50%; Fisher's, p= 0.550). This indicates responses to this item were representative of the whole DD sample.

The prevalence of positive responses to item 68 in the DD group (60%) was closer to that in our bipolar sample (71%) and major depression sample (80%) (figures not reported in the Jones *et al.*(2005) paper) than in the unaffected relatives group (33%). The vast majority of individuals in the mood disorders sample had a past history of depression (excluding individuals with bipolar disorder who had only experienced manic episodes). Recurrent thoughts of death, suicidal ideation and suicide attempts are a diagnostic symptom of major depression. This suggests that the group differences are due to individuals with DD having a higher prevalence of past thoughts of wishing they were dead rather than the unaffected relatives group being biased towards individuals with no history of such thoughts. This finding is consistent with previous studies reporting a high prevalence of suicidal ideation and attempts among individuals with DD (Denicoff *et al.*, 1990; Ringpfeil *et al.*, 2001).

12.2.3.4 TEMPS-A Temperament Questionnaire

Individuals with DD had significantly higher scores on the *cyclothymic* subscale of the TEMPS-A temperament questionnaire compared to their unaffected relatives (see Table 11.9 pg. 241). However this group difference disappeared after controlling for current depression. Individuals with DD also had non-significantly higher scores on the depressive dimension and lower scores on the hyperthymic dimension compared to their unaffected relatives. Scores on the irritable and generalised anxious dimensions were similar in both groups. The TEMPS-A questionnaire was included in the current study to investigate whether individuals with DD had a particular vulnerability to any specific type of mood and/or anxiety disorder compared to their unaffected relatives. Although initial comparisons suggested individuals with DD had higher scores on the temperaments measuring mood, in particular the cyclothymic temperament, this difference did not remain significant after controlling for current depression. It is therefore not possible to make any conclusions from the comparisons of TEMPS-A scores in the current study.

12.2.3.5 Kings Schizotypy Questionnaire (KSQ)

Individuals with DD had significantly higher scores on the *total, ideas of reference* and *recurrent illusions 1* scales of the KSQ than their unaffected relatives (see Table 11.10 pg. 242). After controlling for current depression, the differences between the groups on the *total* and *ideas of reference* scales remained significant, although would not after corrections for multiple testing. Individuals *with* a participating unaffected relative had lower KSQ scores than individuals *without* a participating relative (median *total* scores; 10 vs. 13; U=119, n=41, p=0.175), (median *ideas of reference* scores; 1 vs. 2; U=131, n=41, p=0.310) and (median *recurrent illusion* scores; 0 vs. 1; U=97.5, n=41, p=0.040). This suggests the KSQ scores of

the DD group may not be representative of the scores of the whole DD sample. It is also possible that the unaffected relatives group was biased towards individuals with lower KSQ scores. This is supported by the fact that the mean KSQ score of the unaffected relatives in the current study (7.64) is lower than the mean KSQ scores of a previously reported sample of controls without a personal or family history of psychiatric illness (10.18) (Jones *et al.*, 2000). Despite these potential group biases, the findings do indicate individuals with DD had higher *total, ideas of reference* and *recurrent illusions 1* scale scores than their unaffected relatives. However, it is possible that individuals with DD may have related a small number of the KSQ items directly to their DD, for example, item 10 " Do you feel self-conscious in public?" and item 24 " Do people look at you strangely?". These responses may have contributed to the higher *total* score in the DD group. These items form part of the *social anxiety* subscale and there was a trend for individuals with DD to have higher scores on this scale. DD individuals also had significantly higher scores on the *ideas of reference* subscale. A higher proportion of individuals with DD compared to their unaffected relatives responded yes to item 7 in this subscale "Do you quite often get the feeling that other people are taking notice of you in the street or on a bus or in a restaurant?" (54.5% vs. 9.1%, Fisher's p=0.063). In contrast, an equal proportion of individuals within the two groups responded yes to the item 14 in the subscale "Do people often seem to drop hints about you or say things with a double meaning?" (9.1% vs. 9.1%; Fisher's, p=1.00). These findings suggests the higher KSQ scores among the DD group are reflecting feelings of self consciousness around others rather than a vulnerability to functional psychosis.

12.2.4 Low Self-Esteem, Neuroticism and Mood Disorders

The current findings suggest individuals with DD have lower self-esteem and higher levels of neuroticism compared to their unaffected relatives. These personality characteristics have been identified as risk factors for developing mood disorders (Brown *et al.*, 1990;

Duggan *et al.*, 1995; Kendler *et al.*, 2006). Neuroticism in particular has been shown to reflect a genetically determined trait underlying vulnerability to develop major depression (Duggan *et al.*, 1995; Kendler *et al.*, 2006). Associations between self-esteem, neuroticism and depression may also be due to these features being a consequence or symptom of past or current depression. For example, Farmer *et al.*(2002) suggest neuroticism mainly reflects residual symptoms of depression. The group differences in neuroticism and self-esteem remained after controlling for current depression, suggesting this did not account for the group differences. Thirty-nine percent of the DD group had a lifetime diagnosis of major depression. However, the prevalence in the unaffected relatives group is unknown since a proportion of the sample were not interviewed. It is possible that a higher prevalence of past depression in the DD group could contribute to the increased neuroticism and lower self-esteem in this group. Low self-esteem may also be a consequence of the distressing symptom of malodour experienced by individuals with DD in addition to the detrimental impact the disorder has on many aspects of individuals' personal and social lives. These findings suggest that individuals with DD may benefit from psychological therapy since feelings of low self-esteem in particular are likely to have a long-term detrimental impact on many aspects of their lives.

Due to the cross-sectional design of the study, it is not possible to determine whether the increased neuroticism and lowered self-esteem scores in the DD group reflect an increased genetic predisposition to these personality characteristics or whether they are a consequence of past episodes of depression and/or the symptoms of DD. Ideally, longitudinal studies assessing these personality characteristics in the children of individuals with DD before they may or may not develop DD themselves should be carried out. Such studies would greatly improve the understanding of the association between neuroticism, self-esteem, mood disorders and DD.

12.2.5 Summary of Comparisons of Individuals with DD and their Unaffected Relatives

The findings of the current study show an overall trend for an increased prevalence of neuropsychiatric features and psychopathology among individuals with DD compared to their unaffected relatives. Key findings are that a much higher proportion of individuals with DD had received treatment for psychiatric illness and reported that they had wished that they were dead at some point in their life. In addition they had lower self-esteem and higher levels of neuroticism. It is possible that the group differences are due to group biases. In particular, unaffected relatives with a history of psychiatric illness may have been less likely to participate in the study. However, this is difficult to assess. The group differences may also reflect the psychosocial consequences of DD or an increased genetic vulnerability to low self-esteem, neuroticism and psychiatric illness in individuals with DD.

This study has a number of limitations. Firstly the small group sizes mean that power to detect significant difference was limited. Secondly, as mentioned above, it is possible that the unaffected relatives group was biased towards individuals with fewer neuropsychiatric features. Finally the majority of unaffected relatives were not interviewed face to face, which affects the quality of the data obtained. The findings of the study require replication in larger samples. However, obtaining such samples may be difficult.

The following chapter (Chapter 13) is the final chapter. This chapter will summarise the key findings of the investigations, discuss the limitations and implications of these findings, and makes suggestions for further research.

13 FINAL CONCLUSIONS, IMPLICATIONS, LIMITATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This final chapter summarises the main findings and final conclusions of the investigations into the neuropsychiatric phenotype in DD presented in this thesis. This is followed by a discussion of the implications of these findings and the limitations of the study. Finally, suggestions for further research are described.

13.1 Main Findings and Final Conclusions

The presence of neuropsychiatric features among individuals with DD has long been reported and discussed in the literature. Explanations for this association have also been debated, particularly following the discovery that the disorder is caused by mutations in the *ATP2A2* gene (Sakuntabhai *et al.*, 1999b). One important possibility requiring thorough investigation is that mutations in *ATP2A2* have pleiotropic effects in the skin and brain. This suggestion would be supported by firstly, a population-level association between neuropsychiatric features and DD and secondly, genotype-phenotype correlations between mutations in *ATP2A2* and the presence of neuropsychiatric phenotypes. These relationships have not been consistently or robustly found in previous studies of DD. However, these studies have had methodological problems as well as small sample sizes. The limitations of these previous studies led to the three main aims of this thesis. The main findings of the investigations relating to each of these aims are summarised below.

13.1.1 First Aim

To conduct a systematic investigation of the neuropsychiatric characteristics in a sample of 100 unrelated individuals with DD using a battery of standardised neuropsychiatric measures.

This thesis reported the findings of the first systematic investigation into the neuropsychiatric phenotype in DD using a battery of standardised neuropsychiatric measures. The results of the investigation found that the prevalence of mood disorders, including major depression, suicide attempts and suicidal thoughts are significantly higher in individuals with DD than in the general population. This finding is consistent with previous studies reporting an increased prevalence of depression and suicidal ideation in DD (Denicoff *et al.*, 1990; Ringpfeil *et al.*, 2001). The prevalence of bipolar disorder and epilepsy in the sample was also found to be non-significantly higher than the prevalence in the general population suggesting that a subset of individuals/families with DD may have an increased risk of developing these disorders.

Further investigations indicated that psychosocial factors alone could not account for the observed increased prevalence of mood disorders, suicidal thoughts and suicide attempts. Only a small proportion of individuals reported that they felt all the episodes of psychiatric illness they had experienced were due to the symptoms of DD. Furthermore, among 12 out of the 13 individuals who had made a suicide attempt, there was no subjective or objective temporal relationship between their suicide attempts and the symptoms of DD. A further interesting finding was the increased prevalence of psychiatric illness and history of suicidal thoughts and attempts among individuals reporting a positive family history DD, which

potentially provides support for the hypothesis that mutations in *ATP2A2* have pleiotropic effects in the skin and brain.

There was also a high prevalence of soft neurological symptoms in the sample, in particular episodes of loss of consciousness and headaches. However, no general population comparison data for the prevalence of these soft neurological features could be obtained. A large proportion of the sample was found to have no qualifications. There are a number of possible reasons for this finding including the possibility that individuals with DD may be impaired in specific aspects of learning. However, difficulties were encountered in assessing true prevalence of learning difficulties in the sample. Individuals were not found to differ from the general population in terms of their general level of intellectual functioning.

13.1.2 Second Aim

To investigate possible genotype-phenotype correlations between the type and/or locations of pathogenic mutations detected in the ATP2A2 gene and neuropsychiatric features observed in a large sample of unrelated individuals with DD.

The main finding of these investigations was that mutations found among individuals with similar neuropsychiatric phenotypes clustered in certain locations within the SERCA2b protein. A number of these observations were further supported when the findings of the current study were combined with the previous literature. In particular, strong evidence was found to suggest that mutations within the S4-M4 domain of the protein may confer increased susceptibility to more severe psychiatric symptoms and bipolar disorder. Another interesting finding was that three individuals with the same mutation at one of the Ca^{2+} binding sites of the protein all had a diagnosis of a mood disorder. A mutation previously shown to result in a

'mutant' SERCA2b pump with unique functioning was found in two individuals both with severe DD and a personal and family history of neuropsychiatric features.

A further main finding of these investigations was that the prevalence and severity of all the lifetime neuropsychiatric phenotypes measured was higher among individuals with genetically confirmed DD compared to those in whom a pathogenic mutation was not detected. A number of explanations could account for this finding including the possibility that a proportion of the individuals in whom a pathogenic mutation was not detected were phenocopies of DD, i.e. they did not have a pathogenic mutation in *ATP2A2.*

13.1.3 Third Aim

To compare the presence of neuropsychiatric features in a sample of individuals with DD and their first-degree relatives, unaffected by DD.

There was an increased prevalence of neuropsychiatric features and psychopathology among individuals with DD compared to their unaffected relatives. Key findings were that a much greater proportion of individuals with DD had received treatment for psychiatric illness and reported that they had wished that they were dead at some point in their life. In addition, individuals in the DD group had lower self-esteem and higher levels of neuroticism than their unaffected relatives.

13.1.4 Final Conclusions

In conclusion, investigations into the neuropsychiatric phenotype in DD presented in this thesis have found evidence to suggest a potential population-level association between DD and mood disorders, specifically major depression, suicide attempts and suicidal thoughts. The findings of the genotype-phenotype investigations provide strong support for the

hypothesis that mutations in the *ATP2A2* gene have pleiotropic effects in the skin and brain and therefore confer susceptibility to neuropsychiatric features, in particular psychiatric illness, in individuals with DD.

It is highly plausible that mutations in *ATP2A2* could be involved in conferring susceptibility to neuropsychiatric illness since the gene is widely expressed in the brain. The suggestion is also supported by the dual role of the SERCA2b protein in intracellular Ca^{2+} signalling and in the synthesis and post-translational modification of proteins within the ER. It is possible that the skin and brain may have a particular susceptibility to a reduction in SERCA2b activity possibly relating to changes in ER Ca^{2+} concentration and ER functioning.

13.2 Implications

The findings of the investigations presented in this thesis have implications for understanding of the treatment needs of individuals with DD and the identification of genetic factors involved in conferring susceptibility to neuropsychiatric features, in particular psychiatric illness, in individuals *without* DD.

13.2.1 Treatment Implications for Individuals with DD

The high prevalence of mood disorders, suicidal thoughts and suicide attempts observed in the current sample highlights the need for assessment and recognition of psychiatric symptoms in DD. This is particularly important in individuals reporting a positive family history of DD since these individuals were found to have an increased prevalence of psychiatric illness. The lowered self-esteem and higher levels of neuroticism found among individuals with DD compared to their unaffected relatives suggests that individuals with DD may also benefit from psychological therapy. Feelings of low self-esteem in particular are likely to have a long-term detrimental impact on many aspects of individuals' lives.

The current investigations have highlighted the possibility that a proportion of individuals with a clinical diagnosis of DD may be phenocopies of DD. The findings suggest that a lack of family history and having mild clinical features of DD might indicate that an individual is a phenocopy of DD. Further research into this area is needed as the findings of such investigations may have important diagnostic and treatment implications.

13.2.2 Neuropsychiatric Features in Individuals without DD

Support for the suggestion that mutations in *ATP2A2* are involved in conferring susceptibility to neuropsychiatric illness in individuals with DD has implications for the identification of genetic factors involved in conferring susceptibility to these illnesses in individuals without the disorder. Other genes encoding proteins in the same biological system as and/or encoding proteins with a similar function to SERCA2b would be good candidates for involvement in predisposing individuals to developing psychiatric illness, in particular mood disorders.

13.3 Limitations

13.3.1 Lack of Control Group

The main limitation of the current investigations is the lack of a control sample of individuals with another skin disorder of a similar severity to DD. Collecting a control sample was considered when the study was first being designed, however due to time constrictions this was not possible.

13.3.2 Potential Sample Biases

Despite the fact that the majority of individuals in the current study were recruited on the basis of having DD, it is possible that there could be a recruitment bias towards

individuals who had experienced psychiatric symptoms as a consequence of their DD. Conversely, individuals with more severe neuropsychiatric features, including severe psychiatric illness and learning difficulties may have been underrepresented in the sample. Although DD is reported to be equally prevalent among males and females, participants in the current study were predominantly female. It is likely that this female bias will have inflated the prevalence of psychiatric illness in the sample, although this was taken into account when comparisons were made to the reported prevalence of these illnesses in the general population. Finally, it is possible that the sample of unaffected relatives was biased towards individuals with fewer neuropsychiatric features.

13.3.3 Modest Sample Size

A further limitation of these investigations was the modest sample size, which limited the power to detect significant relationships especially when subgroup analysis was carried out. Since DD is rare and individuals with the disorder live all across the UK, it was not possible to collect a larger sample in the given timeframe. As a result, a greater emphasis was placed on looking for trends in the data. A number of statistical tests were carried out however, correcting for multiple testing using the Bonferroni method was felt to be too conservative given the nature of this exploratory study.

13.4 Future Research

Further research into the neuropsychiatric phenotype in DD is required in larger samples of individuals. Because DD is so rare, it is likely that collaborative research will be needed to obtain such samples. The predominance of females in the current study highlights the need for future studies to make an additional effort to recruit male participants. This would enable potential gender differences in the neuropsychiatric phenotype in DD to be investigated.

In order to confirm the suggestion that there is a population-level association between DD and psychiatric illness, a control sample of individuals with another skin disorder of a similar severity to DD needs to be collected. This sample will need to be assessed using a similar battery of standardised neuropsychiatric measures including a psychiatric clinical interview.

Further research is needed into the apparently high prevalence of soft neurological symptoms in the current sample. This needs to be investigated using specific neurological tests including audiograms and neuroimaging, which may confirm the presence of neurological abnormalities in individuals with DD. Further studies are also required into the true prevalence of specific learning difficulties among individuals with DD using standardised neuropsychological measures, such as memory and attention tests. These investigations need to be carried out in a sample of individuals with DD and a suitable age-matched control group, which would ideally be a sample of unaffected relatives.

Evidence that the variable neuropsychiatric phenotypes observed among individuals with DD may be accounted for by certain mutations having specific effects on the functioning of the SERCA2b protein highlights the need for further site-directed mutagenesis functional studies in DD. These studies would enable further genotype-phenotype investigations to be carried out into the association between the functional effects of certain *ATP2A2* mutations and the presence of severe neuropsychiatric features in individuals with DD.

Certain regions of the *ATP2A2* gene were not screened for mutations in the current study including the promoter. This may in part account for the mutation detection rate of 66%. Future research should involve the screening of these regions of the *ATP2A2* gene for pathogenic mutations.

13.5 Summary

This thesis has presented the findings of the first systematic investigation into the neuropsychiatric phenotype in DD. Evidence has been found to support the suggestion that mutations in *ATP2A2,* in addition to causing DD, confer susceptibility to neuropsychiatric features, in particular psychiatric illness in individuals with DD. These findings have implications for management and treatment of individuals with DD as well as the identification of genetic factors involved in conferring susceptibility to these features in individuals without DD. Further research in larger samples using specific neurological and neuropsychological measures is required. There is also a need for more functional studies to be carried out in DD. The findings of such studies are likely to have important clinical and research implications.

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APPENDICES

A Appendices for Chapter 3

A.i Study Invitation Letters, Website, Information Sheet & Consent Form

DERMATOLOGIST INVITATION LETTER

BRIEF INFORMATION SHEET ABOUT
RESEARCH INTO DARIER'S DISEASE

I am writing to ask whether you may be willing to participate in research being conducted by members of the Department of Psychological Medicine at Cardiff University and the Department of Psychiatry at the University of Birmingham. Their aim is to investigate clinical features that may be associated with Darier's Disease. We hope that their study will improve understanding of Darier's Disease and related conditions.

Taking part in the study will involve an interview, a series of short memory and problem-solving tasks, completing some questionnaires and, in some cases, giving a small blood sample from your arm or a saliva sample. In total, this should take approximately 2 and a half hours. A researcher would be more then willing to visit you in your own home. If you would like to receive further information about the study from a member of the research team, I would be extremely grateful if you would complete the enclosed form and return it in the enclosed stamped envelope. If you do decide to participate, I would like to assure you that all the information you provide will be strictly confidential.

Also, you do not have to take part in the study. If you decline, it will not alter the care you receive. If you would like to contact the team directly for further details about the research their telephone number is XXX XXXX

Yours sincerely

Dr
Consultant Dermatologist

DARIER'S SUPPORT GROUP INFORMATION LETTER

DARIER'S DISEASE RESEARCH

I am writing to inform you about research that is currently being conducted by members of the Department of Psychological Medicine at the University of Wales College of Medicine and University of Birmingham. Their aim is to investigate clinical features that may be associated with Darier's Disease and hope their study will improve understanding of the disease itself and related conditions.

Taking part will involve an interview which asks participants about their Darier's Disease and the impact it has had on their everyday lives, a short series of memory and problem solving tasks, completing some questionnaires and in some cases giving a blood sample. If you would be interested in receiving more information about the study from a member of the research team, please fill in the enclosed form and return it in the enclosed stamped envelope. If you do wish to participate, a researcher will be more than willing to visit you in your home at a time convenient for you including evenings and weekends.

If you would like to contact the team directly, please telephone Katherine Gordon-Smith on 0121 678 2352 or email k.m.gordonsmith@bham.ac.uk

Thank you for your time

Jenny Davies
Darier's Disease Support Group

STUDY WEBSITE

Research into Darier's Disease

Department of Psychological Medicine, Cardiff University
Department of Psychiatry, University of Birmingham

What is Darier's Disease?
Darier's disease is a rare inherited skin condition affecting both men and women. The first signs of the condition usually appear somewhere between the ages of 6 and 20. The disease is characterised by a rash which is often present on the chest, neck or upper back at the start but warty bumps may occur on any part of the body. The severity of the condition varies a lot and is unpredictable. The finger nails are usually affected. They tend to be rather fragile and split easily and there may be very obvious long red or white lines running the length of the nails. Nail changes and/or flat "warts" on the backs of the hands are often present in childhood, well before there are any other skin changes. (Adapted from British Association of Dermatologists patient information on Darier's disease)

What is the Research about?
We have funding for 3 years (July 2003-2006) from the Wellcome Trust to conduct research to learn more about features which may be associated with Darier's disease and learn more about the impact that it has on individuals everyday lives. We hope this research will improve understanding of the disease itself and related conditions.

