CANDIDATE GENE STUDIES IN HUMAN ANXIETY DISORDERS

Jonas Donner

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in lecture hall 2, Biomedicum Helsinki, on March 9th 2012, at 12 noon.

Research Programs Unit – Molecular Neurology
and Department of Medical Genetics, Haartman Institute,
University of Helsinki
and
Department of Mental Health and Substance Abuse Services,
National Institute for Health and Welfare

Helsinki, Finland 2012

Helsinki University Biomedical Dissertations Series No. 159
ISSN 1457-8433

Cover graphic: Christina Donner

ISBN 978-952-10-7707-4 (paperback)
ISBN 978-952-10-7708-1 (PDF)
http://ethesis.helsinki.fi/

Unigrafia Oy
Helsinki, Finland 2011

Supervised by

Docent Iiris Hovatta
Research Programs Unit – Molecular Neurology and
Department of Medical Genetics, Haartman Institute
University of Helsinki
and
Department of Mental Health and Substance Abuse Services
National Institute for Health and Welfare
Helsinki, Finland

Reviewed by

Docent Marjo Kestilä
Department of Chronic Disease Prevention
National Institute for Health and Welfare
Helsinki, Finland

and

Professor Erkki Isometsä
Institute of Clinical Medicine, Department of Psychiatry
University of Helsinki
Helsinki, Finland

Opponent

Professor Martin Schalling
Neurogenetics Unit, Center for Molecular Medicine
Karolinska Institute
Stockholm, Sweden

Some of your hurts you have cured,
And the sharpest you still have survived,
But what torments of grief you endured
From the evil which never arrived.

~Ralph Waldo Emerson (1803 – 1882)

Never bear more than one kind of trouble at a time. Some people bear three - all they have had, all they have now, and all they expect to have.

~Edward Everett Hale (1822 – 1909)

The greatest mistake you can make in life is to continually fear you will make one.

~Elbert Hubbard (1856 – 1915)

ABSTRACT

Anxiety disorders are psychiatric disorders, characterized by exaggerated, prolonged and debilitating levels of anxiety. Disorder-specific clinical characteristics of the anxiety are the basis for their subdivision into diseases such as panic disorder (PD), obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), generalized anxiety disorder (GAD), and phobias. Collectively, anxiety disorders form the most common group of mental disorders, annually affecting 14% of Europeans. Together with other mental disorders, they cause significant work disability and loss of quality of life, and are therefore of great importance for public health.

Anxiety disorders are complex diseases with onset influenced by both environmental and genetic factors. Although susceptibility to anxiety disorders is clearly heritable, so far little progress has been made in identifying solid genetic susceptibility factors for them. The main aim of this study was to shed light on the genetic basis of human anxiety disorders by genetic association analyses of selected novel and previously implicated candidate genes in the Finnish population.

Altogether, 333 single nucleotide polymorphism (SNP) markers from 30 genes were tested for genetic association to anxiety disorders. The studied genes were 1) 13 genes selected based on expression levels correlating with anxiety-like behavior in a mouse model of inbred strains with differential innate anxiety; 2) an asthma-linked G-protein coupled receptor (neuropeptide S receptor 1; *NPSR1*) and its neuropeptide ligand (neuropeptide S; *NPS*); and 3) 15 putative anxiety susceptibility genes selected based on previously published associations with anxiety disorders or anxiety-related personality traits. All genes were initially studied in an anxiety disorder sample (N = 974) derived from the Finnish population-based Health 2000 cohort. In this sample, we also evaluated gene-environment interactions by examining whether any of the genotyped SNPs modulate probability for anxiety disorders in interaction with childhood adverse life events, one of the strongest known environmental risk factors for anxiety disorders. In addition to the Health 2000 sample, three other case-control samples from Spain (N = 503), Sweden (N = 2020) and USA (N = 1128) were used for replication attempts or meta-analyses.

First, taking a cross-species approach, we discovered that six of the murine candidate genes (delta-aminolevulinate dehydratase [*ALAD*], cadherin 2 [*CDH2*], erythrocyte membrane protein band 4.1 like 4a [*EPB41L4A*], dynein light chain LC8-type 2 [*DYNLL2*], prosaposin [*PSAP*] and prostaglandin D2 synthase [*PTGDS*]) showed evidence for association with specific anxiety disorders in the Health 2000 sample. The six implicated genes link novel biochemical pathways to influencing anxiety susceptibility, but our findings should be replicated in independent samples as a next step.

Second, both *NPS* and *NPSR1* showed evidence for association with PD in the Health 2000 sample. SNPs in *NPSR1* were further associated with

symptoms of anxiety/depression in 8-year children of a Swedish birth cohort. We further demonstrated that some of the most significantly associated SNPs in *NPSR1* may alter transcription factor binding, and thereby influence the expression level of the gene. We also found that *Npsr1*-deficient mice (*Npsr1*$^{-/-}$) differ from wild types regarding at least some gene expression responses to acute stress. The neurotrophic factor neurotrophin 3 was downregulated in brains of stressed *Npsr1*$^{-/-}$ mice, whereas the stress-related immunotransmitter interleukin-1 beta was upregulated. Taken together, our findings suggest that NPS-NPSR1 signaling modulates predisposition not only to asthma, but also to anxiety disorders.

Third, of the putative anxiety susceptibility genes examined based on previously published human association studies, the gene showing the strongest evidence for association in the Health 2000 sample was glutamate decarboxylase 1 (*GAD1*). This gene encodes the enzyme synthesizing the neurotransmitter gamma-aminobutyric acid (GABA) from glutamate. One specific common risk haplotype spanning the locus of the gene increased risk for phobias. As the identified haplotype was the same as implicated in a prior study, we performed a meta-analysis (N = 1985) incorporating our findings with the published ones. This analysis further supported the role of *GAD1* as a joint risk factor for anxiety- and mood disorders, and neuroticism.

Fourth, SNPs from the neuropeptide Y (*NPY*) gene, accounting for two phylogenetically related risk haplotypes, modulated the effects of the number of experienced childhood adversities on anxiety susceptibility in the Health 2000 sample. Based on previous animal and human studies, *NPY* is a particularly good candidate for influencing individual variation in stress resilience.

In conclusion, altogether ten potential susceptibility genes for anxiety disorders were identified in this study at the $P \leq 0.01$ significance level. The identified genes illustrate the genetic and functional heterogeneity that likely underlies anxiety disorders, as they encode either enzymes, neuropeptides or their receptors, or structural proteins important for synapse formation. Our findings also support the notion that knowledge of the interplay between well established environmental risk factors and genetic variants is of great importance for understanding predisposition to psychiatric disorders.

We suggest that the top candidate genes based on this work be further evaluated in independent study samples, and studied functionally to understand how genetic variation in them influences their transcription, protein properties and ultimately the function of neural circuitry that regulates behavior. Such future studies will be essential for evaluating the therapeutic potential of targeting their biological pathways in treatment of anxiety disorders.

TIIVISTELMÄ

Ahdistuneisuushäiriöt ovat psykiatrisia häiriöitä, joiden tunnusmerkki on liiallinen, pitkittynyt ja toimintakykyä alentava ahdistuneisuustaso. Tautispesifisten kliinisten ahdistuneisuusoireiden perusteella ne jaotellaan useampiin häiriöihin kuten paniikkihäiriö, pakko-oireinen häiriö, traumaperäinen stressihäiriö, yleistynyt ahdistuneisuushäiriö ja fobiat. Ryhmänä ahdistuneisuushäiriöt ovat yleisimpiä mielenterveyshäiriöitämme, joista kärsii 14% eurooppalaisista vuosittain. Yhdessä muiden mielenterveyshäiriöiden kanssa ne ovat merkittävä syy työkyvyttömyyteen ja heikentyneeseen elämänlaatuun. Näistä syistä ahdistuneisuushäiriöiden kansanterveydellinen merkitys on suuri.