We are hoping to interview a sample of at least 100 individuals who suffer from Darier's disease. We would also be interested in seeing other available family members, whether they suffer from Darier's disease or not.

Taking part will involve:
An interview asking individuals about their Darier's disease and the impact it has on their everyday lives
A short series of memory and problem solving tasks
Completing some questionnaires
Giving a small blood sample

If you do wish to participate a researcher will be more than willing to visit you in your home at a time convenient to you.

If you would like to receive more information about the study from a member of the research team, please contact:
Katherine Gordon-Smith
University of Birmingham, Department of Psychiatry, Queen Elizabeth Psychiatric Hospital, Mindelsohn Way, Edgbaston, Birmingham, B15 2QZ, United Kingdom.
Tel: +44 (0)121 678 2352

E-mail: k.m.gordonsmith@bham.ac.uk

PARTICIPANT INFORMATION SHEET

INFORMATION ABOUT RESEARCH INTO DARIER'S DISEASE

Version 2- 21.03.03

INTRODUCTION

I am a member of a team of researchers who work in the Department of Psychological Medicine at Cardiff University and the Department of Psychiatry at the University of Birmingham. We are conducting research (funded by the Wellcome Trust) to learn more about features that may be associated with Darier's Disease.

WHAT IS THE RESEARCH ABOUT?

It is known that sometimes disorders affecting the nervous system (such as epilepsy or depression) occur in individuals with Darier's Disease. However, such disorders are common in the general population and it is not known whether this is just a coincidence or whether there is any relationship to the Darier's Disease itself. The idea of this study is to compare individuals with Darier's Disease with other individuals who do not have the illness in order to discover whether there are any problems that are specifically related to Darier's Disease and also to try to find out what influences their occurrence. This research will help us understand more about Darier's Disease itself and, importantly, the treatment needs of people suffering with the illness. As a sufferer of Darier's Disease, we would be extremely grateful if you would be kind enough to help us with this study.

WHAT DOES TAKING PART INVOLVE?

We are hoping to recruit a sample of at least 100 individuals who suffer from Darier's Disease. We would also be very interested in seeing other available family members, whether they suffer from Darier's Disease or not. Participation involves:
an interview (lasting around 1½ hours) asking you about your Darier's Disease and any medical, neurological and psychiatric conditions you have experienced
a series of short tasks designed to assess cognitive abilities e.g. memory, problem solving skills and attention (¾ hour) completing a set of questionnaires (¼ hour) giving a small blood sample from your arm (approximately 10mls)

If you agree to take part, a researcher will arrange a suitable time to visit you in your home or another place convenient for you. We will only need to see you the once but may ask if you would like to take part in future research. We will keep you informed of any interesting results and of future studies into Darier's Disease.

With your permission, we would like to look at your medical records in strict confidence.

WHAT ARE THE BENEFITS OF TAKING PART ? ARE THERE ANY RISKS?

By taking part in the study you will not gain any direct benefit. However, your help will be of great value in allowing us to learn more about Darier's Disease and this is likely to help improve treatments.
As this study does not include any treatment changes or invasive techniques, there are no real risks of taking part. You may experience mild bruising on you arm after giving a blood sample.
We may check with the doctors involved in your care to ensure it would be appropriate for you to take part in the study.

DECLINING AND WITHDRAWING FROM THE STUDY

Taking part in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason.

A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

CONFIDENTIALITY

All interviews and results will be strictly confidential.

Your blood cells may be kept growing in the laboratory in order to provide samples for future use in this research, by us and by other researchers working with us. The results of your blood test are for research purposes only and will not be available to anybody on an individual basis.

All information given is kept in an anonymous format.

If you have any further questions about this research, we would be delighted to answer them in person or over the telephone. Our address appears above, our telephone number is 0121 678 2352, and our e-mail address is k.m.gordonsmith@bham.ac.uk

Katherine Gordon-Smith - Research Psychologist
Professor Nick Craddock - Head of Department *Dr Lisa Jones*- Research Psychologist

PARTICIPANT CONSENT FORM

AGREEMENT TO TAKE PART IN THE STUDY OF DARIER'S DISEASE

I have read the attached information sheet (version 2, dated 21.03.03) on the above project and have been given a copy to keep. I have had the opportunity to ask questions about the project and understand why the research is being done.

I agree to give a sample of blood for research in the above project.

I understand that participation in this project is voluntary and that I am free to withdraw from the study without giving a reason and without my medical treatment being affected.

I give permission for my medical records to be looked at in strict confidence by responsible people from the research group.

I understand that the tests done as part of this research are not clinically diagnostic and I will not be informed of any specific results.

I understand that I will not benefit personally from taking part in this research.

I understand that the information and blood sample I have donated for this study will be held in a confidential and coded form by the research team and may be made available to researchers at other centres who are carrying out similar work.

I agree that my general practitioner can be told that I am taking part in the research.

I agree that I may be contacted again, in connection with the research, in the future.

I know how to contact the research team if I need to.

NAME _____ SIGNED _____ DATE _____

WITNESSED _____ SIGNED _____ DATE _____

I also agree that other members of my family can be asked to take part in the study.

SIGNED _____ DATE _____

THANK YOU FOR PARTICIPATING IN OUR RESEARCH
YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP

A.ii Clinical Assessment of Darier's Disease

Contact details of dermatologist

_____

Age of onset _____ **Site of onset**_____

DARIER'S SEVERITY _____

Mild: keratotic papules scattered sparsely over the trunk or flexures or disease limited to one or two areas (e.g. hands)
Moderate: more extensive papular lesions or localized verruous plaques
Severe: coalescent verrucous plaques involving most of the trunk or grossly hypertrophic flexured disease

Disease freq/pattern _____
Current status _____
Other Physical symptoms _____

TREATMENT FOR DARIER'S

	Ever used [Y/N]	Helpful [Y/N]	Currently using [Y/N]	Current Dose	Past dose	Side Effects 0 – no, 1-min prob 2 – major problem 3- stopped due to (note side effects)	Years used
Acitretin / Neotigason							
Roaccutane / Isotretinoin							

	Ever used [Y/N]	Helpful [Y/N]	Currently using [Y/N]	Name if known
Emollients				
Sunscreens				
Antihistamines				
Topical Corticosteroids				
Antiseptics				
Topical retinoids				
Topical antibiotics				
Oral antibiotics				
Topical antiviral				
Surgery				
X ray				
Other				

DARIER'S CLINICAL FEATURES

	[Y/N/DK/ N/A]	SITES INVOLVED [PAST AND PRESENT]	[Y/N/DK/ N/A] Past	Cur
Precipitating factors		Scalp		
Stress		Hair loss		
Heat or sweating		Face		
Sun		Neck		
Friction		Chest		
Cold		Upper back		
Pregnancy		Abdomen		
Menstrual cycle		Legs		
Any drug- specify		Low back		
Other		Buttock		
Dairy		Arm		
Clinical Features				
Fluctuation in severity		Flexures		
Static		Groin		
Improving with age		Perineum		
Worse with age		Genitalia		
Sometimes the skin looks normal		Axilla		
		Umbilicus		
Symptoms		Behind Ear		
Itching		Inside Ear		
Pain		Beneath Breasts		
Papules				
Blisters		Hands		
Boils or cysts		Acrokeratosis on back of hand		
Hypo- pigmentation		Finger nail lines (red/white)		
Hyper- pigmentation		Finger nail notches		
Malodour		Palmar Pits		
2ndy infection		Bumps on palms		
Viral				
Bacterial				

LIFETIME RELATED EFFECTS OF DARIER'S DISEASE

Time off work due to Darier's
None __ Days __ Weeks __ Months __ > 1 year __ Stopped work due to __
N/A __

Number of Admissions to hospital due to Darier's ___
Lifetime subjective effect of Darier's on [none/mild/mod/severe/N/A] [0/1//2/3/8]

School	__	Social Activities	__
Work/Career	__	Relationships	__

A.iii Dermatology Life Quality Index

The aim of this questionnaire is to measure how much your skin problem has affected your life **OVER THE LAST WEEK.** Please tick one answer for each question

1.	Over the last week, how **itchy, sore, painful** or **stinging** has your skin been?	Very Much A lot A little Not at all	__ __ __ __	
2.	Over the last week, how **embarrassed** or **self conscious** have you been because of your skin ?	Very much A lot A little Not at all	__ __ __ __	
3.	Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
4.	Over the last week, how much has your skin influenced the **clothes** you wear?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
5	Over the last week, how much has your skin affected any **social** or **leisure** activities ?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
6.	Over the last week, how much has your skin made it difficult for you to do any **sport ?**	Very much A lot A little Not at all	__ __ __ __	Not relevant __
7.	Over the last week, has your skin prevented you from **working** or **studying** ?	Yes No	__ __	Not relevant __
	If "No", over the last week , how much has your skin been a problem at **work** or **studying ?**	A lot A little Not at all	__ __ __	
8.	Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives** ?	Very Much A lot A little Not at all	__ __ __ __	Not relevant __
9.	Over the last week, has your skin caused any **sexual difficulties ?**	Very Much A lot A little Not at all	__ __ __ __	Not relevant __
10.	Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?	Much A lot A little Not at all	__ __ __ __	Not relevant __

The aim of this questionnaire is to measure how much your skin problem has affected your life **IN THE WORST WEEK EVER.** Please tick one answer for each question

1.	In the worst week, how **itchy, sore, painful** or **stinging** was your skin?	Very Much A lot A little Not at all	__ __ __ __	
2.	In the worst week, how **embarrassed** or **self conscious** have you been because of your skin ?	Very much A lot A little Not at all	__ __ __ __	
3.	In the worst week, how much did your skin interfere with you going **shopping** or looking after your **home** or **garden**?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
4.	In the worst week, how much did your skin influenced the **clothes** you wear?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
5	In the worst week, how much did your skin affect any **social** or **leisure** activities ?	Very much A lot A little Not at all	__ __ __ __	Not relevant __
6.	In the worst week, how much did your skin made it difficult for you to do any **sport ?**	Very much A lot A little Not at all	__ __ __ __	Not relevant __
7.	In the worst week, did your skin prevent you from **working** or **studying** ?	Yes No	__ __	Not relevant __
	If "No", in the worst week , how much was your skin a problem at **work** or **studying ?**	A lot A little Not at all	__ __ __	
8.	In the worst week, how much did your skin create problems with your **partner** or any of your **close friends** or **relatives** ?	Very Much A lot A little Not at all	__ __ __ __	Not relevant __
9.	In the worst week, did your skin cause any **sexual difficulties ?**	Very Much A lot A little Not at all	__ __ __ __	Not relevant __
10.	In the worst week, how much of a problem was the **treatment** for your skin , for example by making your home messy, or by taking up time?	Much A lot A little Not at all	__ __ __ __	Not relevant _

A.iv Psychiatric History Screening Questions

1.Have you ever experienced mental health problems in your life? – even if very mild

	Y/N	*Sought help from GP/other health professional*	*Received counselling*	*Prescribed medication*	*Seen psychiatrist + contact details, Hospital admission*	*Current symptoms*
Anxious or panic attacks						
Feeling very low in spirits, depression or low mood						
Feeling much too high in spirits or elated or very irritable without reason, manic depression or bipolar disorder						
Experiencing things that are difficult to explain or understand like hearing voices or seeing things, psychosis or schizophrenia						
OCD- checking things you know you have done, keeping things in a special order						
Eating disorders						
Other						

SECTION 11& 12 –ALCOHOL AND SUBSTANCE

11.001 Screen- ever used alcohol at all [1 – never used, 2 – once or twice in lifetime, 3 – used more than twice] __

Other probes- about how often do/did you take a drink, would it be difficult to do without, have you ever felt you had problems with alcohol

11.002 How often did you take a drink in heaviest year period [0 – not at all, 1 – less and once a month, 2 – once a month,4– 2-3days a month, 4 – 1-2 days a week, 5 – approx. daily any time within one year, 6 - approx. daily repeatedly within one year, 7 – approx, daily for at least one month, 8 - NK, 9 – NA __
11.003 Estimated usual daily amounts [enter number of standard drinks] __

12.001 Screen- [1- never used drugs, 2 – once or twice in a lifetime, 3 – drugs used more than twice] __

12.006-14 Frequency of drug use in year period [0=no use, 1=up to 5 times in period, 2=6-12 times or less than once a month,3=approx. monthly, 4=approx. weekly, 5=approx. daily any time within one year period, 6= approx. daily repeatedly within one year, 7=approx. daily for at least one month

Opioids __ Cannabinoids __ Sedatives, Hypnotics, Anxiolytics __ Cocaine __ Stimulants __ Hallucinogens __PCP __ Volatile Substances __ Other and unknown __

A.v Psychiatric Consensus Rating Form

Study ID _____ Initials _____ DOB____

Rater ____ **Date** _____

Main Diagnosis

DSM-IV	ICD-	RDC

Other diagnosis

DSM-IV	ICD-	RDC

DSM-IV	ICD-	RDC

DSM-IV	ICD-	RDC

DSM-IV	ICD-	RDC

BADDS Dimension Scores:

M ___ ___ ___
D ___ ___ ___
P ___ ___ ___
I ___ ___ ___

GAS scores

Lifetime worst ever episode ___
Lifetime worst in depressive episode ___
Lifetime worst in manic episode ___
Past week ___

Section 2 features ___ **Mood Congruence** ___ **Near Section 2** ___

Predominant manic affect ___ **Dysphoric mania** ___

No. Episodes: Mania ___ Depression ___ Anxiety ___

Longest Duration: Mania ___ Depression ___ Anxiety ___

Age of onset: any psychiatric disorder

Symptom ___ Impairment ___ Contact ___ Admission ___

Age of First

First Depression ____ First Mania ____ First Psychosis____
First anxiety/panic/phobia ____ First obsessional illness ____
First Eating disorder ____ First alcohol or substance ____

Suicidal ideation ___ **Rapid Cycling** ___ **Puerperal** ____

A vi The Bipolar Affective Disorder Dimensional Scale, version 3.0 (BADDS 3.0).

General information

The Bipolar Affective Disorder Dimension Scale (BADDS) has been developed in order to address some of the disadvantages of a purely categorical approach to diagnostic classification of Bipolar Spectrum Disorders.

BADDS is a dimensional rating scheme that retains and builds upon current categorical classifications. It is intended for use in clinical samples from populations over-represented by Bipolar Spectrum illness. It was not developed for use in general population samples.

BADDS has been under development since 1996 and has now been used by a variety of researchers within our group on more than 1100 cases. It has proved to be user friendly and has excellent reliability, even on sets of diagnostically challenging cases.

BADDS comprises 4 dimensions: M: Mania; D: Depression; P: Psychosis; I: Incongruence. Each dimension is rated using integer scores on a 0 – 100 scale. Ratings are made after review of all available clinical data on a subject (eg. case records, semi-structured psychiatric interview and information from an informant) and can be performed as a simple addition to the conventional consensus lifetime psychiatric diagnostic procedures already in use by many research groups. Each rating reflects a mixture of severity and frequency of clinical features. Guidelines are provided that define anchor points in the rating scales and specify how ratings should be made.

Researchers and clinicians are welcome to use BADDS providing that an appropriate literature citation acknowledging its use is included in any published work that arises.

We welcome comments and suggestions about BADDS and will consider these when making revisions to the scale and rating guidelines. Please direct any correspondence to:

Professor Nick Craddock
Neuropsychiatric Genetics Unit
Department of Psychological Medicine
University of Wales College of Medicine
Heath Park
Cardiff CF14 4XN
United Kingdom
Email: craddockn@cardiff.ac.uk

BADDS: General rating guidelines

1) Do not rate a dimension if there is insufficient information - just leave the dimension blank.

2) Use all available information to make the best judgement for each rating.

3) It is expected that when used for research BADDS will be used within the accepted framework of the lifetime best-estimate consensus diagnostic procedure.

4) All ratings should be made using integers in the range 0 - 100.

5) Ratings for M and D are a mixture of severity and frequency. Generally the severity of the most severe episode identifies a range in which the rating will be made and the frequency determines the score assigned within the range. In assigning a rating, start at the lowest score in the range and then add points according to any relevant psychopathology over and above that of the most severe episode according to the following guidelines:
a) In general each additional episode *of that level of severity* will add a score of 2 in a 20 point range and 1 in a 10 point range.
b) Scores in the identified severity range can and should be modified according to severity and duration of total episodes – but with a substantial down-weighting for episodes of lower severity.
c) For episodes that are one level of severity lower than the rating range, add 0.25 points for each episode of lower severity for a score in a 10 point range and 0.5 points for each episode of lower severity for a score in a 20 point range.
d) For episodes that are more than one level of severity lower than the rating range the total adjustment should not normally exceed 1 or 2 points.

6) For the P and I dimensions anchor points are given in these guidelines. Judgment is used to assign scores between anchor points.

7) Under very exceptional circumstances a score can be rated outside the severity range. However, this should always be agreed by at least two raters and the rating should lie in the interval 0 - 100. Such a rating should be indicated by an asterisk (*) following the rating for that dimension. An example of the applicability of this rule is the rating up of an episode in which the balance of evidence clearly suggests a severe illness that is not adequately supported by the documented evidence *because of poor documentation.* Another example would be the rating down of an episode if the balance of evidence strongly suggests that the formal evidence clearly over-represents the clinical significance of the episode.

1) Mania dimension (M)

The rating reflects severity and frequency.
Use ICD10 to define symptom and duration criteria for hypomanic and manic syndromes.
Sub-hypomanic features in the ranges 1 - 19 and 20 - 39 should be rated using judgement according to the balance of number and duration of symptoms.
No impairment criterion is used for hypomania.
The impairment criteria for mania are one or more of:

Disrupts work or social life more or less completely
Markedly inappropriate overspending that is reckless within the context of the
Subject's financial position
Fights
Lost job
Police involvement
Family split up
Received specific treatment (including dose increase of mood stabilizer) *for acute mania*
Psychotic features

Incapacitating mania refers to a severe manic episode that includes the presence of one or more of the following features: incoherence, disorientation, loss of contact with reality (which includes psychotic features), frenzied or bizarre psychomotor activity.
Mixed episodes are rated on the M dimension. If *all* manic episodes are mixed, add "m" to the rating (eg. 65m).

Key points and ranges on the M dimension

0	No manic features.
1 - 19	Mild sub-hypomanic features. Elation/irritability and less than 3 symptoms.
20 - 39	Sub-hypomanic features. Elation/irritability and 3+ symptoms for at least 1 day.
40 - 59	Hypomanic features. At least one hypomanic episode.
60 - 79	Manic features. At least one manic episode.
80 - 100	Severe manic features. At least one episode of incapacitating mania.

2) Depression (D)

Rating reflects severity and duration.
Use ICD10 to define depressive syndromes. This includes 10 symptoms of depression that count for the purposes of diagnosis:

A Depressed mood
Loss of interest/pleasure
Loss of energy

B Suicidal ideation
Pathological guilt
Loss of confidence/self esteem
Loss of concentration
Slowed activity
Change of appetite or weight
Change in sleep pattern

Depression severity: Mild - 4+ symptoms (2+ from A); moderate - 6+ symptoms (2+ from A); severe - 8+ symptoms (3 from A). Refer to ICD10 for full definition of syndromes and symptoms.
Duration criterion for Major Depressive Episode is 2 + weeks. If 1- 2 weeks, classify as Minor Depression.
Rate depression as severe if (a) ICD10 criteria fulfilled, or (b) criteria for major depression are fulfilled and there has been a serious suicide attempt, ECT treatment or hospital admission for depression.
Minor depression refers to at least 1 week of low mood accompanied by 2 or more depression items or to brief episodes that would otherwise meet criteria for Major Depression.
Incapacitating depression refers to severe major depression that includes presence of one or more of the following features: stupor; mutism; loss of contact with reality (including psychotic features).
If psychotic features are present, a depressive episode can be rated as incapacitating if the minimum criteria for major depression are satisfied (ie. 4 items).