Ahdistuneisuushäiriöt ovat monitekijäisiä tauteja, joiden puhkeamiseen vaikuttavat sekä ympäristö- että geneettiset tekijät. Huolimatta siitä, että alttius ahdistuneisuushäiriöille on perinnöllinen, vankkoja geneettisiä alttiustekijöitä tunnetaan toistaiseksi heikosti. Tämän tutkimuksen päätavoitteena oli selvittää ahdistuneisuushäiriöiden perinnöllistä taustaa geneettisillä assosiaatioanalyyseillä, joissa tutkittiin sekä uusia että aiemmin ahdistuneisuuteen yhdistettyjä ehdokasgeenejä suomalaisväestössä.

Arvioimme yhteensä 333 SNP:n eli yhden nukleotidin polymorfismin (30 eri geenistä) mahdollista geneettistä assosiaatiota ahdistuneisuushäiriöihin. Tutkitut geenit olivat: 1) 13 geeniä joiden ilmenemistaso korreloi ahdistuneisuuskäyttäytymisen kanssa sisäsiittoisissa hiirikannoissa, joilla synnynnäinen ahdistuneisuustaso eroaa toisistaan; 2) astmaan yhdistetty G-proteiinikytkentäinen reseptori (neuropeptidi S reseptori 1; *NPSR1*) ja sen ligandi, neuropeptidi S (*NPS*); sekä 3) 15 ehdokasgeeniä, jotka valittiin perustuen aikaisempiin assosiaatiolöydöksiin ahdistuneisuushäiriöissä tai ahdistuneisuutta mittaavissa persoonallisuuspiirteissä. Kaikkia geenejä tutkittiin ensin ahdistuneisuushäiriöaineistossa (N = 974), joka oli peräisin suomalaisesta Terveys 2000 (T2000) väestötutkimuksesta. Kyseisessä aineistossa tutkimme myös geeni-ympäristövuorovaikutuksia selvittämällä muokkaavatko jotkut SNP:stä alttiutta ahdistuneisuushäiriöille yhdessä lapsuusiän vastoinkäymisten kanssa. Lapsuusiän vastoinkäymiset ovat vahvimpia tunnettuja ympäristöllisiä riskitekijöitä ahdistuneisuushäiriöille. T2000 näytteen lisäksi käytimme kolmea muuta tapaus-verrokkiaineistoa Espanjasta (N = 503), Ruotsista (N = 2020) ja Yhdysvalloista (N = 1128) replikaatioaineistona tai meta-analyyseissä.

Lajeja yhdistävän lähestymistavan avulla havaitsimme, että kuusi hiirimallien perusteella valittua ehdokasgeeniä (delta-aminolevulinaatti dehydrataasi [*ALAD*], kadheriini 2 [*CDH2*], erytrosyyttikalvoproteiinibändi 4.1 kuten 4a [*EPB41L4A*], dyneiinin kevyt ketju LC8-tyyypi 2 [*DYNLL2*], prosaposiini [*PSAP*] ja prostaglandiini D2 syntaasi [*PTGDS*]) assosioituivat spesifisiin ahdistuneisuushäiriöihin T2000-aineistossa. Nämä kuusi geeniä yhdistävät uusia biologisia reittejä ahdistuneisuusalttiuteen, mutta löydöksemme tulee seuraavaksi toistaa riippumattomissa aineistoissa.

Sekä *NPS* että *NPSR1* assosioituivat paniikkihäiriöön T2000-aineistossa. SNPt *NPSR1*:stä assosioituivat myös ahdistuneisuuden/masennuksen oireisiin 8-vuotiailla lapsilla ruotsalaisessa syntymäkohortissa. Osa merkitsevimmin assosioituvista SNP:stä *NPSR1*-geenissä saattavat vaikuttaa transkriptiofaktoreiden sitoutumiseen ja siten geenin ilmenemistasoon. Havaitsimme myös, että hiirillä joilta *Npsr1* puuttuu (*Npsr1*$^{-/-}$*),* on ainakin osittain poikkeava geenien ilmenemisvaste akuuttiin stressiin. Neurotrofinen tekijä neurotrofiini 3 ilmentyi alhaisemmalla tasolla stressattujen *Npsr1*$^{-/-}$ hiirten aivoissa, kun taas stressivasteeseen yhdistetyn immunovälittäjän interleukini-1 betan ilmentyminen oli korkeammalla tasolla. Löydöksemme viittaavat siihen, että NPS-NPSR1 signalointi muuntelee altiutta paitsi astmalle myös ahdistuneisuushäiriöille.

Aikaisemmissa tutkimuksissa ahdistuneisuuteen assosiaatioanalyysein yhdistetyistä geeneistä merkitsevimmät löydöksemme T2000-aineistossa olivat glutamaattidekarboksylaasi 1 (*GAD1*) geenistä. Tämä geeni koodittaa entsyymiä, joka syntetisoi hermovälittäjäaine gamma-aminovoihappoa (GABA) glutamaatista. Tutkimuksessamme yksi yleinen koko geenilokuksen kattava riskihaplotyyppi lisäsi riskiä sairastua fobioihin. Tunnistettu riskhaplotyyppi oli sama kuin aikaisemmassa amerikkalaisaineistossa, joten teimme meta-analyysiin (N = 1985) jossa yhdistimme aineistomme siihen. Myös tämä analyysi tuki *GAD1*:n merkitystä jaettuna riskitekijänä ahdistuneisuus- ja mielialahäiriöille sekä neuroottisuudelle.

SNP:t neuropeptidi Y (*NPY*)-geenistä, edustaen kahta fylogeneettisesti sukua olevaa riskihaplotyyppiä, muuntelivat koettujen lapsuusiän vastoinkäymisten määrän vaikutusta ahdistuneisuusaltiuteen T2000-aineistossa. Aikaisemmat eläin- ja ihmistutkimukset tukevat *NPY*:n roolia yksilöllisen stressinsietokyvyn muuntelussa.

Yhteenvetona tunnistimme tässä tutkimuksessa yhteensä kymmenen potentiaalista alttiusgeeniä ahdistuneisuushäiriöille merkitsevyystasolla $P \leq 0.01$. Tunnistetut geenit havainnollistavat ahdistuneisuushäiriöihin vaikuttavaa geneettistä ja toiminnallista monimuotoisuutta, sillä ne koodittavat joko entsyymejä, neuropeptidejä tai synapsien muodostumiselle tärkeitä rakenneproteiineja. Löydöksemme tukevat myös käsitystä, että vakiintuneiden ympäristöllisten riskitekijöiden ja geneettisten varianttien vuorovaikutusten tunteminen on tärkeää psykiatristen tautien alttiuden ymmärtämisessä.

Ehdotamme, että parhaimpia tässä työssä tunnistettuja ehdokasgeenejä tutkitaan jatkossa riippumattomissa näytteissä sekä toiminnallisin kokein. Näin voimme ymmärtää, miten geneettinen variaatio vaikuttaa näiden geenien ilmenemiseen, proteiinien ominaisuuksiin ja lopulta käyttäytymistä säätelevien hermoverkkojen toimintaan. Tämänkaltaisia tutkimuksia tarvitaan jatkossa, jotta voidaan arvioida voisivatko tässä tutkimuksessa tunnistettujen geenien biologiset reitit olla sopivia kohteita ahdistuneisuushäiriöiden lääkehoidossa.