Key points and ranges on D dimension

0	No features of depression during lifetime. .
1 – 19	Sub-Minor depression.
20 - 39	Minor depression.
40 - 49	Mild major depression.
50 - 59	Moderate major depression.
60 - 79	Severe depression.
80 - 100	Incapacitating depression

3) Psychotic features (P)

Psychotic features refers to delusions, hallucinations, positive formal thought disorder, catatonia or grossly disorganized behaviour (but see exclusions below).
Ratings on this dimension exclude stupor or excitement during an affective episode or positive formal thought disorder during mania.
Lifetime occurrence of psychotic features is rated.
Near psychotic schizotypal features refers to the following DSMIV schizotypal items: ideas of reference; odd beliefs or magical thinking that influences behaviour and is inconsistent with sub-cultural norms; unusual perceptual experiences including bodily illusions; odd thinking and speech; suspiciousness or paranoid ideation; behaviour or appearance that is odd eccentric or peculiar. Depersonalization and derealization are not classified as near psychotic features.
The period of illness considered refers to all affective and non-affective periods of psychopathology.
Rating should take account of both number and duration of episodes with and without psychotic features. If in doubt, "rate up" the psychotic features. Examples:
If there have been two 1 week long affective psychotic episodes and a 1 year non-psychotic depressive episode, rate 60 (ie. approx. 2/3 of illness *episodes*).
If there have been nine 1 month non-psychotic affective episodes, one 1 month psychotic affective episode and 4 years of chronic hallucinations outside affective episodes, rate 80 (ie. approx. 80% of illness *duration*).
The Uncertain category (P = 1) is used for situations in which insufficient information is available to determine if sign or symptom meets criteria for near psychotic feature.

Key points and ranges on P dimension

0 Absent.
1 Uncertain.
2 - 9 Near psychotic features: occasional at low end of range, frequent at high end of range. Occurrence of true psychotic symptoms should not be rated in this range.
10 - 20 Brief clear-cut psychotic symptom that are not a prominent feature of illness.
10 – Single.
20 – Multiple.
21 – 100 Psychotic symptoms that are a prominent feature in one of more episodes of illness.
25 - present for 25% of illness.
50 - present for 50% of illness.
75 - present for 75% of illness.
100 - prominent psychotic features present throughout illness.

4) Mood incongruence (I)

DSMIV definitions of congruence and incongruence are used.
Rate incongruence of lifetime occurrence of psychotic features.
For convenience, the set of psychotic symptoms recognized as having special weight in the diagnosis of schizophrenia and schizoaffective disorder (thought echo, insertion, withdrawal or broadcasting; passivity experiences; hallucinatory voices giving running commentary, discussing subject in third person or originating in some part of the body; bizarre delusions; catatonia) are denoted in the guidelines as the "S set".
If Psychosis Features dimension, P < 10, leave I blank.

Key points on I dimension

0 -40 Psychotic symptoms occur only during affective episodes and do not include any of the S set.
Rating 0 – virtually completely mood congruent.
Rating 20 – approximate balance between mood congruent and incongruent.
Rating 40- virtually completely mood incongruent
43 Psychotic symptoms occur only during affective episodes and include one or more of the S set which have not definitely been present for 2 weeks.
47 Psychotic symptoms occur only during affective episodes and include one or more of the S set which have definitely been present for 2 weeks.
50 - 59 Psychotic symptoms probably present for at least 2 weeks either side of an affective episode.
Rating 50 – on at least one occasion.
Ratings of 51-59 used to reflect recurrence and/or certainty.
60 - 100 Psychotic symptoms definitely present for at least 2 weeks either side of an affective episode.
Rating 60 – on at least one occasion.
Rating 80- on many occasions.
Rating 100 – Psychotic symptoms predominate illness and occur chronically outside (or in absence of) affective episodes.

A.vii Neurological Screen

Ask if experienced symptoms from the list- if respond yes complete table

Head injury _____
Headaches _____
Fits, faints, funny turns, epilepsy or seizure, lost consciousness/ seem to loose contact with reality/Strange feelings in behaviour/perception/ feelings

_____
_____
_____

Memory difficulties or diagnosis of "dementia"_____
Weaknesses of the limbs _____
Loss of sensation in the limbs _____
Tremors or shaking _____

Difficulty walking _____
Giddiness or difficulty with balance_____

Strokes, brain haemorrhages, (TIAs) _____

Brain tumour or cancer affecting brain or NS _____

Infections of the brain or NS _____

Problems with vision _____
Problems with hearing _____

Developmental delay or development problem relating to the brain or nervous system
_____

Any other symptoms or treatment related to problems with the brain or nervous system
___ _____

Treatments or investigations
Ever been seen by a neurologist? **[0/1]** __
Ever had neurological procedures such as Brain scans (CT, MRI), brain wave recordings (EEG), lumbar punctures? **[0/1]**
Details

_____
_____
_____

Neurological Problem	Brief details	Dates-	Seen GP	Referred to specialist	Treatment	Impairment- mild/moderate/ severe

A.viii Adult Dyslexia Checklist

Please tick **Yes** or **No** to each question. If in doubt, tick the answer that you feel is true most often

	Yes	No
Do you find difficulty telling left from right?		
Is map reading or finding your way to a strange place confusing?		
Do you dislike reading aloud?		
Do you take longer than you should to read a page of a book?		
Do you find it difficult to remember the sense [gist/general idea] of what you have read?		
Do you dislike reading long books?		
Is your spelling poor?		
Is your writing difficult to read?		
Do you get confused if you have to speak in public?		
Do you find it difficult to take messages on the telephone and pass them on correctly?		
When you say a long word, do you sometimes find it difficult to get all the sounds in the right order?		
Do you find it difficult to do sums in your head without using your fingers or paper?		
When using the telephone, do you tend to get the numbers mixed up when you dial?		
Do you find it difficult to say the months of the year forwards quickly and without mistakes?		
Do you find it difficult to say the months of the year backwards?		
Do you mix up dates and times and miss appointments?		
When writing cheques do you frequently find yourself making mistakes?		
Do you find forms difficult and confusing?		
Do you mix up bus numbers like 95 and 59?		
Did you find it hard to learn your multiplication tables at school?		

B Appendices for Chapter 4

Table B-1 DLQI Last Week and Worst Week Median Domain Scores as a Percentage of Total Domain Scores

		DLQI Last Week		DLQI Worst Week	
Domain	***Score out of***	**Median**	**% of total domain score**	**Median**	**% of total domain score**
Symptoms and feelings	6	2	33	5	83
Daily Activities	6	1	17	4	67
Leisure	6	0	0	3	50
Work and School	3	0	0	1	33
Personal relationships	6	0	0	2	33
Treatment	3	0	0	1	33

Table B-2 Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Attempts

	No DSM-IV	**Any DSM-IV**	**Unknown DSM-IV**	**Total**
No suicidal ideation or suicide attempts	37 (100%)	20 (36%)	6 (75%)	63
Suicidal Thoughts	0 (0%)	17 (31%)	1 (12.5%)	18
Suicide Attempts	0 (0%)	12 (22%)	1 (12.5%)	13
Unknown	0 (0%)	6 (11%)	0 (0%)	6
Total	37	55	8	100

Table B-3 Age of Onset of Psychiatric Illness –Descriptives

	N	**Median**	**Range**	**Interquartile Range**
Age of onset of any psychiatric illness	39	25.00	14-58	15
Age of onset of depressive disorder	35	25.00	14-58	14
Age of onset of bipolar disorder	4	23.00	15-52	32

Table B-4 Number of Episodes of Depression (D) and Mania (M) among Individuals with a Lifetime DSM-IV Mood Disorder-Descriptives

		N	Median	Range	Inter-quartile Range
Major Depressive Disorder (N=30)	D	30	4.00	1-20	4
	M*	2	1.50	1-2	-
Dysthymic Disorder (N=2)	D*	2	1	1-1	-
	M	0	-	-	-
Depression NOS (N=13)	D*	9	2.00	1-130	10
	M	0	-	-	-
Bipolar Disorder (N=4)	D	3	10.00	1-50	-
	M	4	6.00	1-20	14
Cyclothymia (N=1)	D*	**	-	-	-
	M*	**	-	-	-

* Episodes did not reach full DSM-IV criteria an episode hypomania/mania (M) or major depression (D), ** number of episodes could not be determined.

Table B-5 Longest Duration of Episodes of Depression (D) and Mania (M) in Weeks among Individuals with a Lifetime DSM-IV Mood Disorder -Descriptives

		N	Median	Range	Inter-quartile Range
Major Depressive Disorder (N=30)	D	29	26.00	2-260	42
	M*	2	2.50	2-3	-
Dysthymic Disorder (N=2)	D*	2	546.00	312-780	-
Depression NOS (N=13)	D*	9	3.00	0.4-130	18
Bipolar I Disorder (N=4)	D	2	13.50	12-15	-
	M	4	5.00	2-50	37

*Episodes did not reach full DSM-IV criteria an episode hypomania/mania (M) or major depression (D).

Table B-6 IQ Scores and Gender

	Male	Female	t*	p
Mean	100.62	100.27	0.111	0.912
Std. Deviation	14.873	12.441		
95% CI	94.61-106.62	97.03-103.51		
Range	70-125	67-125		
N	26	59		

*T test.

Table B-7 First-degree Relatives with a History of Psychiatric Illness and DD-Frequencies

First-degree Relatives with Psychiatric History and DD		First-degree Relatives with Psychiatric History Without DD		First-degree Relatives with Psychiatric History Unsure DD	
N	%	N	%	N	%
34	53.1	23	35.9	7	10.9

Figures are the numbers and percentages of first-degree relatives.

Table B-8 First-degree Relatives with a History of Epilepsy and DD - Frequencies

First-degree Relatives Epilepsy and DD		First -degree Relatives with Epilepsy without DD		First-degree Relatives with Epilepsy unsure DD	
N	%	N	%	N	%
6	75	1	12.5	1	12.5

Figures are the numbers of first-degree relatives.

Table B-9 Relationship between Lifetime Neuropsychiatric Features, IQ Scores and Family History of Psychiatric Illness

	Investigations for Blackouts, Loss of Consciousness or Fainting Episodes [No=81, Yes=13]			Lifetime Diagnosis of Epilepsy [No=97, Yes=3]			Family History of Psychiatric Illness [No=52, Yes=43]			IQ Score Below Average [No=68, Yes=17]			Learning Difficulties [No=79, Yes=21]		
	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p	χ^2	df	p
Lifetime DSM-IV Diagnosis [No= 37, Yes= 55]	**4.411**	**1**	**0.036**	**	1	0.159	2.051	1	0.152	0.002	1	0.960	0.743	1	0.389
Learning Difficulties	**	1	0.722	**	1	0.511	1.077	1	0.299	**	**1**	**0.003**			
IQ Score Below Average	**	1	0.341	**	1	0.493	0.047	1	0.828						
FH of Psychiatric Illness	**3.781**	**1**	**0.052**	**	1	0.249									
Lifetime Diagnosis of Epilepsy	**	1	1.000												

Chi-square tests, **Fisher's exact tests. Significant relationships are reported in **bold.**

Table B-10 Relationship between History of Investigations for Blackouts, Loss of Conscious or Fainting Episodes and Lifetime DSM-IV Diagnosis and Family History of Psychiatric Illness

		Any Lifetime DSM-IV Diagnosis					Family History of Psychiatric Illness				Total
		No		Yes			No		Yes		
		N	%	N	%	N	N	%	N	%	
Investigations for blackouts, loss of consciousness or fainting episodes	**NO**	34	47	39	53	73	46	60	31	40	77
	YES	2	15	11	85	13	4	31	9	69	13
Total N		36		50		86	50		40		90
		(χ^2= 4.411, df=1, p=0.036)					**(χ^2= 3.781, df=1, p=0.052)**				

Chi-square tests.

Table B-11 Relationship between Below Average IQ Scores and Learning Difficulties

		IQ Score Below Average				Total
		No		Yes		
		N	%	N	%	N
Learning Difficulties	**No**	57	88	8	12	65
	Yes	11	55	9	45	20
Total N		68		17		85
		(Fishers, p=0.003)				

Fisher's exact tests.

Table B-12 Subjective Lifetime and Temporal Relationships between DD and Depression-Frequencies and Percentages

	Yes		No		Unsure		Total
	N	%	N	%	N	%	N
Lifetime Relationship	3	6	39	78	8	16	50
Temporal Relationship	19	38	24	48	7	14	50

Table B-13 Age of Onset and Duration of DD and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Attempts

	Any Lifetime DSM-IV Diagnosis				History of Suicidal Thoughts and Suicide Attempts				
	No	Yes	U*	p	None	Suicidal Thoughts	Suicide Attempts	H**	p
Age of onset of DD									
Median	14.00	13.00	840.5	0.250	14.00	14.00	14.50	0.783	0.676
Range	6-48	0-48			0-48	0-48	6-37		
Interquartile Range	9	10			11	8	15		
N***	37	53			61	17	12		
Duration of DD									
Median	30.00	31.00	911.5	0.571	29.00	36.00	32.00	2.262	0.323
Range	5-53	3-60			5-59	3-60	5-49		
Interquartile Range	20	22			23	16	18		
N***	37	53			61	17	12		

*Mann-Whitney U Tests, ** Kruskal-Wallis H Tests.
*** Missing cases are individuals where age of onset of DD and/or lifetime DSM-IV diagnosis was unknown and/or history of suicidal thoughts and suicide attempts was unknown.

Table B-14 Age of Onset and Duration of DD-Correlations with Key Psychiatric Clinical Variables

	Age of Onset Of DD	Duration of DD
Age of onset of any psychiatric illness		
*rho	-0.034	-
p	0.843	-
N**	37	-
Number of Episodes of Depression		
*rho	0.234	0.113
p	0.136	0.476
N**	42	42
Longest Duration of Depression		
*rho	-0.139	0.157
p	0.394	0.334
N**	40	40

*Spearman's rho correlation coefficients. **Missing cases are individuals where age of onset of DD was unknown and/or cases where there was not enough information to make a rating on the key psychiatric clinical variables.

Table B-15 Relationship between DD Severity and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

	Any Lifetime DSM-IV Diagnosis					History of Suicidal Thoughts and Suicide Attempts						
	No		Yes			None		Suicidal Thoughts		Suicide Attempts		
DD Severity	**N**	**%**	**N**	**%**	**N**	**N**	**%**	**N**	**%**	**N**	**%**	**N**
Mild	10	50	10	50	**20**	16	80	2	10	2	10	**20**
Moderate	23	37	39	63	**62**	42	67	11	17	10	16	**63**
Severe	4	40	6	60	**10**	5	45.5	5	45.5	1	9	**11**
Total N*	37		55		**92**	63		18		13		**94**
	χ^2=1.047, df=2, p=0.592					**χ^2= 6.79, df=4, p=0 .143						

Chi-square tests, ** exact significance test for Pearson's chi-square.
* Missing cases are individuals where lifetime DSM-IV diagnosis was unknown and/or where history of suicidal thoughts and suicide attempts was unknown.

Table B-16 DD Severity and Key Psychiatric Clinical Variables

		DD Severity				
		Mild	**Moderate**	**Severe**	**H***	**p**
Age of onset of any psychiatric illness	Median	25.00	26.00	26.50	0.229	0.892
	Range	17-44	14-58	19-40		
	Interquartile Range	10	17	18		
	N**	7	28	4		
Number of Episodes of Depression	Median	2.50	3.00	5.00	0.030	0.985
	Range	1-9	1-130	1-6		
	Interquartile Range	6	8	4		
	N**	8	31	5		
Longest Duration of Depression	Median	11.00	21.00	3.50	2.525	0.283
	Range	1-52	0.4-780	2-104		
	Interquartile Range	41	41	77		
	N**	8	30	4		

*Kruskal-Wallis H Tests. ** Missing cases are individuals where there was not enough information to make a rating on the key psychiatric clinical variables.

Table B-17 Relationship between Pain and Malodour Reported as a Symptom of DD and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Attempts

		Any Lifetime DSM-IV Diagnosis					History of Suicidal Thoughts and Suicide Attempts						
		No		Yes		Total	None		Suicidal Thoughts		Suicide Attempts		Total
		N	%	N	%	N	N	%	N	%	N	%	N
Pain	**No**	19	50	19	50	38	27	73	5	13.5	5	13.5	37
	Yes	15	30	35	70	50	32	60	13	25	8	15	53
Total N		34		54		88*	59		18		13		90**
		χ^2=3.643, df=1, p=0.056					χ^2= 1.887, df=2, p= 0.389						
Malodour	**No**	15	45	18	55	33	26	79	4	12	3	9	33
	Yes	19	34.5	36	65.5	55	33	58	14	24.5	10	17.5	57
Total N		34		54		88*	59		18		13		90**
		χ^2= 1.035, df=1, p= 0.309					χ^2= 4.043 df=2, p= 0.132						

Chi-square tests. * Missing cases are individuals where lifetime DSM-IV diagnosis was unknown and/or where individuals were unsure whether they had experienced pain and/or malodour as symptom of DD. **Missing cases are individuals where history of suicidal thoughts and suicide attempts was unknown and/or where individuals were unsure whether they had experienced pain and/or malodour as symptom of DD.

Table B-18 Pain and Malodour Reported as Symptom of DD and Key Psychiatric Clinical Variables

	Pain Reported as a Symptom of DD				Malodour Reported as a Symptom of DD			
	No	Yes	U*	p	No	Yes	U*	p
Age of onset of any psychiatric illness								
Median	25.00	27.00	145.00	0.590	25.00	26.00	131.00	0.431
Range	15-52	14-58			15-44	14-58		
Interquartile Range	15	18			12	21		
N**	13	25			12	26		
Number of Episodes of Depression								
Median	5.00	3.00	157.50	0.232	2.50	4.00	182	0.913
Range	1-50	1-130			1-10	1-130		
Interquartile Range	7	4			7	5		
N**	14	29			12	31		
Longest Duration of Depression								
Median	15.50	13.00	167.00	0.543	12.00	26.00	151.500	0.425
Range	1-156	0.4-780			1-104	0.4-780		
Interquartile Range	21	49			34.5	47.3		
N**	14	27			12	30		

*Mann-Whitney U Tests. ** Missing cases are individuals who were unsure whether they had experienced pain and/or malodour as a symptom of DD and/or where there was not enough information to make a rating on the key psychiatric clinical variables.

Table B-19 Relationship between Subjective Impact of having DD on School, Work/Career, Relationships and Social Activities and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

		Any Lifetime DSM-IV Diagnosis					History of Suicidal Thoughts and Suicide Attempts						
		No		Yes		Total	None		Suicidal Thoughts		Suicide Attempts		Total
		N	%	N	%	N	N	%	N	%	N	%	N
School	**None**	14	46.7	16	53.3	30	24	77.4	4	12.9	3	9.7	31
	Mild	4	36.4	7	63.6	11	5	50.0	3	30.0	2	20.0	10
	Mod/Sv	4	23.5	13	76.5	17	10	58.8	5	29.4	2	11.8	17
	N/A	15	44.1	19	55.9	34	24	66.7	6	16.7	6	16.7	36
Total N*		37		55		92	63		18		13		94
		χ^2 = 2.771, df=3, p= 0.428					**χ^2 = 4.325 df=6, p= 0.653						
Work/ Career	**None**	25	43.1	33	56.9	58	43	76.8	9	16.2	4	7.1	56
	Mild	5	50	5	50	10	6	54.5	1	9.1	4	36.4	11
	Mod/Sv	6	28.6	15	71.4	21	12	50	8	33.3	4	16.7	24
Total N*		36		53		89	61		18		12		91
		χ^2 = 1.778, df=2, p= 0.411					**χ^2 = 11.595, df=4, p= 0.021						
Relation-ships	**None**	26	41.9	36	58.1	62	43	68.3	13	20.6	7	11.1	63
	Mild	8	53.3	7	46.7	15	12	80	2	13.3	1	6.7	15
	Mod/Sv	3	23.1	10	76.9	13	7	50	3	21.4	4	28.6	14
Total N*		37		53		90	62		18		12		92
		χ^2 = 2.689, df=2, p= 0.261					**χ^2 = 4.585, df=4, p= 0.337						
Social Activities	**None**	20	50	20	50	40	34	79.1	4	9.3	5	11.6	43
	Mild	11	36.7	19	63.3	30	17	60.7	7	25	4	14.3	28
	Mod/Sv	6	28.6	15	71.4	21	12	54.5	7	31.8	7	13.6	22
Total N*		37		54		91	63		18		12		93
		χ^2 = 2.916, df=2, p= 0.233					**χ^2 = 6.161, df=4, p= 0.193						

Chi-square tests, **exact significance tests for Pearson's chi-square. Mod/Sv; moderate/severe DD.
*Missing cases are individuals where DSM-IV diagnosis was unknown and/or where history of suicidal thoughts and suicide attempts was unknown and/or where the effects of DD on the domain of life could not be established and/or where the lifetime effect on the domain of life was not applicable.

Table B-20 Scores on the Dermatology Life Quality Index and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

	Any Lifetime DSM-IV Diagnosis				History of Suicidal Thoughts and Suicide Attempts				
	No	Yes	U*	p	None	Suicidal Thoughts	Suicide Attempts	H**	p
DLQI- Last Week									
Median	3.00	5.00	846.000	0.215	3.00	5.00	5.00	2.146	0.342
Range	0-20	0-28			0-26	0-28	0-12		
Iq R	6	7			7	11	6		
N***	37	54			63	17	13		
DLQI- Worst Week									
Median	14.00	16.50	841.500	0.203	14.00	20.00	16.00	7.120	**0.028**
Range	0-30	2-30			0-30	2-30	10-30		
Iq R	10	14			11	11	12		
N***	37	54			63	17	13		

*Mann-Whitney U Tests, ** Kruskal-Wallis H Tests. Iq R; interquartile range.