CONTENTS

ABBREVIATIONS

5HT	serotonin
ACTH	adrenocorticotropic hormone
ALAD	δ-aminolevulinate dehydratase
ANOVA	analysis of variance
BAMSE	Barn Allergi Miljö Stockholm Epidemiologi
bp	base pairs
cAMP	cyclic adenosine monophosphate
CD-CV	common disease – common variant
CD-RV	common disease – rare variant
CDH2	cadherin 2
CNS	central nervous system
CNV	copy number variation
COMT	catechol-*O*-methyltransferase
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
DYNLL2	dynein light chain LC8-type 2
e.g.	exempli gratia
EMSA	electrophoretic mobility shift assay
EPB41L4A	erythrocyte membrane protein band 4.1 like 4a
EPQ	Eysenck Personality Questionnaire
EQ-5D	EuroQol-5D Questionnaire
FKBP5	FK506-binding protein 5
GABA	γ-aminobutyric acid
GAD	generalized anxiety disorder
GAD1	glutamate decarboxylase 1
GLO1	glyoxalase 1
GWA	genome-wide association
GxE	gene x environment
HPA	hypothalamic-pituitary-adrenal
i.e.	id est
IL1B	interleukin 1 beta
kb	kilobase
LD	linkage disequilibrium
LOD	logarithm of the odds
LRT	likelihood-ratio test
MAF	minor allele frequency
M-CIDI	Munich Composite International Diagnostic Interview

MDD	major depressive disorder
mRNA	messengerRNA
miRNA	microRNA
NE	norepinephrine
NEO	Neuroticism-Extraversion-Openness Personality Inventory
NOS	not otherwise specified
NPS	neuropeptide S
NPSR1	neuropeptide S receptor 1
NPY	neuropeptide Y
NTF3	neurotrophin 3
OCD	obsessive-compulsive disorder
OR	odds ratio
PCR	polymerase chain reaction
PD	panic disorder
PFC	prefrontal cortex
PSAP	prosaposin
PTGDS	prostaglandin D2 synthase
PTSD	post-traumatic stress disorder
RGS2	regulator of G-protein signaling 2
RNA	ribonucleic acid
SLC6A4	serotonin transporter
SNP	single nucleotide polymorphism
SSRI	selective serotonin re-uptake inhibitor
STAI	State Trait Anxiety Inventory
TPQ/TCI	Tridimensional Personality Questionnaire /Temperament and Character Inventory
UTR	untranslated region
VATSPSUD	Virginia Adult Twin Study of Psychiatric and Substance Use Disorders
qRT-PCR	quantitative real-time polymerase chain reaction
QTL	quantitative trait locus

Abbreviations appearing at least three times in the text are listed.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their roman numerals:

I **Donner J**, Pirkola S, Silander K, Kananen L, Terwilliger JD, Lönnqvist J, Peltonen L, Hovatta I. An association analysis of murine anxiety genes in humans implicates novel candidate genes for anxiety disorders. *Biological Psychiatry* 2008; 64:672-680.

II **Donner J**, Haapakoski R, Ezer S, Melén E, Pirkola S, Gratacòs M, Zucchelli M, Anedda F, Johansson LE, Söderhäll C, Orsmark-Pietras C, Suvisaari J, Martín-Santos R, Torrens M, Silander K, Terwilliger JD, Wickman M, Pershagen G, Lönnqvist J, Peltonen L, Estivill X, D'Amato M, Kere J, Alenius H, Hovatta I. Assessment of the neuropeptide S system in anxiety disorders. *Biological Psychiatry* 2010; 68:474-483.

III **Donner J**, Sipilä T, Ripatti S, Kananen L, Chen X, Kendler KS, Lönnqvist J, Pirkola S, Hettema JM, and Hovatta I. Support for involvement of glutamate decarboxylase 1, and neuropeptide Y in anxiety susceptibility. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*; in press.

1 INTRODUCTION

Anxiety and fear are a part of life. Whereas we *fear* an immediate and definite threat, such as an approaching stranger in a dark alley, we feel *anxious* in anticipation of future possible dangers and intangible threats. Anxiety is accompanied by emotional manifestations of uneasiness, worry, restlessness, despair, horror and panic. Somatically, anxiety is distinguished by a fight-or-flight response triggered by activation of the sympathetic nervous system. Increased heart rate, accelerated breathing, and sweating are meant to help the body perform better under threat. However, this comes with downsides like dizziness, choking sensations, tightness in the chest, chills, and dry mouth.

Thus, normal anxiety is an adaptive response to potentially harmful situations. On the other hand, exaggerated anxiety may manifest as clinically diagnosable anxiety disorders, such as panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, generalized anxiety disorder, and phobias (American Psychiatric Association, 2000). The core feature of these mental disorders is excessive, prolonged and debilitating anxiety. Other specific features regarding focus, course, and onset of the anxiety are the basis for the clinical subdivision. A recent large cross-European study reported that 14.0% of Europeans annually suffer from anxiety disorders (Wittchen et al., 2011), making them not only detrimental to the individual, but also a common and costly burden for society via health care costs and work disability (Sillanpää et al., 2008).

Evidence from twin- and family studies indicate a heritable, partially genetic basis for anxiety disorders (Hettema et al., 2001). Consequently, much effort has been put into identifying the specific genetic determinants of these complex disorders with the aim to obtain a better understanding of their biology and discover new potential targets for safer and more efficient anxiolytics. Although a great deal of knowledge has been gained regarding specific brain regions and neurotransmitter systems regulating anxiety, only a handful of solid susceptibility genes have been identified thus far. The main aim of this study was to investigate whether genetic variation in several putative candidate genes, both unexamined and previously implicated ones, predisposes to human anxiety disorders in the Finnish population.

The following review of the literature describes the origins of human genetic variation and how it may manifest as disease phenotypes. Genetic mapping methods for identification of genes predisposing to complex disorders are reviewed, followed by a summary of the characteristics and genetics of anxiety disorders with a special emphasis on work that has lead to the identification of novel potential susceptibility genes for anxiety-related phenotypes.

2 REVIEW OF THE LITERATURE

2.1 GENETICS OF DISEASES

2.1.1 THE HUMAN GENOME

Genetic information is stored in the base sequence of DNA, a linear double helical molecule composed of a sugar-phosphate backbone and the bases adenine (A), cytosine (C), guanine (G) and thymine (T). The sequence of the bases contains hereditary genetic instructions for the construction of proteins, and functional ribonucleic acid molecules.

The human genome consists of around 3 billion base pairs (bp) of DNA, carried by 22 autosomal chromosome pairs and the sex-determining X and Y chromosomes in each cellular nucleus. In addition to the nuclear linear chromosomes, all mitochondria carry their own genome on a small 16.6 kilobase (kb) circular DNA molecule. While inheritance of the nuclear genome is mediated by transmission of one copy of each homologous chromosome from the mother, and another copy from the father, inheritance of the mitochondrial genome is maternal.

Both publically and privately funded consortia raced to sequence the human genome, with draft versions from both projects published simultaneously ahead of schedule in 2001 (Lander et al., 2001; Venter et al., 2001). Availability of the sequence, and subsequent work, has lead to major insights regarding the organization of the human genome. We now know that it contains an estimated 20000 genes (Clamp et al., 2007), i.e. much less than previously thought. The complexity of humans and other vertebrates compared to worms or flies is rather the result of more complex gene structure and regulation, and alternative splicing of gene products. In fact, only around 2% of the genome encodes for protein-coding genes. Another 2% represents evolutionarily conserved non-coding regions that might be of functional importance (Dermitzakis et al., 2005). A large proportion of what was previously called non-coding “junk” DNA is now thought to encode functionally important non-protein coding RNA molecules (Wright and Bruford, 2011). Our genome is also rich in repeat sequences of varying length, and about half of it consists of sequences derived from transposable elements (Lander, 2011).

2.1.2 HUMAN GENETIC VARIATION

The genome sequence of any two individuals is identical to 99.9%. The remaining varying 0.1% must thus be of particular importance in that it, together with environmental factors, determines individual variation in disease susceptibility.

Genetic variation between individuals arises through three main mechanisms: random combination of male and female gametes during fertilization, independent assortment of chromosomes with crossovers during meiosis, and mutations. Of these mechanisms, mutations create novel alleles, whereas the others create unique combinations of alleles.