*** Missing cases are individuals where age of onset of DD and/or lifetime DSM-IV diagnosis was unknown and/or history of suicidal thoughts and suicide attempts was unknown and one individual who did not complete the DLQI.

Table B-21 Subjective Impact of Having DD on School and Work/ Career and Key Psychiatric Variables

	Subjective Impact of DD on School						Subjective Impact of DD on Work/Career				
	None	Mild	Moderate/ Severe	Not Applicable	*H	p	None	Mild	Moderate/ Severe	*H	p
Age of onset of any psychiatric illness											
Median	28.50	16.00	29	24.50	1.790	0.617	25.00	17.00	29.40	1.310	0.519
Range	15-52	14-48	15-41	18-58			15-52	14-58	15-41		
Interquartile Range	9	25	22	10			12	34	22		
N	12	7	8	12			24	4	10		
Number of Episodes of Depression											
Median	4.00	4.00	1.5	3.50	4.160	0.245	2.50	5.00	3.00	0.581	0.748
Range	1-50	1-6	1-10	1-130			1-50	1-9	1-130		
Interquartile Range	8	5	9	7			7	7	5		
N	12	6	10	16			26	4	13		
Longest Duration of Depression											
Median	9.00	65.00	19	14.50	6.241	0.10	12.50	19.50	26.00	0.581	0.748
Range	1.0-104.0	13.0-260.0	1-780	0.4-156.0			1.0-780	13.0-52	0.4-312		
Interquartile Range	20.0	139.8	238.3	35.0			45	32.5	102		
N	12	6	8	16			26	4	11		

*Kruskal-Wallis H Tests.

Table B-22 Subjective Impact of Having DD on Relationships and Social Activities and Key Psychiatric Variables

	Subjective Impact of having DD on Relationships					Subjective Impact of having DD on Social Activities				
	None	Mild	Moderate/Severe	*H	p	None	Mild	Moderate/Severe	*H	P
Age of onset of any psychiatric illness										
Median	25.00	20.50	31.00	1.588	0.542	29.50	25.00	23.00	4.01	0.135
Range	15-58	15-29	14-52			15-58	14-48	15-40		
Interquartile Range	14	13	24			18	12	14		
N	27	4	7			14	14	10		
Number of Episodes of Depression										
Median	2.50	5.50	3.00	1.514	0.469	2.50	2.00	5.00	0.456	0.796
Range	1-20	2-10	1-130			1-130	1-10	1-20		
Interquartile Range	4	6	27			7	6	5		
N	30	4	9			16	16	11		
Longest Duration of Depression										
Median	13.00	26.00	15.00	0.652	0.722	21.00	12.00	26.00	1.276	0.528
Range	1.0-780	12.0-52	0.4-312			0.4-780	1.0-130	1-312		
Interquartile Range	47.3	30	25.0			36.8	48.0	139		
N	30	4	7			16	15	10		

*Kruskal-Wallis H Tests.

Table B-23 DLQI Last Week and Worst Week Scores - Correlations with Key Psychiatric Clinical Variables

	DLQI Last Week	DLQI Worst Week
Age of onset of any psychiatric illness		
*rho	0.087	-0.202
p	0.604	0.223
n	38	38
Number of Episodes of Depression		
*rho	0.080	.082
p	0.611	0.601
n	43	43
Longest Duration of Depression		
*rho	0.130	0.086
p	0.418	0.591
n	41	41

Spearman's rho correlation coefficients.

Table B-24 Relationship between having a Family History of DD and Lifetime DSM-IV Diagnosis and History of Suicidal Thoughts and Suicide Attempts

		Any Lifetime DSM-IV Diagnosis					History of Suicidal Thoughts and Suicide Attempts						
		No		Yes		Total	None		Suicidal Thoughts		Suicide Attempts		Total
		N	%	N	%	N	N	%	N	%	N	%	N
Family History of DD	No	15	60	10	40	25	23	92	0	0	2	8	25
	Yes	19	31	42	69	61	36	57	17	27	10	16	63
Total N*		34		52		*86	59		17		12		**88
		χ^2=6.175, df=1, p=0.013					**χ^2=10.803, df=2, p=0.003						

Chi-square test , ** exact significance test for Pearson's chi-square. * Missing cases are individuals where lifetime DSM-IV diagnosis was unknown and/or where history of suicidal thoughts or suicide attempts was unknown and/or family history of DD was unknown.

Table B-25 Family History of DD and Key Psychiatric Clinical Variables

	Negative Family History of DD	Positive Family History of DD	U*	p
Age of onset of any psychiatric illness				
Median	18.50	27.00	74.000	0.433
Range	16-39	14-58		
Interquartile Range	18	9		
N**	6	31		
Number of Episodes of Depression				
Median	2.00	3.00	89.500	0.562
Range	1-6	1-130		
Interquartile Range	4	8		
N**	6	35		
Longest Duration Depression				
Median	10.00	16.00	80.500	0.470
Range	1-780	0.4-312		
Interquartile Range	211	43		
N**	6	33		

*Mann-Whitney U Tests. ** Missing cases are individuals where family history of DD was unknown and/or where there was not enough information to make a rating on the key psychiatric clinical variables.

Table B-26 Age of Onset of DD and History of Learning Difficulties

	Learning Difficulties			
	No	Yes	U*	p
Age of onset of DD				
Median	14.00	13.00	605.0	0.244
Range	0-48	5-33		
Interquartile Range	10	5		
N**	77	19		

*Mann-Whitney U Test. ** Missing cases are individuals where age of onset of DD was unknown.

Table B-27 Relationship between DD Severity and History of Learning Difficulties

		Learning Difficulties				Total
		No		Yes		
		N	%	N	%	N
Severity index	**Mild**	17	77.3	5	22.7	22
	Moderate	54	80.6	13	19.4	67
	Severe	8	72.7	3	27.3	11
Total N		79		21		100
		χ^2=0.403, df=2, p=0.805				

Exact significance test for Pearson's chi-square.

Table B-28 Relationship between Subjective Impairment of DD on School Life and History of Learning Difficulties

		Learning Difficulties				
		No		Yes		Total
		N	%	N	%	N
Impact on School	**None**	25	76	8	24	33
	Mild	8	73	3	27	11
	Mod/Sv	13	76	4	24	17
	N/A	33	85	6	15	39
Total N		79		21		100
		$\chi^2 = 1.277$, df=3, p= 0.758				

Exact significance tests for Pearson's chi-square. Mod/Sv; moderate/severe DD.

Table B-29 Relationship between Family History of DD and History of Learning Difficulties

		Learning Difficulties				Total
		No		Yes		
		N	%	N	%	N
Family History of DD	**No**	22	84.6	4	15.4	26
	Yes	52	76.5	16	23.5	68
Total N*		74		20		94
		χ^2=0.745, df=1, p=0.388				

Chi-square test. *Missing cases are individuals where family history of DD was unknown.

Table B-30 DD Severity and IQ Scores

	DD Severity				
	Mild	**Moderate**	**Severe**	**H***	**p**
Median	106.50	101.50	92.00	5.256	0.072
Range	70-118	67-125	76-108		
Interquartile Range	14	19	13		
N**	20	56	9		

*Kruskal-Wallis H Test. ** Missing cases are individuals who did not complete the IQ measurement.

Table B-31 Relationship between Subjective Impairment of DD on School Life

	Subjective Impact of DD on School					
	None	**Mild**	**Moderate/Severe**	**Not Applicable**	***H**	**p**
IQ						
Median	105.00	99.50	96.00	105.00	2.955	0.399
Range	70-118	84-18	67-114			
Interquartile Range	20	15	14	23		
N**	28	10	14	33		

*Kruskal-Wallis H Tests. ** Missing cases are individuals who did no complete the IQ measurement

Table B-32 Family History of DD and IQ Scores

	Negative Family History of DD	Positive Family History of DD	U*	p
IQ Score				
Median	102.00	104.00	636.500	0.935
Range	70-118	67-125		
Interquartile Range	19	21		
N**	23	56		

*Mann-Whitney U Test. ** Missing cases are individuals where family history of DD was unknown and/or individuals who did not complete the IQ measurement.

C Appendices for Chapter 7

C.i DNA Extraction Protocols for Blood and Oragene Saliva Samples

PHENOL-CHLOROFORM DNA EXTRACTION PROTOCOL FOR 10ML HUMAN PERIPHERAL BLOOD:

First label 50ml centrifuge tubes (Elkay Laboratory Products Ltd) with the same IDs used on the tubes with the blood (Greiner Bio-one).

1. Put the blood in the 50ml centrifuge tubes adding Lysis buffer to a total volume of 40 ml.
The lysis buffer removes the erythrocytes from the peripheral blood by lysing them.
2. Place the blood-lysis mix on ice for 30 minutes.
3. Centrifuge the samples in the centrifuge (Sorvall® Legend*RT*, Kendro Laboratory products) at 3000 rpm for 15 minutes.
4. Tip off the supernatant retaining the cell pellet (the pellet should consist of leucocytes) at the bottom of the tube. The supernatant should be disposed off into a Virkon solution (Antec International Ltd) for decontamination before disposal down the sink.
5. Add lysis buffer to the cell pellet to a total volume of 30 ml.
6. Vortex the samples until the cell pellet is resuspended.
7. Centrifuge the samples at 3000 rpm for 15 minutes.
8. Tip off most of the supernatant.
9. Invert the tubes on to a tissue paper to remove the excess liquid.

The pellets are ready for digestion.
Cells (leukocytes from the blood) have to be digested overnight with:
-500 µl SET buffer
-50 µl 10% SDS (Sodium Dodecyl Sulfate Solution, Gibco™)
Adding the 10% SDS (strong anionic detergent) helps to disrupt the cell membranes, distributes the extraction buffer through the tissue, and helps the Proteinase K to denature the proteins.
-50 µl Proteinase K (from Tritirachium album, Sigma®)
Proteinase K is added to remove the proteins bound to the DNA and to destroy the cellular enzymes that would otherwise digest the DNA, thus maximizing the amount of intact DNA that is extracted.

Incubate overnight at 55°C in a water bath (Grant Instruments Cambridge Ltd.).

The 2ml tubes (Bioquote Limited) that will be used must be labelled before the beginning of the extraction.

1. Transfer the sample from the 50ml centrifuge tube to a 2ml tube (already labelled).
Add 1ml Tris-saturated Phenol (pH 8.0, Sigma®).
Vortex vigorously and centrifuge at 3000rpm for 5 minutes (Biofuge 13, Heraeus Sepatech).
Phenol is an organic solvent, hydrophobic and it disrupts the 3D structure of the proteins, precipitates and denatures them. The denatured proteins become soluble in the organic interphase, while the DNA remains in the aqueous phase.
Phenol does not denature the DNA because it is hydrophobic and DNA is hydrophilic.

2. Transfer the top (aqueous) layer to fresh prelabelled 2ml tube containing 500ul chloroform (BDH Laboratory Supply)/isoamyl (24:1) alcohol (BDH Laboratory Supply) and 500µl Tris-saturated phenol (pH8.0). Vortex and spin at 3000rpm for 5 minutes.
The chloroform helps the phenol in the denaturing of the proteins. It aids in the removal of the lipids as well, thus improving separation of nucleic acid into the aqueous phase. Phase separation is also

enhanced, which assists in the removal of the aqueous phase with minimal cross contamination from the organic phase. The isoamyl alcohol is added to the phenol and the chloroform to reduce foaming.

3. Transfer the top layer trying not to disturb the white interface (it predominantly consists of proteins) to a fresh tube containing 1ml chloroform.
Vortex and spin at 3000rpm for 5 minutes.
The chloroform is used to remove residual phenol from the aqueous phase, which is slightly soluble and a known PCR inhibitor.

4. Transfer the top layer without the interface to a fresh 2ml tube. Add 1ml ethanol (cold, absolute, Fischer scientific) to precipitate the DNA.

5. Spool the DNA, rinse it with 70% ethanol and put it into 1ml 1% TE buffer.

The tubes with the DNA must be put on a rotator (VWR International Ltd) at 4^0C at least for 3 days in order the DNA to be diluted entirely in the TE buffer.

6. Measure the concentration of the DNA, using Quant-it ™ PicoGreen® ds DNA assay kit (Invitrogen).

7. Test if the DNA is degraded.

8. Prepare PCR for known working assay and test if the DNAs are viable on 0.8% agarose gel (Sigma®).

Lysis buffer:
Ammonium chloride (BDH Laboratory Supply)-8g
Potassium bicarbonate (Sigma®)–1g
0.5 M EDTA-200μl
Make up to 1l with sterile ddH_2O (Fresenius Kabi Ltd)

SET buffer:

NaCl-1.461 g (10ml 5M NaCl)
1M TRIS-Cl (pH 8.0)-10ml
0.5M EDTA (pH 8.0)-5ml
Make up to 500 ml with sterile ddH_2O (Fresenius Kabi Ltd)

TE buffer-1%:
1M TRIS-Cl (pH 8.0)-10ml
1mM EDTA (pH 8.0)-2 ml
Make up to 1l with sterile ddH_2O (Fresenius Kabi Ltd)

ORAGENE KITS FOR PURIFYING DNA FROM SALIVA (DNA GENOTEK INC):

DNA purification steps:

1. Incubate overnight the Oragene/saliva sample in the Oragene vial at 55°C in a water bath (Grant Instruments Cambridge Lid.).

2. Divide the total 4 ml Oragene/saliva sample into four 1.5 ml centrifuge tubes (Bioquote Limited), each containing approximately 1 ml of sample.

3. Add 40 µl (1/25th volume) of Oragene Purifer (supplied with the kit) to each tube and mix gently by inversion. The sample will become turbid as impurities are precipitated.

4. Incubate the four tubes on ice for 10 minutes.

5. Centrifuge the four tubes for 3 minutes at 15,000 × *g* (13,000 rpm) (centrifuge-Biofuge 13, Heraeus Sepatech) at room temperature. Carefully pipet the clear supernatant from each tube and combine them all into one 15 ml centrifuge tube (Elkay Laboratory Products Ltd) without disturbing the pellets. Discard the pellets.

6. Add 4 ml (equal volume) of room-temperature 95% ethanol (Sigma®) to the supernatant and mix gently by inversion. Invert at least 5 times. A clot of DNA may be visible.

7. Let the solution stand for 10 minutes at room temperature, so that the DNA is fully precipitated. Do not incubate at -20°C because impurities may co-precipitate with the DNA.

8. Centrifuge for 10 minutes at 1,100 × *g* (3,500 rpm) at room temperature (Sorvall® Legend*RT*, Kendro Laboratory products).

9. Discard the supernatant without disturbing the DNA pellet (may or may not be visible). Remove ethanol as thoroughly as possible.

10. Once all of the ethanol has been removed, dissolve the DNA pellet in 500 µl of 1% TE buffer. The expected concentration of the rehydrated DNA is 20 to 200 ng/µl.

11. The tubes with the DNA must be put on a rotator (VWR International Ltd) at 4^0C at least for 3 days in order the DNA to be diluted entirely in the TE buffer.

12. Measure the concentration of the DNA, using Quant-it ™ PicoGreen® ds DNA assay kit (Invitrogen).

13. Test if the DNA is degraded.

14. Prepare PCR for known working assay and test if the DNAs are viable on 0.8% agarose gel (Sigma®).

D Appendices for Chapter 8

D.i Comparison of Type and Location of Mutations Detected Compared to Previously Reported Mutations in ATP2A2

Table D-1 Frequencies and Percentage of Types of Pathogenic Mutations in the Present Study Compared to Mutations Previously Reported in the Literature

Mutation Type	Frequencies of *mutations* in present study		All *mutations* previously in literature		ALL MUTATIONS	
	N	%	N	%	N	%
Nonsense	3	5	14	10	16	9
Frameshift (insertion/deletion)	12	20	33	23	43	23
Missense (non-synonomous)	30	50	71	50	87	47
In-frame (insertion/deletion)	4	7	9	6	13	7
Splice Site	8	13	12	8	20	11
Other	3	5	4	3	6	3
Total number of mutations	60	100	143	100	185	100

18 mutations detected/known in the present study that had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL MUTATIONS column.

Table D-2 Frequencies and Percentages of Individuals in the Present Study according to Mutation Type Compared to Unrelated Individuals Previously Reported in the Literature

Mutation Type	Frequencies of unrelated *individuals* in present study		Frequencies of unrelated *individuals* previously in the literature		ALL UNRELATED INDIVIDUALS	
	N	%	N	%	N	%
Nonsense	4	6	15	9	19	8.1
Frameshift (insertion/deletion)	12	18	34	19.4	45	19.3
Missense (non-synonomous)	37	54	98	56	128	54.9
In-frame (insertion/deletion)	4	6	11	6	14	6
Splice Site	8	12	13	7.4	21	8.7
Other	3	4	4	2	6	2.6
Total number of unrelated individuals	68	100	175	100	233	100

10 individuals in the present study whose mutations had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL UNRELATED INDIVIDUALS column.

Table D-3 Frequencies and Percentages of the Exon/Intron Locations of Pathogenic Mutations in the Present Study Compared to Mutations Previously Reported in the Literature

Mutation Location	Frequencies of *Mutations* in present study		All *mutations* previously in the literature		ALL MUTATIONS	
	N	%	N	%	N	%
Exon 1	1	1.7	7	5	7	3.8
Intron 1	0	0	1	0.7	1	0.5
Exon 2	0	0	5	3.5	5	2.7
Intron 2	1	1.7	0	0	1	0.5
Exon 3	3	5	4	2.8	6	3.2
Intron 3	0	0	0	0	0	0
Exon 4	0	0	2	1.4	2	1.1
Intron 4	1	1.7	0	0	1	0.5
Exon 5	0	0	6	4	6	3.24
Intron 5	1	1.7	0	0	1	0.5
Exon 6	2	3.3	5	3.5	7	3.8
Intron 6	0	0	1	0.7	1	0.5
Exon 7	0	0	7	5	7	3.8
Intron 7	0	0	1	0.7	1	0.5
Exon 8	12	20	15	10.5	24	13
Intron 8	1	1.7	0	0	1	0.5
Exon 9	0	0	3	2	3	1.6
Intron 9	0	0	0	0	0	0
Exon 10	1	1.7	2	1.4	3	1.6
Intron 10	0	0	1	0.7	1	0.5
Exon 11	3	5	2	1.4	4	2.2
Intron 11	0	0	0	0	0	0
Exon 12	2	3.3	7	5	8	4.3
Intron 12	0	0	1	0.7	1	0.5
Exon 13	3	5	6	4	9	4.9
Intron 13	1	1.7	1	0.7	2	1.1
Exon 14	4	6.7	11	7.7	14	7.6
Intron 14	1	1.7	1	0.7	2	1.1
Exon 15	8	13.3	19	13	22	11.9
Intron 15	1	1.7	0	0	1	0.5
Exon 16	3	5	8	5.6	10	5.4
Intron 16	0	0	0	0	0	0
Exon 17	2	3.3	1	0.7	2	1.1
Intron 17	0	0	1	0.7	1	0.5
Exon 18	6	10	10	7	14	7.6
Intron 18	0	0	0	0	0	0
Exon 19	3	5	8	5.6	10	5.4
Intron 19	0	0	1	0.7	1	0.5
Exon 20	0	0	2	1.4	2	1.1
Intron 20	0	0	1	0.7	1	0.5
Exon 20b	0	0	3	2	3	1.6
Total number of mutations	**60**	**100**	**143**	**100**	**185**	**100**

18 mutations detected/known in the present study that had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL MUTATIONS column.

Table D-4 Frequencies and Percentages of Individuals in the Present Study according to Exon/Intron Mutation Location Compared to Unrelated Individuals Previously Reported in the Literature

Mutation Location	Frequencies of unrelated *individuals* in present study		Frequencies of unrelated *individuals* previously in the literature		ALL UNRELATED INDIVIDUALS	
	N	%	N	%	N	%
Exon 1	1	1.5	13	7.4	14	6.0
Intron 1	0	0	1	0.6	1	0.4
Exon 2	0	0	5	2.9	5	2.1
Intron 2	1	1.5	0	0.0	1	0.4
Exon 3	3	4.4	4	2.3	6	2.6
Intron 3	0	0	0	0.0	0	0.0
Exon 4	0	0	2	1.1	2	0.9
Intron 4	1	1.5	0	0.0	1	0.4
Exon 5	0	0	9	5.1	9	3.9
Intron 5	1	1.5	0	0.0	1	0.4
Exon 6	2	2.9	7	4.0	9	3.9
Intron 6	0	0	1	0.6	1	0.4
Exon 7	0	0	7	4.0	7	3.0
Intron 7	0	0	1	0.6	1	0.4
Exon 8	13	19.1	19	10.9	31	13.3
Intron 8	1	1.5	0	0.0	1	0.4
Exon 9	0	0	3	1.7	3	1.3
Intron 9	0	0	0	0.0	0	0.0
Exon 10	1	1.5	2	1.1	3	1.3
Intron 10	0	0	2	1.1	2	0.9
Exon 11	3	4.4	2	1.1	4	1.7
Intron 11	0	0	0	0.0	0	0.0
Exon 12	3	4.4	8	4.6	10	4.3
Intron 12	0	0	1	0.6	1	0.4
Exon 13	3	4.4	7	4.0	10	4.3
Intron 13	1	1.5	1	0.6	2	0.9
Exon 14	4	5.9	14	8.0	17	7.3
Intron 14	1	1.5	1	0.6	2	0.9
Exon 15	13	19.1	26	14.9	37	15.9
Intron 15	1	1.5	0	0.0	1	0.4
Exon 16	3	4.4	9	5.1	12	5.2
Intron 16	0	0	0	0.0	0	0.0
Exon 17	2	2.9	2	1.1	3	1.3
Intron 17	0	0	1	0.6	1	0.4
Exon 18	6	8.8	10	5.7	16	6.9
Intron 18	0	0	0	0.0	0	0.0
Exon 19	4	5.9	10	5.7	12	5.2
Intron 19	0	0	1	0.6	1	0.4
Exon 20	0	0	2	1.1	2	0.9
Intron 20	0	0	1	0.6	1	0.4
Exon 20b	0	0	3	1.7	3	1.3
Total number of unrelated individuals	**68**	**100**	**175**	**100**	**233**	**100**

10 individuals in the present study whose mutations had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL UNRELATED INDIVIDUALS column.

Table D-5 Frequencies and Percentages of the Protein Domain Locations of Pathogenic Mutations in the Present Study Compared to Mutations Previously Reported in the Literature

	Frequencies of *mutations* in present study		All *mutations* previously in the literature		ALL MUTATIONS	
	N	%	N	%	N	%
N Terminus	1	1.7	1	0.7	1	0.5
MI transmembrane/S1	4	6.7	8	5.6	11	5.9
M1-M2 lumenal	0	0.0	0	0.0	0	0.0
M2 transmembrane/ S2	1	1.7	3	2.1	4	2.2
A domain	4	6.7	30	21.0	34	18.4
M3 transmembrane/S3	0	0.0	1	0.7	1	0.5
M3-M4- lumenal	1	1.7	1	0.7	2	1.1
M4 transmembrane/ S4	6	10.0	4	2.8	9	4.9
P domain	12	20.0	25	17.5	33	17.8
N domain	11	18.3	25	17.5	34	18.4
M5 transmembrane/S5	6	10.0	10	7.0	12	6.5
M5-M6 lumenal	0	0.0	0	0.0	0	0.0
M6 transmembrane	3	5.0	6	4.2	8	4.3
M6-M7 cytoplasmic	0	0.0	1	0.7	1	0.5
M7 transmembrane	1	1.7	2	1.4	2	1.1
M7-M8 lumenal	4	6.7	8	5.6	10	5.4
M8 transmembrane	3	5.0	4	2.8	7	3.8
M8-M9 cytoplasmic	3	5.0	4	2.8	6	3.2
M9 transmembrane	0	0.0	3	2.1	3	1.6
M9-10 lumenal	0	0.0	2	1.4	2	1.1
M10 transmembrane	0	0.0	2	1.4	2	1.1
M10-M11 cytoplasmic	0	0.0	0	0.0	0	0.0
M11 transmembrane	0	0.0	3	2.1	3	1.6
Lumenal	0	0.0	0	0.0	0	0.0
Total Number/Percentage of Mutations	**60**	**100**	**143**	**100**	**185**	**100**

18 mutations detected/known in the present study that had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL MUTATIONS column.
Protein Domains: M*n* = transmembrane domains, S*n* =stalk domains, A= Actuator Domain, P= Phosphorylation Domain, N= Nucleotide Binding Domain.

Table D-6 Frequencies and Percentages of Individuals in the Present Study according to SERCA2b Protein Domain Compared to the Frequencies Previously Reported in the Literature

	Frequencies of unrelated *individuals* in present study		Frequencies of unrelated *individuals* previously in the literature		ALL UNRELATED INDIVIDUALS	
	N	%	N	%	N	%
N Terminus	1	1.5	3	1.7	4	1.7
MI transmembrane/S1	4	5.9	8	4.6	11	4.7
M1-M2 lumenal	0	0.0	0	0.0	0	0.0
M2 transmembrane /S2	1	1.5	3	1.7	4	1.7
A domain	4	5.9	39	22.3	43	18.5
M3 transmembrane/S3	0	0.0	1	0.6	1	0.4
M3-M4- lumenal	1	1.5	1	0.6	2	0.9
M4 transmembrane /S4	6	8.8	6	3.4	11	4.7
P domain	14	20.6	31	17.7	44	18.9
N domain	12	17.6	28	16.0	38	16.3
M5 transmembrane/S5	10	14.7	16	9.1	24	10.3
M5-M6 lumenal	0	0.0	0	0.0	0	0.0
M6 transmembrane	3	4.4	6	3.4	9	3.9
M6-M7 cytoplasmic loop	0	0.0	1	0.6	1	0.4
M7 transmembrane	1	1.5	4	2.3	4	1.7
M7-M8 lumenal loop	4	5.9	8	4.6	12	5.2
M8 transmembrane	3	4.4	4	2.3	7	3.0
M8-M9 cytoplasmic loop	4	5.9	5	2.9	7	3.0
M9 transmembrane	0	0.0	4	2.3	4	1.7
M9-10 lumenal	0	0.0	2	1.1	2	0.9
M10 transmembrane	0	0.0	2	1.1	2	0.9
M10-M11 cytoplasmic	0	0.0	0	0.0	0	0.0
M11 transmembrane	0	0.0	3	1.7	3	1.3
Lumenal	0	0.0	0	0.0	0	0.0
Total Number/Percentage of unrelated Individuals	**68**	**100**	**175**	**100**	**233**	**100**

10 individuals in the present study whose mutations had previously been reported in the literature are counted in both the first two columns but only counted once in the ALL UNRELATED INDIVIDUALS column.
Protein Domains: M*n* = transmembrane domains, S*n* =stalk domains, A= Actuator Domain, P= Phosphorylation Domain, N= Nucleotide Binding Domain.

D.ii Comparison of Clinical Features of DD and Neuropsychiatric Phenotypes In Individuals in whom a Pathogenic Mutation Was and Was Not Detected/Known

Table D-7 Relationship between Detection of Pathogenic Mutation, Reported Family History of DD and DD Severity

		Family History of DD					DD Severity						
		No		Definite/Probable		Total	Mild		Moderate		Severe		Total
		N	%	N	%	N	N	%	N	%	N	%	N
Pathogenic Mutation Detected	No	10	43	13	57	23	10	36	14	50	4	14	28
	Yes	14	21	53	79	67	12	18	49	72	7	10	68
Total N*		24		66		90	22		63		11		96
		χ^2=4.465, df=1, p=0.035					χ^2 =4.571, df=2, p= 0.102						

Chi-square tests. *Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or individuals where family history of DD was unknown.

Table D-8 Pathogenic Mutation Detection and Scores on the Dermatology Life Quality Index (DLQI) -Worst Week Ever

	No Pathogenic Mutation Detected	Pathogenic Mutation Detected	U*	p
DLQI Worst Week Ever Score (Max. =30)				
Median	15.50	15.00	849.000	0.467
Range	2-30	0-30		
Interquartile Range	13	14		
N**	28	67		

* Mann-Whitney U Test. ** Missing cases are 4 individuals where no DNA was collected and no mutation information was known and 1 case where the DLQI was not completed.

Table D-9 Relationship Between Detection of Pathogenic Mutation and Presence of Neuropsychiatric Phenotypes

	Any Lifetime DSM-IV Diagnosis					History of Suicide Attempts					History of Psychiatric Contact					Learning Difficulties					Investigations for Blackouts, Loss of Consciousness or Fainting Episodes				
	No		Yes			No		Yes			No		Yes			No		Yes			No		Yes		
Pathogenic Mutation Detected	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N
No	11	44	14	56	**25**	25	96	1	4	**26**	24	86	4	14	**28**	24	86	4	14	**28**	24	92	2	8	**26**
Yes	25	40	38	60	**63**	54	84	10	16	**64**	46	69	21	31	**67**	51	75	17	25	**68**	54	84	10	16	**64**
Total N*	36		52		**88**	79		11		**90**	70		25		**95**	75		21		**96**	78		12		**90**
	χ^2=0.138, df=1, p=0.710					Fisher's, p=0.166					χ^2=2.963, df=1, p= 0.085					χ^2=1.332, df=1,p= 0.248					Fisher's, p=0.497				

Chi-square tests, Fisher's; Fisher's exact test. *Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or cases where lifetime history of a specific neuropsychiatric phenotype was unknown.

Table D-10 Relationship between Detection of Pathogenic and BADDS Mania (M) and Depression (D) Scores and Age of First Psychiatric Contact

		No Pathogenic Mutation Detected	Pathogenic Mutation Detected	U*	p
BADDS M	Median (Iq R)	4.00 (10)	8.00 (14)	344.500	**0.014**
	Range	0-40	0-90		
	N**	23	47		
BADDS D	Median (Iq R)	45.00 (40)	54.00 (25)	148.0	0.132
	Range	10-70	7-95		
	N**	13	32		
Age of onset of Psychiatric Illness (Years)	Median (Iq R)	25.00 (19)	27.00 (14)	115	0.696
	Range	18-44	15-58		
	N**	9	28		

*Mann-Whitney U Tests. BADDS; Bipolar Affective Disorder Dimensional Scale, Iq R; Interquartile Range.

**Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or individuals not given a BADDS M and/or D score and/or individuals with no psychiatric illness or where age of onset of psychiatric illness was unknown.

D.iii Investigations of Neuropsychiatric Genotype-Phenotype Correlations- Schematic Diagram of SERCA2b