Mutations, or changes in the base sequence of DNA, are the ultimate introducers of novel genetic variation into a population, and the raw material of evolution. It was estimated that each parent passes on an average of 30 *de novo* germline mutations to their offspring, although great variability exists (Conrad et al., 2011). Mutations can be the result of intracellular events such as errors in DNA replication and repair, or caused by external factors such as radiation or mutagenic chemicals. All mutations are originally rare, and most are neutral or deleterious in effect rather than beneficial. However, novel alleles can increase in frequency and even eventually become fixed in a population by evolutionary processes such as natural selection and genetic drift. When the least common allele of a mutation has a frequency of more than 1% in the population, it is no longer considered as an abnormal deviation from the normal DNA sequence. It is then called a polymorphism, a variant of the standard DNA sequence.

Mutations range in type from point mutations affecting only a single base pair in the genome, to larger chromosomal rearrangements and polyploidy of entire chromosome sets. Point mutations can be classified as substitutions, insertions or deletions of single base pairs, whereas larger chromosomal alterations are typically duplications, deletions, inversions or translocations of larger genomic segments.

It has become apparent that the human genome is rich in both sequence (e.g., single nucleotide polymorphisms; SNPs) and structural genetic variation (e.g., copy number variations; CNVs) originally created by mutations and shaped by evolutionary processes. Although we call most of it normal polymorphic genetic variation between populations and individuals, its potential importance for disease predisposition is evident. Although most genetic variation is not disease-causing in itself, it represents a valuable tool for genetic mapping of actual disease-predisposing variants as variant alleles can be used as genetic markers representing their surrounding genomic region (discussed in section 2.2.1).

2.1.3 THE GENETIC BASIS OF HUMAN DISEASE

Gregor Mendel (1822-1884), an Austrian monk often called the father of genetics, was the first one to form and document descriptive laws explaining how traits are inherited from parents to offspring. His studies were limited to traits regulated by one or a few genes that follow basic genetic rules of what we now call, in his honour, Mendelian inheritance. Today diseases are classified as either monogenic (Mendelian), or complex, based on their pattern of inheritance (Figure 1).

Monogenic disorders

Monogenic disorders are caused by defects in single genes. In general, they can be classified as either dominant or recessive, depending on whether one or two copies of the mutated gene are required to cause the disease. Monogenic disorders are also classified as either autosomal or X-linked, based on the chromosomal location of the causal gene. Examples of recessive disorders include the well studied cystic fibrosis, phenylketonuria and sickle cell anemia, and many of the disorders of the Finnish disease heritage such as aspartylglucosaminuria (AGU), Salla disease, and RAPADILINO syndrome (Online Mendelian Inheritance in Man [OMIM], www.ncbi.nlm.nih.gov/omim). Examples of dominantly inherited disorders include Huntington's disease, neurofibromatosis, and Marfan syndrome.

Diseases caused by mutations in mitochondrial DNA display mitochondrial inheritance. As only maternal mitochondria are transmitted to the embryo, only females pass on mitochondrial diseases, whereas affected males do not. The clinical phenotype of mitochondrial diseases varies depending on the percentage of cellular mitochondria that carry mutated DNA. Examples of mitochondrially inherited disorders are MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome) and CPEO (chronic progressive external ophthalmoplegia).

Complex disorders

In contrast to monogenic disorders, complex (multifactorial) diseases are caused by the joint effects of genetic variants in multiple genes combined with lifestyle and environmental factors. On a population level, they are significantly more common than monogenic ones, and therefore of great socioeconomical importance. Examples include well known diseases such as asthma, diabetes, cardiovascular diseases, cancers, and psychiatric disorders.