Figure D-1 Genotype-Phenotype Correlation: BADDS Mania Scores

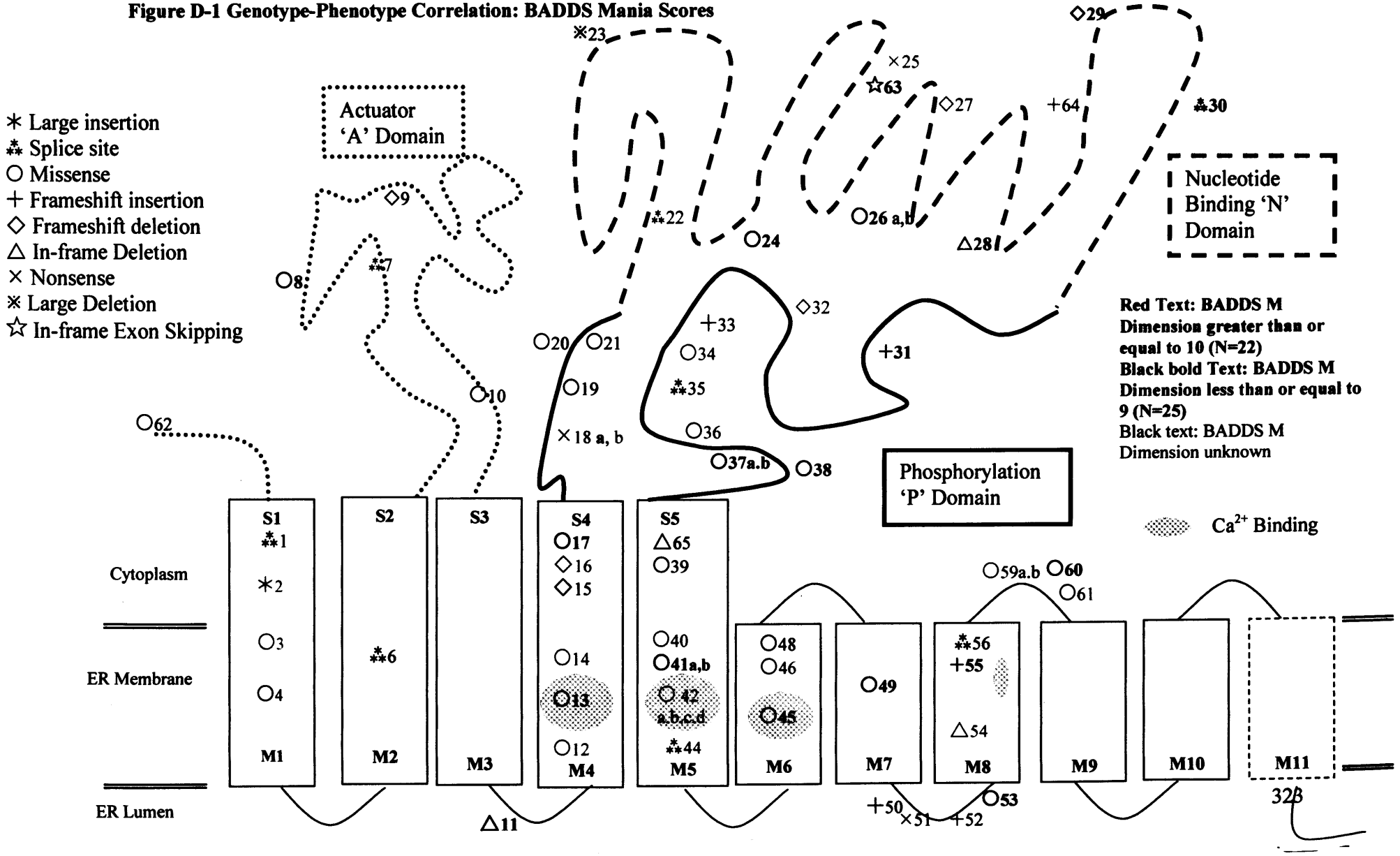


Figure D-2 Genotype-Phenotype Correlation: History of Suicide Attempts

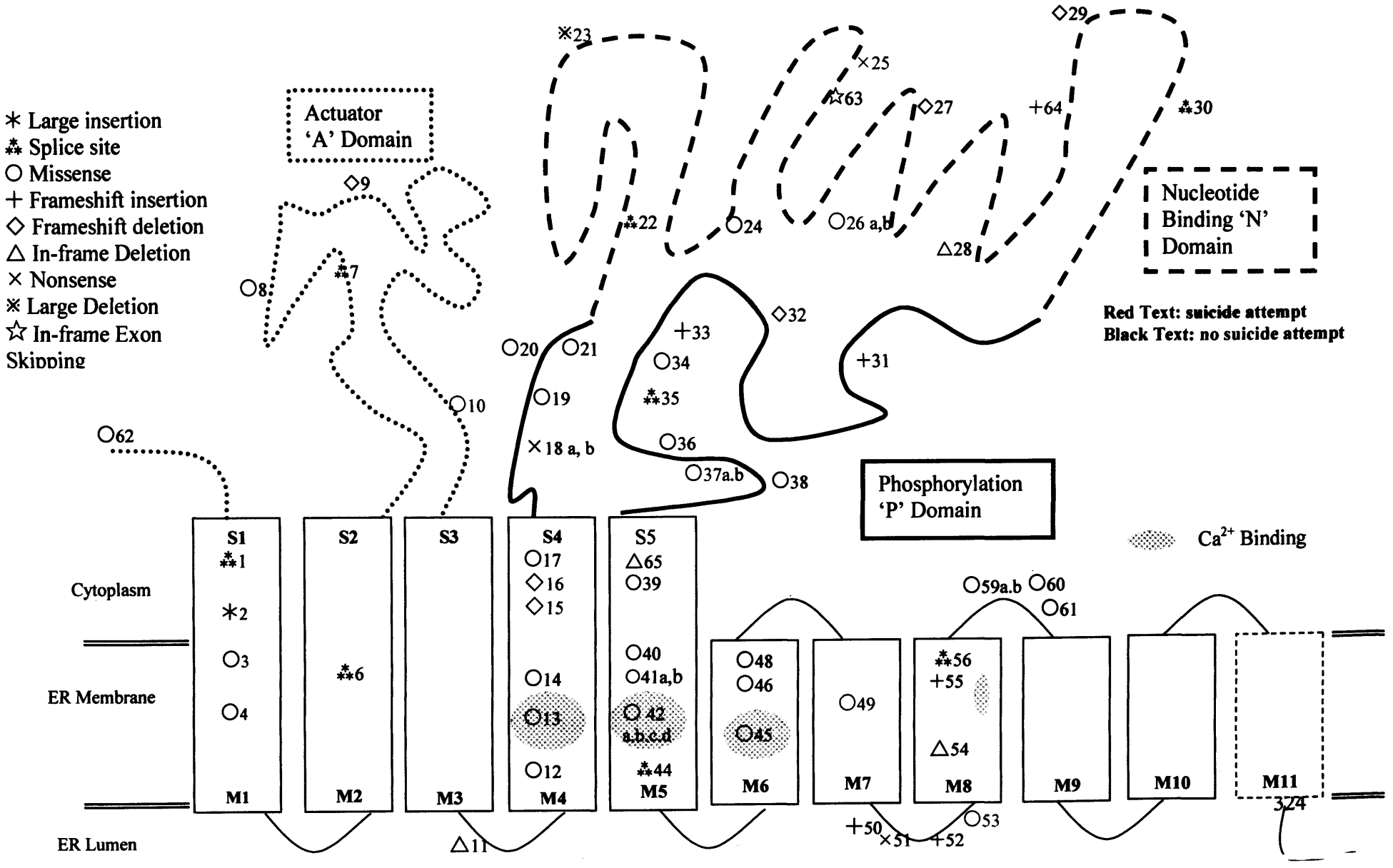


Figure D-3 Genotype-Phenotype Correlation: History of DSM-IV Lifetime Diagnosis

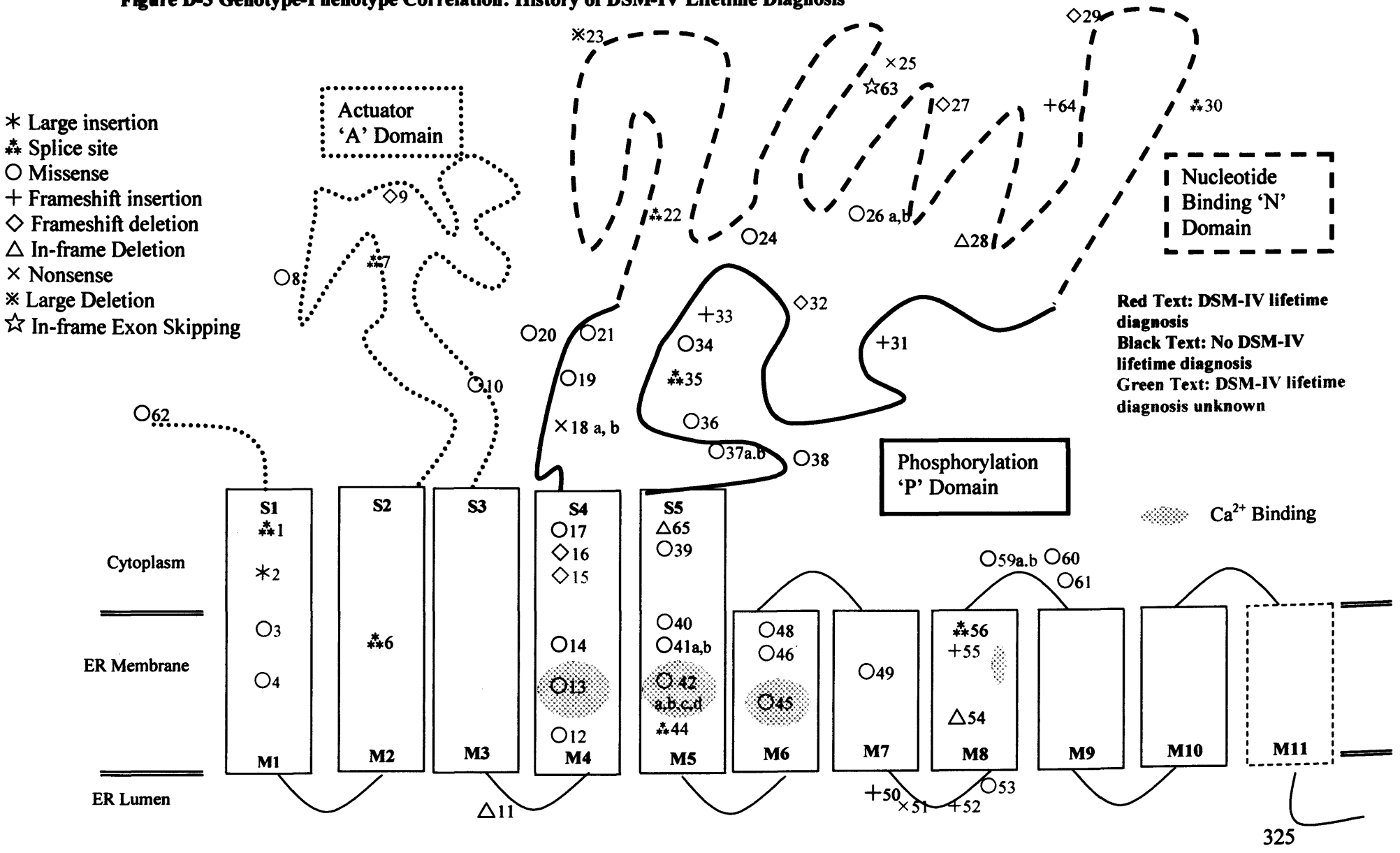


Figure D-4 Genotype-Phenotype Correlation: Learning Difficulties

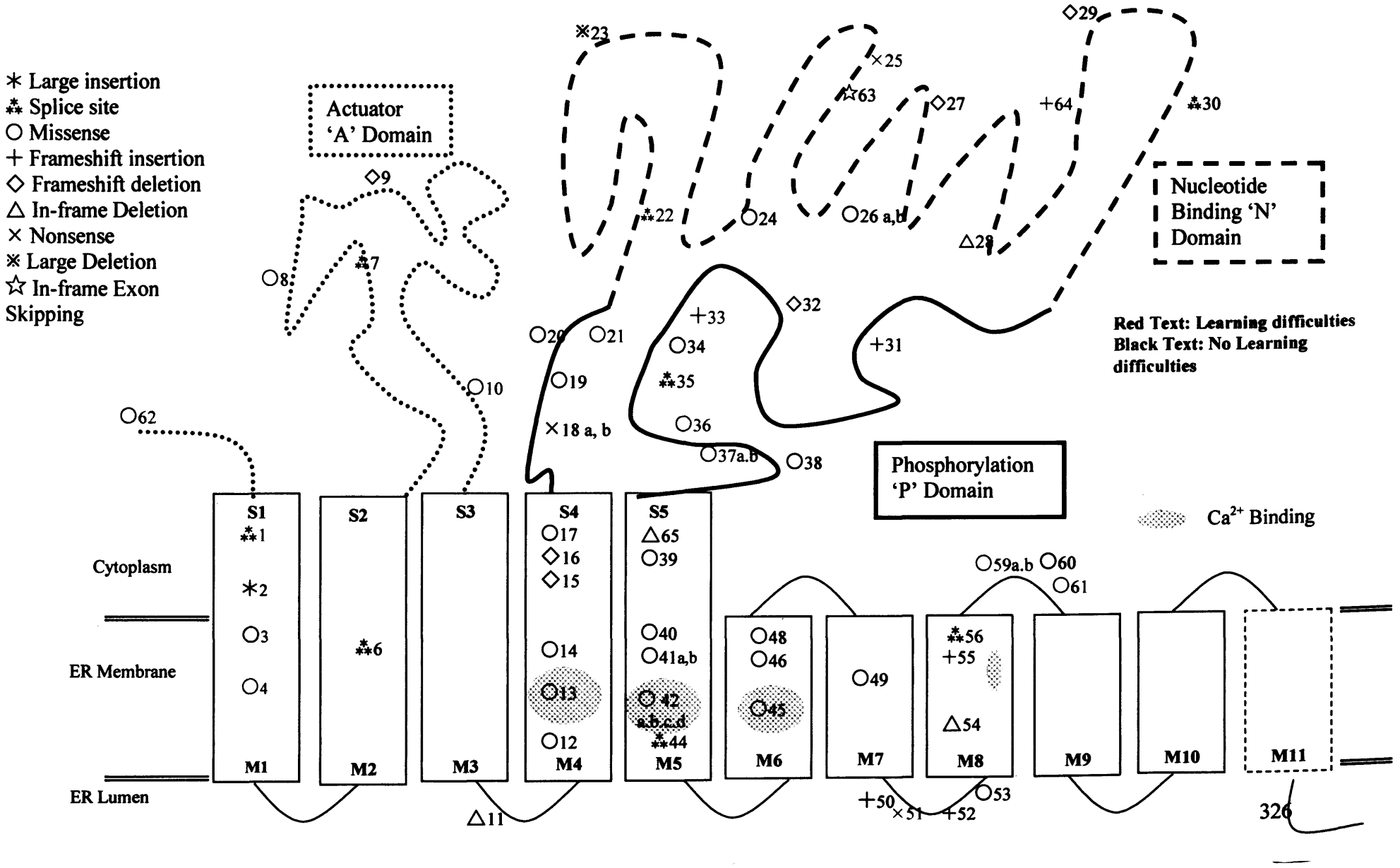


Figure D-5 Genotype-Phenotype Correlation: Investigations for Blackouts, Loss of Consciousness or Fainting Episodes

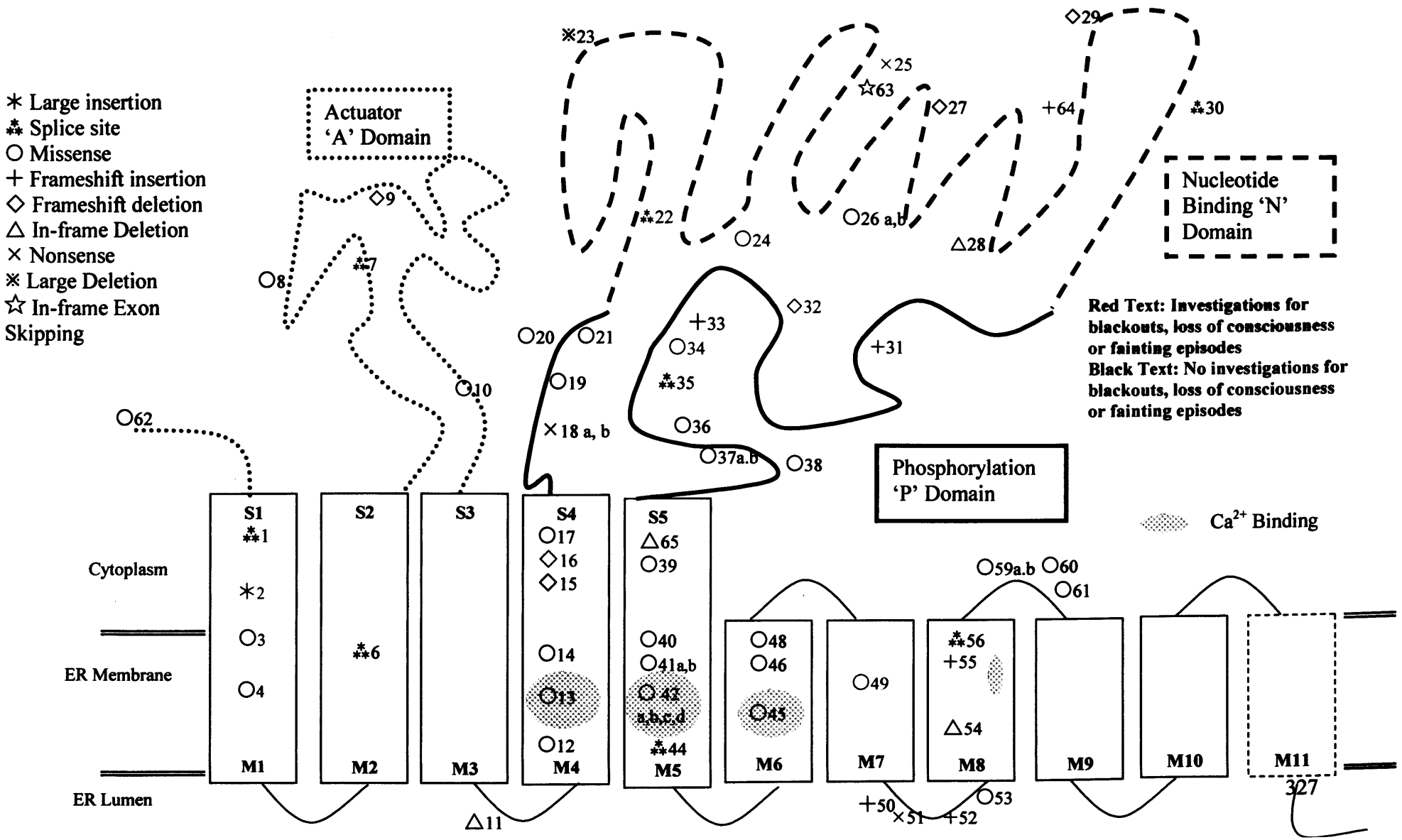
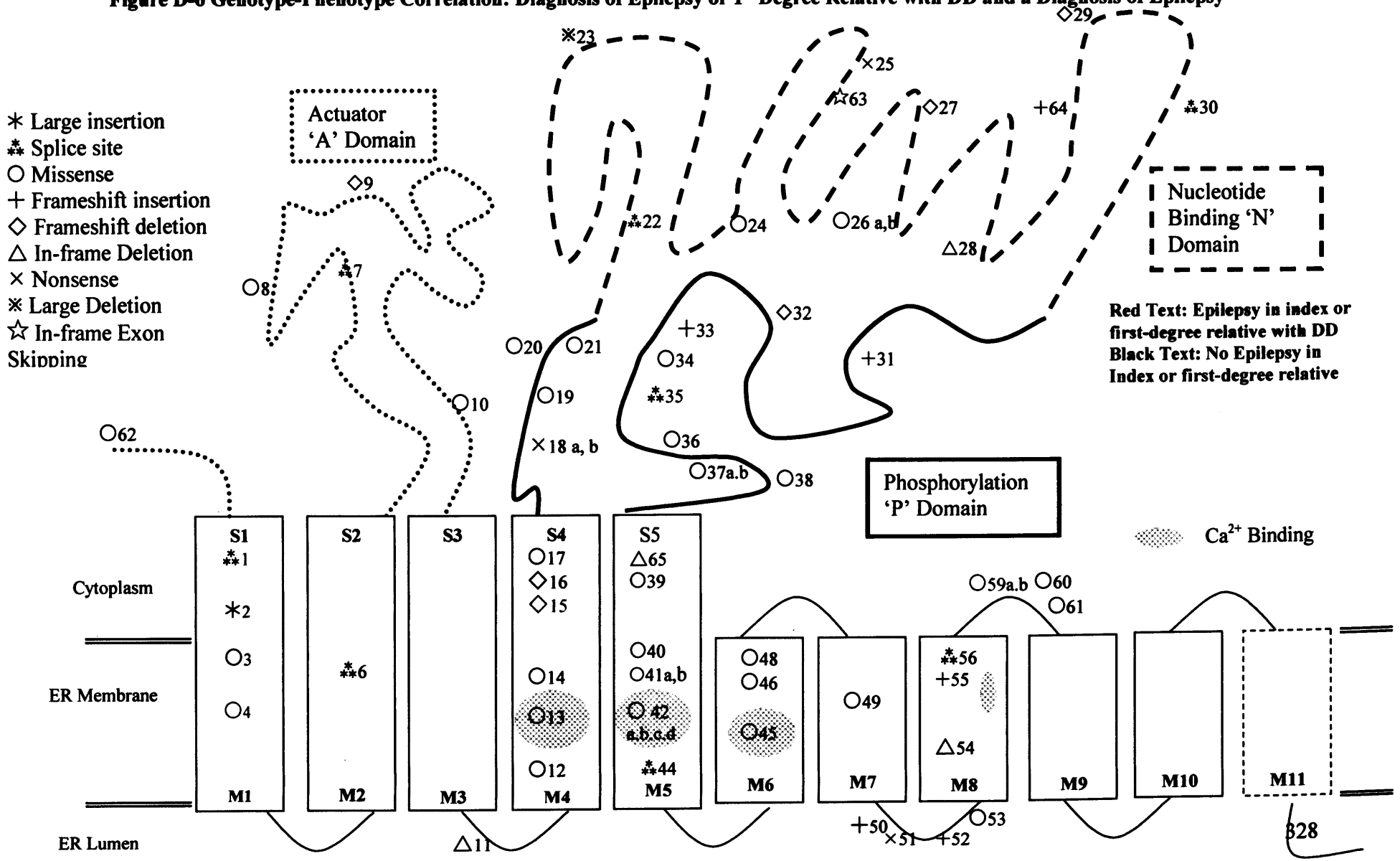


Figure D-6 Genotype-Phenotype Correlation: Diagnosis of Epilepsy or 1st Degree Relative with DD and a Diagnosis of Epilepsy



D.iv Investigations of Genotype-Phenotype Correlations- Mutation Type

Table D-11 Relationship Between Mutation Type and Neuropsychiatric Phenotypes (1)

	Any Lifetime DSM-IV Diagnosis					History of Suicide Attempts					History of Psychiatric Contact					Learning Difficulties					Investigations for Blackouts, Loss of Consciousness or Fainting Episodes				
	No		Yes			No		Yes			No		Yes			No		Yes			No		Yes		
MUTATION	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N
Frameshift	2	18	9	82	**11**	7	70	3	30	**10**	5	50	5	50	**10**	10	83	2	17	**12**	8	73	3	27	**11**
In-frame	4	100	0	0	**4**	4	100	0	0	**4**	4	100	0	0	**4**	3	75	1	25	**4**	4	100	0	0	**4**
Missense	13	37	22	63	**35**	30	86	5	14	**35**	27	73	10	27	**37**	27	73	10	27	**37**	32	89	4	11	**36**
Nonsense	1	33	2	67	**3**	3	75	1	25	**4**	2	50	2	50	**4**	2	50	2	50	**4**	2	67	1	33	**3**
Other	1	33	2	67	**3**	2	67	1	33	**3**	1	33	2	67	**3**	3	100	0	0	**3**	2	67	1	33	**3**
Splice Site	4	57	3	43	**7**	8	100	0	0	**8**	6	75	2	25	**8**	6	75	2	25	**8**	6	86	1	14	**7**
Total N*	25		38		**63**	54		10		**64**	45		21		**66**	51		17		**68**	54		10		**64**
	χ^2=9.291, df=5, p= 0.090					χ^2=4.817, df=5, p= 0.441					χ^2=6.242, df=5, p= 0.284					χ^2=2.859, df=5, p= 0.760					χ^2=3.866, df=5, p= 0.527				

Exact significance tests for Pearson's chi-square. *Missing cases are individuals where lifetime history of a specific neuropsychiatric phenotype was unknown.

Table D-12 Relationship between Mutation Type and Neuropsychiatric Phenotypes (2)

	Index with Bipolar Disorder or 1st Degree Relative with DD and Bipolar Disorder					Index with Epilepsy or 1st Degree Relative with DD and Epilepsy				
	No		Yes			No		Yes		
MUTATION	N	%	N	%	N	N	%	N	%	N
Frameshift	11	92	1	8	**12**	11	92	1	8	**12**
In-frame	4	100	0	0	**4**	4	100	0	0	**4**
Missense	35	95	2	5	**37**	35	95	2	5	**37**
Nonsense	4	100	0	0	**4**	4	100	0	0	**4**
Other	2	67	1	33	**3**	3	100	0	0	**3**
Splice Site	7	88	1	12	**8**	6	75	2	25	**8**
Total N	63		5		**68**	63		5		**68**
	χ^2=3.570, df=5, p= 0.657					χ^2=4.753, df=5, p= 0.413				

Exact significance tests for Pearson's chi-square.

Table D-13 Relationship between Mutation Types and BADDS Mania (M) and Depression (D) Scores and Age of First Psychiatric Illness

		Frameshift	In-frame	Missense	Nonsense	Other	Splice Site	H*	p
BADDS M	Median (Iq R)	12.00 (14)	4.00 (11)	8.00 (11)	17.00	41.00	13.50(46)	2.620	0.758
	Range	0-90	2-16	0-70	1-17	1-81	6-65		
	N**	7	4	27	3	2	4		
BADDS D	Median (Iq R)	61.50 (38)	-	51.00 (24)	63.50	59.50	41.00	3.369	0.498
	Range	7-95	-	10-70	55-72	50-69	30-70		
	N**	6	0	19	2	2	3		
First Psychiatric Illness (years)	Median (Iq R)	27 (29)		27 (16)	29.5	24.5	25	0.424	0.981
	Range	15-52	-	15-58	29-30	18-31	15-48		
	N**	4	0	17	2	2	3		

*Kruskal-Wallis H Tests. BADDS; Bipolar Affective Disorder Dimensional Scale, Iq R; Interquartile Range.

**Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or individuals not given a BADDS M and/or D score and/or individuals with no psychiatric illness or where age of onset of psychiatric illness was unknown.

D.v Investigations of Genotype-Phenotype Correlations -Mutations Located in Functional Domains of SERCA2b and Neuropsychiatric Phenotypes

Table D-14 Relationship between Mutation Location in the A, N, P and Ca^{2+} Binding Domains of SERCA2b and Neuropsychiatric Phenotypes (1)

	Any Lifetime DSM-IV Diagnosis					History of Suicide Attempts					History of Psychiatric Contact					Learning Difficulties					Investigations for Blackouts, Loss of Consciousness or Fainting Episodes				
	No		Yes			No		Yes			No		Yes			No		Yes			No		Yes		
DOMAIN	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N
A No	25	42	34	58	59	51	85	9	15	60	45	73	17	27	62	48	94	3	6	51	50	93	4	7	54
Yes	0	0	4	100	4	3	75	1	25	4	0	0	4	100	4	16	94	1	6	17	10	100	0	0	10
Total N*	25		38		63	54		10		64	45		21		66	64		4		68	60		4		64
	Fisher's, p= 0.145					Fisher's, p= 0.502					**Fisher's, p= 0.008**					Fisher's, p=1					Fisher's, p=1				
N No	22	42	30	58	52	46	88	6	12	52	38	70	16	30	54	42	82	9	18	51	46	85	8	15	54
Yes	3	27	8	73	11	8	67	4	33	12	7	58	5	42	12	14	82	3	18	17	6	60	4	40	10
Total N*	25		38		63	54		10		64	45		21		66	56		12		68	52		12		64
	Fisher's, p= 0.503					Fisher's, p= 0.082					Fisher's, p=0.499					Fisher's, p=1					Fisher's, p=0.082				
P No	17	34	33	66	50	41	82	9	18	50	33	63	19	37	52	41	80	10	20	51	43	80	11	20	54
Yes	8	62	5	38	13	13	93	1	7	14	12	86	2	14	14	13	76	4	24	17	8	80	2	20	10
Total N*	25		38		63	54		10		64	45		21		66	54		14		68	51		13		64
	χ^2=3.3269, df=1, p= 0.071					Fisher's, p= 0.437					Fisher's, p=0.127					Fisher's, p=0.737					Fisher's, p=1				
Ca^{2+} binding No	17	40	26	60	43	37	82	8	18	45	28	62	17	38	45	31	61	20	39	51	36	67	18	33	54
Yes	8	40	12	60	20	17	89	2	11	19	17	81	4	19	21	15	88	2	12	17	8	80	2	20	10
Total N*	25		38		63	54		10		64	45		21		66	46		22		68	44		20		64
	χ^2=0.001, df=1, p= 0.972					Fisher's, p= 0.451					χ^2=2.315, df=1, p=0.128					**χ^2=4.390, df=1, p=0.036**					Fisher's, p=0.486				

Chi-square tests, Fisher's; Fisher's exact tests. *Missing cases are where the lifetime history of a specific neuropsychiatric phenotype was unknown.

Table D-15 Relationship between Mutation Location in the A, N, P and Ca^{2+} Binding Domains of SERCA2b and Neuropsychiatric Phenotypes (2)

		Index with Bipolar Disorder or 1st Degree Relative with DD and Bipolar Disorder					Index with Epilepsy or 1st Degree Relative with DD and Epilepsy				
		No		Yes			No		Yes		
SERCA2b Domain		N	%	N	%	N	N	%	N	%	N
A	**No**	60	95	3	5	63	60	95	3	5	63
	Yes	4	80	1	20	5	4	80	1	20	5
	Total *N	64		4		68	64		4		68
		Fisher's, p=0.269					Fisher's, p=0.269				
N	**No**	52	83	11	17	63	51	81	12	19	63
	Yes	4	80	1	20	5	5	100	0	0	5
	Total *N	56		12		68	56		12		68
		Fisher's, df=1, p=1					Fisher's, p=0.577				
P	**No**	49	78	14	22	63	49	78	14	22	63
	Yes	5	100	0	0	5	5	100	0	0	5
	Total *N	54		14		68	54		14		68
		Fisher's, p=0.575					Fisher's, p=0.575				
Ca^{2+} binding	**No**	43	68	20	32	63	42	67	21	33	63
	Yes	3	60	2	40	5	4	80	1	20	5
	Total *N	46		22		68	46		22		68
		Fisher's, p=0.656					Fisher's, df=1, p=1				

Fisher's; Fisher's exact tests. *Missing cases are where lifetime history of a specific neuropsychiatric phenotype was unknown.

Table D-16 Relationship between Mutation Location in the A, N, P and Ca^{2+} Binding Domains of SERCA2b and BADDS Mania (M) and Depression (D) Scores and Age of First Psychiatric Contact

Functional Domain		Individuals with mutations within domain	Individuals with mutations not within domain	U*	P
BADDS Mania Scores					
N domain	Median (Iq R)	36.5 (-)	8 (14)	25.5	0.303
	Range	8-65	0-90		
	N*	2	45		
N domain	Median (Iq R)	7.5 (13)	8 (14)	180	0.908
	Range	0-90	0-81		
	N*	10	37		
P domain	Median (Iq R)	5.5 (14)	9 (12)	162	0.549
	Range	1-17	0-90		
	N*	10	37		
Ca binding domains	Median (Iq R)	10 (14)	7.5 (13)	235.5	0.649
	Range	0-70	0-90		
	N*	17	30		
BADDS Depression Scores					
A domain	Median (IqR)	56.5 (25)	53 (25)	51.5	0.797
	Range	39-70	7-95		
	N*	4	28		
N domain	Median (IqR)	63 (28)	52 (24)	71	0.276
	Range	7-95	10-74		
	N*	8	24		
P domain	Median (IqR)	55.5 (20)	54 (26)	52	0.819
	Range	39-62	7-95		
	N*	4	28		
Ca binding domains	Median (IqR)	51 (32)	55 (24)	93.5	0.675
	Range	10-74	7-95		
	N*	9	23		
Age of Onset of Psychiatric Illness (Years)					
A domain	Median (IqR)	23 (22)	27 (13)	40.5	0.622
	Range	15-40	15-58		
	N*	4	24		
N domain	Median (IqR)	34 (-)	27 (13)	72	0.936
	Range	22-41	15-58		
	N*	3	25		
P domain	Median (IqR)	28 (12)	27 (16)	24	0.315
	Range	15-52	15-58		
	N*	7	21		
Ca binding domains	Median (IqR)	24 (25)	28 (12)	75.5	0.622
	Range	15-58	15-52		
	N*	9	19		

*Mann-Whitney U Tests. BADDS; Bipolar Affective Disorder Dimensional Scale, Iq R; Interquartile Range.
**Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or individuals not given a BADDS M and/or D score and/or individuals with no psychiatric illness or where age of onset of psychiatric illness was unknown.

D.vi Mutation Location within *ATP2A2* and Neuropsychiatric Phenotypes

Table D-17 Relationship between Mutation Location in *ATP2A2* and Neuropsychiatric Phenotypes (1)

	Any Lifetime DSM-IV Diagnosis					History of Suicide Attempts					History of Psychiatric Contact					Learning Difficulties					Investigations for Blackouts, Loss of Consciousness or Fainting Episodes				
	No		Yes			No		Yes			No		Yes			No		Yes			No		Yes		
ATP2A2 Position	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N
First Third	8	47	9	53	17	16	84	3	16	19	11	58	8	42	19	15	79	4	21	19	17	89	2	11	19
Middle	7	39	11	61	18	15	79	4	21	19	14	74	5	26	19	14	74	5	26	19	12	67	6	34	18
Last Third	10	36	18	64	28	23	88	3	12	26	20	71	8	29	28	22	73	8	27	30	25	93	2	7	27
Total N*	25		38		63	54		10		64	45		21		66	51		17		68	54		10		64
	χ^2=0.575, df=2, p= 0.750					χ^2=0.754, df=2, p= 0.763					χ^2=1.328, df=2, p= 0.515					χ^2=0.220, df=2, p= 0.939					**χ^2=6.039, df=2, p=0.048**				

Exact significance tests for Pearson's chi-square. *Missing cases are where lifetime history of a specific neuropsychiatric phenotype was unknown.

Table D-18 Relationship between Mutation Location in *ATP2A2* and Neuropsychiatric Phenotypes (2)

	Index with Bipolar Disorder or 1st Degree Relative with DD and Bipolar Disorder					Index with Epilepsy or 1st Degree Relative with DD and Epilepsy				
	No		Yes			No		Yes		
ATP2A2 Position	N	%	N	%	N	N	%	N	%	N
First Third	16	84	3	16	19	15	79	4	21	19
Middle	18	95	1	5	19	19	100	0	0	19
Last Third	29	97	1	3	30	29	97	1	3	30
Total N	63		5		68	63		5		68
	χ^2=2.818, df=2, p= 0.371					**χ^2=7.454, df=2, p=0.039**				

Exact significance tests for Pearson's chi-square.

Table D-19 Relationship between Mutation Location and BADDS Mania and Depression Domains

		First Third	Middle Third	Last Third	H*	P
BADDS Mania	Median (Iq R)	13.5 (23)	7.5 (13)	7 (15)	1.209	0.546
	Range	1-81	0-90	0-70		
	N**	12	14	21		
BADDS Depression	Median (Iq R)	56.50 (18)	61.50 (22)	46.00 (31)	3.206	0.201
	Range	39-74	7-95	10-70		
	N**	8	10	14		
Age of Onset of Psychiatric Illness (years)	Median (Iq R)	27 (15)	29 (13)	25 (19)	0.267	0.875
	Range	15-40	15-52	15-58		
	N**	7	8	13		

*Kruskal-Wallis H Tests. BADDS; Bipolar Affective Disorder Dimensional Scale, Iq R; Interquartile Range.

** Missing cases are 4 individuals where no DNA was collected and no mutation information was known and/or individuals not given a BADDS M and/or D score and/or individuals with no psychiatric illness or where age of onset of psychiatric illness was unknown.

E Appendices for Chapter 10

E.i Unaffected Relatives Invitation Letter, Information Sheet and Consent Form

UNAFFECTED RELATIVES INVITATION LETTER

Dear

I am a member of a team of psychiatrists and psychologists who work in the Department of Psychological Medicine at Cardiff University and Department of Psychiatry at the University of Birmingham. We are conducting research into features that may be associated with Darier's Disease. I enclose with this letter a short information sheet about our study.

If you may be interested in taking part, or would like further details, I would be very grateful if you would complete the enclosed form and return it in the enclosed stamped addressed envelope.

Many thanks in anticipation of your help,

Katherine Gordon-Smith
Research Psychologist

UNAFFECTED RELATIVES INFORMATION SHEET

INFORMATION ABOUT RESEARCH INTO DARIER'S DISEASE

INTRODUCTION

I am a member of a team of researchers who work in the Department of Psychological Medicine at Cardiff University and Department of Psychiatry at the University of Birmingham. We are conducting research (funded by the Wellcome Trust) to learn more about features that may be associated with Darier's Disease.

WHAT IS THE RESEARCH ABOUT?

It is known that sometimes disorders affecting the nervous system (such as epilepsy or depression) occur in individuals with Darier's Disease. However, such disorders are common in the general population and it is not known whether this is just a coincidence or whether there is any relationship to the Darier's Disease itself. The idea of this study is to compare individuals with Darier's Disease with other individuals who do not have the illness in order to discover whether there are any problems that are specifically related to Darier's Disease and also to try to find out what influences their occurrence. This research will help us understand more about Darier's Disease itself and, importantly, the treatment needs of people suffering with the illness. As an unaffected family member of a sufferer of Darier's Disease, we would be extremely grateful if you would be kind enough to help us with this study.

WHAT DOES TAKING PART INVOLVE?

We are hoping to recruit a sample of at least 100 individuals who suffer from Darier's Disease. We would also be very interested in seeing other available family members, whether they suffer from Darier's Disease or not. Participation as an unaffected family member involves:
an interview (lasting around 1½ hours) asking you about any medical, neurological and psychiatric conditions you have experienced
a series of short tasks designed to assess cognitive abilities e.g. memory, problem solving skills and attention (¾ hour)
completing a set of questionnaires (¼ hour)
giving a small blood sample from your arm (approximately 10mls).

If you agree to take part, a researcher will arrange a suitable time to visit you in your home or another place convenient for you. We will only need to see you the once but may ask if you would like to take part in future research. We will keep you informed of any interesting results and of future studies into Darier's Disease.

With your permission, we may ask to look at your medical records in strict confidence.

WHAT ARE THE BENEFITS OF TAKING PART ? ARE THERE ANY RISKS?

By taking part in the study you will not gain any direct benefit. However, your help will be of great value in allowing us to learn more about Darier's Disease and this is likely to help improve treatments.

As this study does not include any treatment changes or invasive techniques, there are no real risks of taking part. You may experience mild bruising on you arm after giving a blood sample.

DECLINING AND WITHDRAWING FROM THE STUDY

Taking part in the research is entirely voluntary. It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason.

A decision to withdraw at any time, or a decision not to take part, will not affect the standard of any care you may receive.

CONFIDENTIALITY

All interviews and results will be strictly confidential.

Your blood cells may be kept growing in the laboratory in order to provide samples for future use in this research, by us and by other researchers working with us. The results of your blood test are for research purposes only and will not be available to anybody on an individual basis.

All information given is kept in an anonymous format.

If you have any further questions about this research, we would be delighted to answer them in person or over the telephone. Our address appears above, our telephone number is 0121 678 2352, and our e-mail address is k.m.gordonsmith@bham.ac.uk

Katherine Gordon-Smith - Research Psychologist
Professor Nick Craddock - Head of *Department*
Dr Lisa Jones- Research Psychologist

UNAFFECTED RELATIVES CONSENT FORM

AGREEMENT TO TAKE PART IN THE STUDY OF DARIER'S DISEASE

I have read the attached information sheet (Version 4 07.06.05) on the above project and have been given a copy to keep. I have had the opportunity to ask questions about the project and understand why the research is being done.

I understand that participation in this project is voluntary and that I am free to withdraw from the study without giving a reason.

I understand that the tests done as part of this research are not clinically diagnostic and I will not be informed of any specific results.

I understand that I will not benefit personally from taking part in this research.

I understand that the information I have donated for this study will be held in a confidential and coded form by the research team and may be made available to researchers at other centres who are carrying out similar work.

I know how to contact the research team if I need to.

NAME _____ SIGNED _____

DATE _____

THANK YOU FOR PARTICIPATING IN OUR RESEARCH
THIS COPY IS FOR YOU TO KEEP

E.ii Unaffected Relatives Questionnaire

A. GENERAL INFORMATION

Name _____ **Sex** Male ☐ Female ☐ (please tick)

Date of Birth (DD/MM/YY) _____

Name of family member who passed on this questionnaire pack

_____

What is your relationship to this family member (brother, sister, mother, father, son, daughter)

_____

Please give your address if you are willing to be sent a saliva collection kit in the post (it should take less than 5 minutes to do)

_____

_____

_____

_____

Please tick the following box if you would be willing to receive a brief follow up telephone call to discuss your some of the information you have provided in this questionnaire pack ☐

Telephone number _____

Please state any days or times when it would be most convenient to contact you

_____

B. PERSONAL MEDICAL AND PSYCHIATRIC HISTORY

1. Have you ever been diagnosed with Darier's disease yes __ no __ (please tick one)

2. Have you ever experienced any other skin conditions/problems (e.g. eczema, psoriasis) yes__ no__ (please tick one)

If yes please give brief details _____
_____
_____
_____

3.Do you currently suffer from any physical illnesses yes __ no __ (please tick one)

If yes please give brief details _____
_____
_____
_____

4. Are you currently on any medication yes __ no __ (please tick one)

If yes please give brief details _____
_____
_____

_____

5. Have you ever been a regular smoker: (please tick one)

□ No □ Yes in past now stopped
□ Yes, currently

6. Have you ever experienced any of the following problems in your life? – even if very mild (please tick one for each)

	Yes	No
Anxiety or panic attacks	□	□
Feeling very low in spirits, depression or low mood	□	□
Feeling much too high in spirits or elated or very irritable without reason, manic depression or bipolar disorder	□	□
Experiencing things that are difficult to explain or understand like hearing voices or seeing things, psychosis or schizophrenia	□	□
Checking things you know you have done keeping things in a special order, obsessive compulsive disorder	□	□
Eating disorders	□	□
Problems due to alcohol or other substances	□	□

7. If you answered YES to any of the items above, please could you indicate any treatment you have had:
(tick as many as are relevant)

	Sought help from GP or other health professional	*Prescribed Medication*	*Received Counselling*	*Seen Psychiatrist*	*Hospital Admission*	*None*
Anxiety or panic attacks	□	□	□	□	□	□
Depression or low mood	□	□	□	□	□	□
Feeling much too high in spirits or elated or very irritable without reason, manic depression or bipolar disorder	□	□	□	□	□	□
Experiencing things that are difficult to explain or understand like hearing voices or seeing things, psychosis or schizophrenia	□	□	□	□	□	□
Checking things you know you have done, keeping things in a special order, obsessive compulsive disorder	□	□	□	□	□	□
Eating disorders	□	□	□	□	□	□
Problems due to alcohol or other substances	□	□	□	□	□	□

8. If you have responded 'yes' to any of items in question 6 or ticked any of the items in question 7 please could you provide brief details such as the symptoms you have experienced, how often they have occurred, how long they lasted, how severe they have been and the treatment you have received.

C. NEUROLOGICAL SYMPTOMS

1. Have you ever experienced any of the following symptoms? (please tick)

Head injury	☐	Severe Headaches	☐
Fits, faints, funny turns, epilepsy or seizure, loss of consciousness	☐	Memory difficulties or diagnosis "dementia"	☐
Weaknesses of the limbs	☐	Loss of sensation in the limbs	☐
Tremors or shaking	☐	Difficulty walking	☐
Giddiness or difficulty with balance	☐	Stokes, brain haemorrhages, (TIAs)	☐
Brain tumour or cancer affecting brain or nervous system	☐	Infections of the brain or nervous system	☐
Problems with vision	☐	Problems with hearing	☐

Developmental delay or development problem relating to the brain or nervous system ☐
Any other symptoms or treatment related to problems with the brain or nervous system ☐

2. Have you ever been seen by a neurologist? Please tick **No** ☐ **Yes** ☐ **Unsure** ☐

3. Have you ever had neurological procedures such as brain scans (CT, MRI), brain wave recordings (EEG), lumbar punctures? Please tick No ☐ Yes ☐ Unsure ☐

If you have ticked any of the items in question 1 or answered YES to either question 2 or 3 please could you provide brief details of the symptoms and procedures you have had

D. PARTICIPANT QUESTIONNAIRE

1. Please tick which of the following qualifications you have obtained (*please tick as many as necessary):*

- ☐ None
- ☐ 11+
- ☐ O-Level/GCSE
- ☐ A-Level/HND/BTEC
- ☐ Degree
- ☐ Post-graduate degree

2. Please tick which of the following types of jobs have you have had (*tick as many as necessary):*

- ☐ Never worked
- ☐ Never worked due to sickness/disablement

- ☐ Legislator/senior official/manager
- ☐ Technician/associate professional/civil servant
- ☐ Service worker/shop worker/market worker
- ☐ Skilled agricultural /fishery worker
- ☐ Plant & machinery operator/assembler
- ☐ Other (*please specify*) _____
- ☐ Other professional
- ☐ Clerk
- ☐ Craft and related trade worker
- ☐ Armed forces
- ☐ Homemaker

3. Please tick which of the following types of jobs <u>your father</u> has had (*tick as many as necessary):*

- ☐ Unknown
- ☐ Never worked
- ☐ Never worked due to sickness/disablement

- ☐ Legislator/senior official/manager
- ☐ Technician/associate professional/civil servant
- ☐ Service worker/shop worker/market worker
- ☐ Skilled agricultural /fishery worker
- ☐ Plant & machinery operator/assembler
- ☐ Other (*please specify*) _____
- ☐ Other professional
- ☐ Clerk
- ☐ Craft and related trade worker
- ☐ Armed forces
- ☐ Homemaker

4. Please tick which of the following types of jobs <u>your mother</u> has had (*tick as many as necessary):*

- ☐ Unknown
- ☐ Never worked
- ☐ Never worked due to sickness/disablement

- ☐ Legislator/senior official/manager
- ☐ Technician/associate professional/civil servant
- ☐ Service worker/shop worker/market worker
- ☐ Skilled agricultural /fishery worker
- ☐ Plant & machinery operator/assembler
- ☐ Other (*please specify*) _____
- ☐ Other professional
- ☐ Clerk
- ☐ Craft and related trade worker
- ☐ Armed forces
- ☐ Homemaker

5. Please tick which of the following best describes your current occupational status:

- ☐ Employed full time
- ☐ Employed part time
- ☐ Unemployed – receiving benefits
- ☐ Unemployed – not receiving benefits
- ☐ Homemaker
- ☐ Student
- ☐ Retired

6. Have you been married/ lived with a partner as though married?

- ☐ Yes
- ☐ No

7. Please tick which of the following best describes your current social circumstances:

- ☐ Living alone
- ☐ Living with partner at least one year but not married
- ☐ Living in own home with spouse and /or children
- ☐ Living in home with parents or children
- ☐ Living in home of siblings
- ☐ Living in shared home with other relatives or friends
- ☐ Living in residential treatment facility
- ☐ Other

8. Place of birth (please tick)

- ☐ England – Midlands
- ☐ England – North
- ☐ England – South
- ☐ Wales – North
- ☐ Wales – South
- ☐ Scotland – North
- ☐ Scotland – South
- ☐ Northern Ireland
- ☐ Republic of Ireland
- ☐ Other Europe
- ☐ Asia
- ☐ Africa
- ☐ North America
- ☐ South America
- ☐ Australasia
- ☐ Other
- ☐ Unknown

9. Ethnic Origin (please tick)

□ UK/Irish Caucasian □ Asian
□ West European Caucasian □ Mixed race
□ Other Caucasian □ Unknown
□ Afro-Caribbean

10. Which hand do you write with (please tick) Right □ Left □ Ambidextrous □

11. Did you experience any problems in the following areas at school (Please tick)

Reading	No □	Mild problems □	Major Problems □	Unsure □
Spelling	No □	Mild problems □	Major Problems □	Unsure □
Writing	No □	Mild problems □	Major Problems □	Unsure □
Sums/numbers	No □	Mild problems □	Major Problems □	Unsure □
Coordination/ clumsiness	No □	Mild problems □	Major Problems □	Unsure □

12. Have you ever had any of the following diagnoses (Please tick)

Dyslexia	No □	Yes □	Unsure □
Dyspraxia	**No** □	**Yes** □	**Unsure** □

13. Did you receive any help extra help at school (Please tick)

No □ Yes □ Unsure □

E.iii Personality and Temperament Questionnaires

1.BECK DEPRESSION INVENTORY (BDI)

On this questionnaire are groups of statements. Please read each group of statements carefully, circle the number (0, 1, 2 or 3) next to the one statement in each group which ***best*** describes how you feel ***today***. If several statements within a group seem to apply equally well, circle each one. ***Be sure to read all the statements in each group before making your choice.***

1	0	I do not feel sad.
	1	I feel sad.
	2	I am sad all the time and I can't snap out of it.
	3	I am so sad or unhappy that I can't stand it.
2	0	I am not particularly discouraged about the future.
	1	I feel discouraged about the future.
	2	I feel I have nothing to look forward to.
	3	I feel that the future is hopeless and that things cannot improve.
3	0	I do not feel like a failure.
	1	I feel I have failed more than the average person.
	2	As I look back on my life, all I can see is a lot of failures.
	3	I feel I am a complete failure as a person.
4	0	I get as much satisfaction out of things as I used to.
	1	I don't enjoy things the way I used to.
	2	I don't get real satisfaction out of anything anymore.
	3	I am dissatisfied or bored with everything.
5	0	I don't feel particularly guilty.
	1	I feel guilty a good part of the time.
	2	I feel quite guilty most of the time.
	3	I feel guilty all of the time.
8	0	I don't feel I am worse than anyone else.
	1	I am critical of myself for my weaknesses or mistakes.
	2	I blame myself all the time for my faults.
	3	I blame myself for everything bad that happens.
9	0	I don't have any thoughts of killing myself.
	1	I have thoughts of killing myself, but I would not carry them out.
	2	I would like to kill myself.
	3	I would kill myself if I had the chance.
10	0	I don't cry any more than usual.
	1	I cry more now than I used to.
	2	I cry all the time now.
	3	I used to be able to cry, but now I can't cry even though I want to.
11	0	I am no more irritated now than I ever am.
	1	I get annoyed or irritated more easily than I used to.
	2	I feel irritated all the time now.
	3	I don't get irritated at all by the things that used to irritate me.
12	0	I have not lost interest in other people.
	1	I am less interested in other people than I used to be.
	2	I have lost most of my interest in other people.
	3	I have lost all of my interest in other people.

6	0	I don't feel I am being punished.
	1	I feel I may be punished.
	2	I expect to be punished.
	3	I feel I am being punished.
7	0	I don't feel disappointed in myself.
	1	I am disappointed in myself.
	2	I am disgusted with myself.
	3	I hate myself.
13	0	I make decisions about as well as I ever could.
	1	I put off making decisions more than I used to.
	2	I have greater difficulty in making decisions than before.
	3	I can't make decisions at all anymore.
14	0	I don't feel I look any worse than I used to.
	1	I am worried that I am looking old or unattractive.
	2	I feel that there are permanent changes in my appearance that make me look unattractive.
	3	I believe that I look ugly.
19	0	I haven't lost much weight, if any, lately.
	1	I have lost more than 5 pounds.
	2	I have lost more than 10 pounds.
	3	I have lost more than 15 pounds.
		I am purposely trying to lose weight by eating less. Yes No (please circle)
15	0	I can work about as well as before.
	1	It takes an extra effort to get started at doing something.
	2	I have to push myself very hard to do anything.
	3	I can't do any work at all.
20	0	I am no more worried about my health than usual.
	1	I am worried about physical problems such as aches and pains; or upset stomach; or constipation.
	2	I am very worried about physical problems and it's hard to think of much else.
	3	I am so worried about my physical problems that I cannot think of anything else.
16	0	I can sleep as well as usual.
	1	I don't sleep as well as I used to.
	2	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
	3	I wake up several hours earlier than I used to and cannot get back to sleep.
21	0	I have not noticed any recent change in my interest in sex.
	1	I am less interested in sex than I used to be.
	2	I am much less interested in sex now.
	3	I have lost interest in sex completely.
17	0	I don't get more tired than usual.
	1	I get tired more easily than I used to.
	2	I get tired from doing almost anything.
	3	I am too tired to do anything.

18	0	My appetite is no worse than usual.	
	1	My appetite is not as good as it used to be.	
	2	My appetite is much worse now.	
	3	I have no appetite at all anymore.	

2.ALTMAN SELF-RATING SCALE FOR MANIA(ARSM)

1. On this questionnaire are groups of five statements. Please read each group of statements carefully.
2. Choose one statement in each group that ***best*** describes how you feel ***today***.
3. Circle the number next to the statement you have picked.
4. Please not the word '*occasionally*' when used here means once or twice. '*Often*' means several times or more. '*Frequently*' means most of the time.

1 0 I do not feel happier or more cheerful than usual.
1 I occasionally feel happier or more cheerful than usual.
2 I often feel happier or more cheerful than usual.
3 I feel happier or more cheerful than usual most of the time.
4 I feel happier or more cheerful than usual all of the time.

2 0 I do not feel more self-confident than usual.
1 I occasionally feel more self-confident than usual.
2 I often feel more self-confident than usual.
3 I feel more self-confident than usual most of the time.
4 I feel more self-confident than usual all of the time.

3 0 I do not need less sleep than usual.
1 I occasionally need less sleep than usual.
2 I often need less sleep than usual.
3 I frequently need less sleep than usual.
4 I can go all day and night without any sleep and still do not feel tired.

4 0 I do not talk more than usual.
1 I occasionally talk more than usual.
2 I often talk more than usual.
3 I frequently talk more than usual.
4 I talk constantly and cannot be interrupted.

5 0 I have not been more active (either socially, sexually, at work, home or school) than usual.
1 I have occasionally been more active than usual.
2 I have often been more active than usual.
3 I have frequently been more active than usual.
4 I am constantly active or on the go all the time.

3. ROSENBERG SELF-ESTEEM SCALE(RSE)

This is a short questionnaire to measure thoughts about yourself.
Please indicate whether you strongly agree, agree, disagree or strongly disagree with each statement by ticking the appropriate box.

	Strongly agree	**Agree**	**Disagree**	**Strongly disagree**
1. On the whole I am satisfied with myself.				
2. At times I think I am no good at all.				
3. I feel I have a number of good qualities.				
4. I am able to do things as well as most people.				
5. I feel I do not have much to be proud of.				
6. I certainly feel useless at times.				
7. I feel I am a person of worth, at least equal to others.				
8. I wish I could have more respect for myself.				
9. All in all, I am inclined to feel I am a failure.				
10. I take a positive attitude towards myself.				

4. EYSENCK PERSONALITY QUESTIONNAIRE (EPQ)

Please answer each of the following questions by ticking the 'yes' or 'no' box following the question.
There are no right or wrong answers, and no trick questions.
Work quickly and do not think too long about the exact meaning of the questions.

Please complete all questions.

	Yes	No
1. Do you have many different hobbies?	☐	☐
2. Do you stop to think things over before doing anything?	☐	☐
3. Does your mood often go up and down?	☐	☐
4. Have you ever taken the praise for something you knew someone else had really done?	☐	☐
5. Are you a talkative person?	☐	☐
6. Would being in debt worry you?	☐	☐
7. Do you ever feel 'just miserable' for no reason?	☐	☐
8. Were you ever greedy by helping yourself to more than your fair share of anything?	☐	☐
9. Do you lock up your house carefully at night?	☐	☐
10. Are you rather lively?	☐	☐
11. Would it upset you a lot to see a child or animal suffer?	☐	☐
12. Do you often worry about things you should not have done or said?	☐	☐
13. If you say you will do something, do you always keep your promise no matter how inconvenient it might be?	☐	☐
14. Can you usually let yourself go and enjoy yourself at a lively party?	☐	☐
15. Are you an irritable person?	☐	☐
16. Have you ever blamed someone for doing something you knew was really your fault?	☐	☐

17. Do you enjoy meeting new people? ☐ ☐

18. Do you believe insurance schemes are a good idea? ☐ ☐

19. Are your feelings easily hurt? ☐ ☐

20. Are *all* your habits good and desirable ones? ☐ ☐

21. Do you tend to keep in the background on social occasions? ☐ ☐

22. Would you take drugs which may have strange or dangerous effects? ☐ ☐

23. Do you often feel 'fed-up'? ☐ ☐

24. Have you ever taken anything (even a pin or button) that belonged to someone else? ☐ ☐

25. Do you like going out a lot? ☐ ☐

26. Do you enjoy hurting people you love? ☐ ☐

27. Are you often troubled about feelings of guilt? ☐ ☐

28. Do you sometimes talk about things you know nothing about? ☐ ☐

29. Do you prefer reading to meeting new people? ☐ ☐

30. Do you have enemies who want to harm you? ☐ ☐

31. Would you call yourself a nervous person? ☐ ☐

32. Do you have many friends? ☐ ☐

33. Do you enjoy practical jokes that can sometimes really hurt people? ☐ ☐

34. Are you a worrier? ☐ ☐

35. As a child, did you do as you were told immediately and without grumbling? ☐ ☐

36. Would you call yourself happy-go-lucky? ☐ ☐

37. Do good manners and cleanliness matter much to you? ☐ ☐

38. Do you worry about awful things that might happen? ☐ ☐

39. Have you ever broken or lost something belonging to someone else? ☐ ☐

40. Do you usually take the initiative in making new friends? ☐ ☐

41. Would you call yourself tense or 'highly-strung'? ☐ ☐

42. Are you mostly quiet when you are with other people? ☐ ☐

43. Do you think marriage is old-fashioned and should be done away with? ☐ ☐

44. Do you sometimes boast a little? ☐ ☐

45. Can you easily get some life into a rather dull party? ☐ ☐

46. Do people who drive carefully annoy you? ☐ ☐

47. Do you worry about your health? ☐ ☐

48. Have you ever said anything bad or nasty about anyone? ☐ ☐

49. Do you like telling jokes and funny stories to your friends? ☐ ☐

50. Do most things taste the same to you? ☐ ☐

51. As a child, were you ever cheeky to your parents? ☐ ☐

52. Do you like mixing with people? ☐ ☐

53. Does it worry you if you know there are mistakes in your work? ☐ ☐

54. Do you suffer from sleeplessness? ☐ ☐

55. Do you always wash before a meal? ☐ ☐

56. Do you nearly always have a 'ready answer' when people talk to you? ☐ ☐

57. Do you like to arrive at appointments in plenty of time? ☐ ☐

58. Have you often felt listless and tired for no reason? ☐ ☐

59. Have you ever cheated at a game? ☐ ☐

60. Do you like doing things in which you have to act quickly? ☐ ☐

61. Is (or was) your mother a good woman? ☐ ☐

62. Do you often feel that life is very dull? ☐ ☐

63. Have you ever taken advantage of someone? ☐ ☐

64. Do you often take on more activities than you have time for? ☐ ☐

65. Are there several people who keep trying to avoid you? ☐ ☐

66. Do you worry a lot about your looks? ☐ ☐

67. Do you think people spend too much time safeguarding their future with savings and insurances? ☐ ☐

68. Have you ever wished you were dead? ☐ ☐

69. Would you dodge paying taxes if you were sure you could never be found out? ☐ ☐

70. Can you get a party going? ☐ ☐

71. Do you try not to be rude to people? ☐ ☐

72. Do you worry too long after an embarrassing experience? ☐ ☐

73. Have you ever insisted on having your own way? ☐ ☐

74. When you catch a train do you often arrive at the last minute? ☐ ☐

75. Do you suffer from 'nerves'? ☐ ☐

76. Do your friendships break up easily without it being your fault? ☐ ☐

77. Do you often feel lonely? ☐ ☐

78. Do you always practise what you preach? ☐ ☐

79. Do you sometimes like teasing animals? ☐ ☐

80. Are you easily hurt when people find fault with you or the work you do? ☐ ☐

81. Have you ever been late for an appointment or work? ☐ ☐

82. Do you like plenty of bustle and excitement around you? ☐ ☐

83. Would you like people to be afraid of you? ☐ ☐

84. Are you sometimes bubbling over with energy and sometimes very sluggish? ☐ ☐

85. Do you sometimes put off until tomorrow what you ought to do today? ☐ ☐

86. Do other people think of you as being very lively? ☐ ☐

87. Do people tell you a lot of lies? ☐ ☐

88. Are you touchy about some things? ☐ ☐

89. Are you always willing to admit it when you have made a mistake? ☐ ☐

90. Would you feel very sorry for an animal caught in a trap? ☐ ☐

5. TEMPERAMENT EVALUATION OF MEMPHIS, PISA, PARIS AND SAN DIEGO-AUTOQUESTIONNAIRE VERSION (TEMPS-A)

Please read the following statements and
Circle T true for all the items that are true about you for much of your life
Circle F false for all the rest that don't apply to you for much of your life

T F My ability to think varies greatly from sharp to dull for no apparent reason
T F I constantly switch between being lively and sluggish
T F I get sudden shifts in mood and energy
T F The way I see things is sometimes vivid, but at other times lifeless
T F My mood often changes for no reason
T F I go back and forth between being outgoing and being withdrawn from others
T F My moods and energy are either high or low, rarely in between
T F I go back and forth between feeling overconfident and feeling unsure of myself
T F My need for sleep varies a lot from just a few hours to more than 9 hours
T F I sometimes go to bed feeling great, and wake up in the morning feeling life is not worth living.
T F I can really like someone a lot, and then completely lose interest in them
T F I am the kind of person who can be sad and happy at the same time
T F People tell me I am unable to see the lighter side of things
T F I'm the kind of person who doubts everything
T F I am a very skeptical person
T F I am by nature a dissatisfied person
T F I'm a sad, unhappy person
T F I think things often turn out for the worst
T F I give up easily
T F I complain a lot
T F People tell me I blow up out of nowhere
T F I can get so furious I could hurt someone
T F I often get so mad that I will just trash everything
T F When crossed, I could get into a fight
T F When I disagree with someone, I can get into a heated argument
T F When angry, I snap at people
T F I am known to swear a lot
T F I have been told that I become violent with just a few drinks
T F I have a gift for speech, convincing and inspiring to others
T F I often get many great ideas
T F I love to tackle new projects, even if risky
T F I like telling jokes, people tell me I am humorous
T F I have abilities and expertise in many areas
T F I am totally comfortable, even with people I hardly know
T F I love to be with a lot of people
T F I am the kind of person who likes to be the boss
T F I am often fearful of someone in my family coming down with a serious disease
T F I am always thinking someone might break bad news to me about a family member
T F When someone is late coming home, I fear they have had an accident

KINGS SCHIZOTYPY QUESTIONNAIRE(KSQ)

INSTRUCTIONS: The following questions are about the way you feel or act. Please answer each question
by putting a circle around the 'yes' or 'no' following the question. There are no right or wrong answers. Please work quickly and do not spend too long on any one question.

1.	Do you ever get the feeling that familiar surroundings seem strange?	Yes	No
2.	Given the choice would you prefer to live with other people than by yourself?	Yes	No
3.	Are you a friendly, outgoing person?	Yes	No
4.	Would you say you're a superstitious person?	Yes	No
5.	Have you ever felt that you looked unreal when you looked at yourself in the mirror?	Yes	No
6.	Do you like authority?	Yes	No
7.	Do you quite often get the feeling that other people are taking notice of you in the street or on a bus or in a restaurant?	Yes	No
8.	Do you often feel muddled or unable to think clearly for long periods of time for no reason?	Yes	No
9.	Do you like mixing with people socially?	Yes	No
10.	Do you feel self-conscious in public?	Yes	No
11.	Do you have a sixth sense?	Yes	No
12.	Have you ever felt unreal, that you were not a real person, not in the living world?	Yes	No
13.	Are there several people who keep trying to avoid you?	Yes	No
14.	Do people often seem to drop hints about you or say things with a double meaning?	Yes	No
15.	Are your thoughts often in a fog?	Yes	No
16.	Do you feel remote from other people?	Yes	No
17.	Do people say they find it easy to get on with you?	Yes	No

18.	Are you clairvoyant or able to see into the future?	Yes	No
19.	Do you often feel puzzled for an hour or more for no apparent reason?	Yes	No
20.	Do people tell you a lot of lies?	Yes	No
21.	Are there people about who are not what they seem to be?	Yes	No
22.	Have you ever had the feeling for hours at a time that things around you were unreal?	Yes	No
23.	Do people find you easy to get to know?	Yes	No
24.	Do people often look at you strangely?	Yes	No
25.	Can other people read your mind?	Yes	No
26.	Do you ever get the feeling that something odd is going on around you that you cannot explain?	Yes	No
27.	Have you ever felt that someone was after you trying to hurt you either mentally or physically?	Yes	No
28.	Do you quite often get the feeling you are being blamed unjustifiably for something or even accused of something?	Yes	No
29.	Do you ever have strange lifeless feelings?	Yes	No
30.	Do you usually want to stay away from other people?	Yes	No
31.	Are you relaxed about meeting people?	Yes	No
32.	Do you ever wonder if other people or forces can influence your actions?	Yes	No
33.	Do you ever hear muttering or whispering when there is no-one there?	Yes	No
34.	Do you feel got at by people in authority such as the police or your superiors at work?	Yes	No
35.	Does there ever seem to be a special meaning in the way things are arranged?	Yes	No
36.	Do parts of your body feel changed or distorted in a way that puzzles you?	Yes	No

37.	Do you prefer having only 1 or 2 close friends?	Yes	No
38.	Are you the type of person who can accept criticism easily?	Yes	No
39.	Do you think other people can feel your feelings?	Yes	No
40.	Have you ever had visions or seen things that other people could not see?	Yes	No
41.	Are some of your neighbours against you?	Yes	No
42.	Does everyone seem to gossip about you?	Yes	No
43.	Do things often seem as though they are almost an imitation of reality?	Yes	No
44.	Are you a quiet, solitary person?	Yes	No
45.	Do you avoid arguments?	Yes	No
46.	Do you often think that there is something like telepathy going on which is directed towards you?	Yes	No
47.	Do you ever hear noises like tapping or music that other people might not hear?	Yes	No
48.	Have you frequently feared that other people might harm you?	Yes	No
49.	Do you ever seem to see special meanings in advertisements or shop windows or on the radio or on T.V.?	Yes	No
50.	Do your surroundings ever feel unreal for hours at a time?	Yes	No
51.	Do your friendships break up easily without it being your fault?	Yes	No
52.	Do you look forward to going out to parties?	Yes	No
53.	Do you sometimes get the idea that other people or forces can control your feelings or your thinking?	Yes	No
54.	Have you ever felt that some part of your body did not belong to you?	Yes	No
55.	Do you have enemies who want to harm you without cause?	Yes	No
56.	Did you ever believe that certain things that		

	happened around you were connected to you, like people talking about you in the streets or following you?	Yes	No
57.	Does the appearance of things or people ever change in a puzzling way?	Yes	No
58.	Do you usually keep yourself to yourself?	Yes	No
59.	Do people have to push you into going out?	Yes	No
60.	Are you in full control of your thoughts?	Yes	No
61.	Do other people seem to be acting instead of being themselves?	Yes	No
62.	Are you suspicious of other people and their intentions?	Yes	No
63.	Do people ever seem to laugh at you or talk about you critically behind your back?	Yes	No

F Appendices for Chapter 11

Table F-1 Demographic Characteristics of Individuals with DD and their Unaffected Relatives- Significance of Differences

	Individuals with Clinical DD and their unaffected relatives N=24 in each group	Individuals with Genetically-confirmed DD and their unaffected relatives N=18 in each group
	p	p
*Age	0.638	0.919
**Female	0.755	0.717
**Lifetime Marital Status	0.731	0.673
***Current Social Circumstances	1.00	0.882
***Current Employment Status	0.875	0.882
***Main Lifetime Occupation	0.479	0.565
*** Highest Level of Educational Qualifications	0.125	0.195

* Paired samples T Tests, **chi-square tests, *** exact significance tests for Pearson's chi-square.

Table F-2 Correlations Personality and Temperament Questionnaire Scores and Current BDI Scores

			*Correlation with current BDI Scores
Total RSE Scores	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	rho= -0.512, n=14, p=0.061
		Unaffected	rho= -0.236, n=14, p=0.417
Negative RSE subscale Score	**Individuals with Clinical DD and their unaffected relatives**	DD	rho= -0.425, n=18, p=0.079
		Unaffected	rho= -0.350, n= 18, p=0.155
	Individuals with Genetically-confirmed DD and their unaffected relatives	DD	rho= -0.414, n=14, p=0.141
		Unaffected	rho=-0.714, n=14, p=0.522
EPQ Neuroticism	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	rho=0.340, n=14, p=0.234
		Unaffected	rho=0.519, n=14, p=0.057
TEMPS-A Cyclothymic	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	**rho=0.779, n=15, p=0.001**
		Unaffected	rho=0.152, n=15, p=0.589
TEMPS-A Hyperthymic	**Individuals with Clinical DD and their unaffected relatives**	DD	**rho=-0.552, n=21, p=0.01**
		Unaffected	rho=-0.098, n=21,p=0.672
Total KSQ	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	rho= 0.155, n=10, p=0.668
		Unaffected	rho=0.508, n=10, p=0.134
KSQ Ideas of Reference	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	rho=0.107, n=10, p=0.768
		Unaffected	rho=0.506, n=10, p=0.135
KSQ Recurrent Illusions 1	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD	rho=0.322, n=10, p=0.364
		Unaffected	rho=0.158, n=10, p=0.663

*Spearman's rho correlation coefficients. Individuals were included where both individuals from a DD-Unaffected pair had completed the BDI and the personality/temperament questionnaire.
BDI; Beck Depression Inventory, RSE; Rosenberg Self-Esteem Scale, EPQ; Eysenck Personality Questionnaire, TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version, KSQ; Kings Schizotypy Questionnaire.

Table F-3 Comparison of Temperament and Personality Questionnaire Score of Individuals with DD and Their Unaffected Relatives (analysis of individuals with scores of <10 on the BDI)

			Mean	Standard Deviation	*t	p
Total RSE Scores	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=8	27.13	4.970	1.510	0.175
		Unaffected**N=8	31.38	5.181		
Negative RSE subscale Score	**Individuals with Clinical DD and their unaffected relatives**	DD**N=10	13.60	3.062	1.686	0.126
		Unaffected**N=10	16.00	3.333		
	Individuals with Genetically-confirmed DD and their unaffected relatives	DD**N=8	13.00	3.117	0.1394	0.206
		Unaffected**N=8	15.50	3.423		
EPQ Neuroticism	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=8	12.25	5.994	**3.656**	**0.008**
		Unaffected**N=8	7.00	4.840		
TEMPS-A Cyclothymic	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=8	2.00	1.690	0.271	0.794
		Unaffected**N=8	2.25	2.053		
TEMPS-A Hyperthymic	**Individuals with Clinical DD and their unaffected relatives**	DD**N=10	3.30	1.829	1.285	0.231
		Unaffected**N=10	4.60	2.221		
Total KSQ	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=6	10.17	5.636	**3.005**	**0.030**
		Unaffected**N=6	6.33	4.179		
KSQ Ideas of Reference	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=6	1.83	1.472	**4.00**	**0.010**
		Unaffected**N=6	0.50	0.837		
KSQ Recurrent Illusions 1	**Individuals with Genetically-confirmed DD and their unaffected relatives**	DD**N=6	0.67	1.211	1.464	0.203
		Unaffected**N=6	0.17	0.408		

*Paired Samples T Tests. **DD-Unaffected pairs were included where both individuals completed the BDI questionnaire and the personality/temperament questionnaire and had a score of <10 on the BDI.
BDI; Beck Depression Inventory, DD; Darier's Disease, RSE; Rosenberg Self-Esteem Scale, EPQ; Eysenck Personality Questionnaire, TEMPS-A; Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire version, KSQ; Kings Schizotypy Questionnaire.