Complex diseases do not follow simple Mendelian patters of inheritance, although they are often described to run in families. Instead, rules of polygenic inheritance first proposed by Ronald Fisher may more accurately

describe their genetics (Fisher, 1918). According to his polygenic theory, quantitative variation in a human trait could be explained by a large number of disease risk loci, each contributing minorly to the phenotype and segrating according to the laws of Mendel. When the number of loci influencing the trait is large, the trait will appear continous and normally distributed (Figure 2A). However, the clinical disease phenotypes of complex diseases that we use are often dichotomous (i.e., affected/healthy) rather than quantitative. The liability-threshold model proposed by Karl Pearson is then useful for understanding disease predisposition from a genetic perspective (Pearson, 1900). According to this theory, genetic liability to a disease follows a normal distribution (Figure 2B). The disease would then manifest when a certain liability-threshold is exceeded, i.e. when an individual carries a large enough number of disease risk alleles.

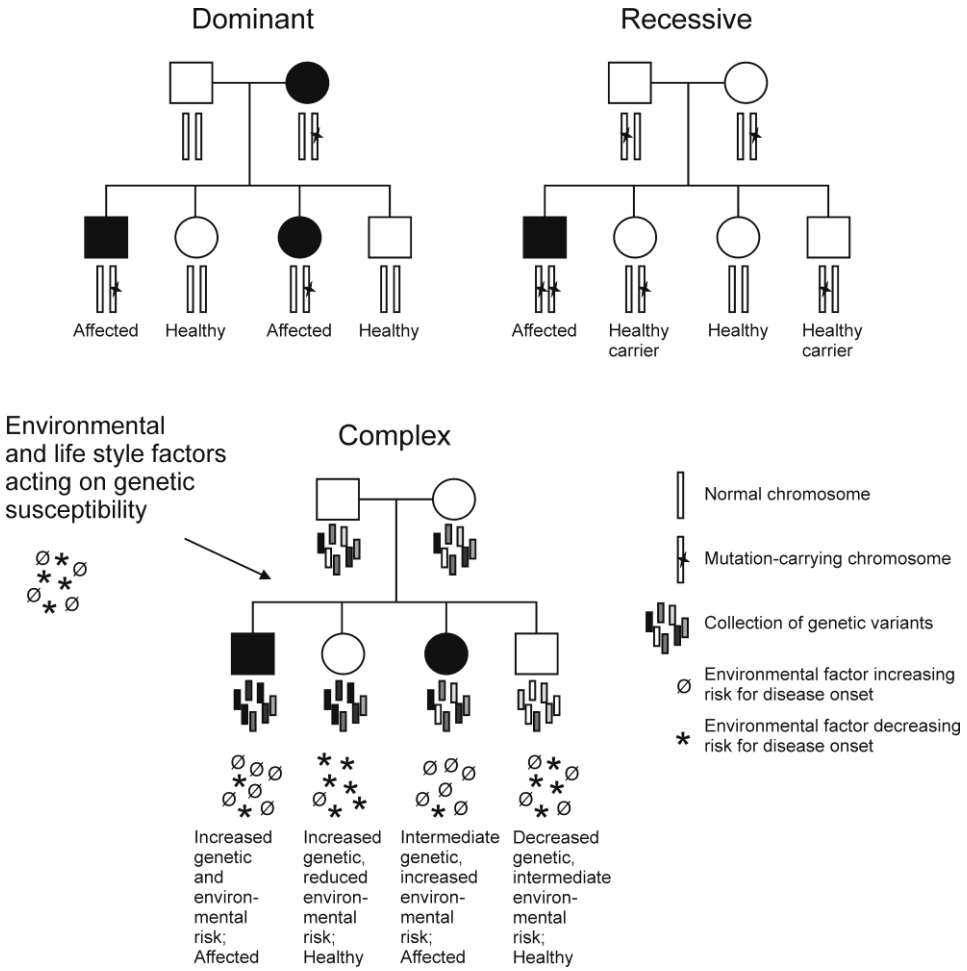


Figure 1 Modes of disease inheritance in pedigrees showing monogenic or complex inheritance. Typically, either one (dominant inheritance) or two (recessive inheritance) copies of a mutated gene are required for disease manifestation. Onset of complex disorders is triggered by multiple genetic variants interacting with environmental factors. In reality, also monogenic diseases show incomplete penetrance, and their course and form is shaped by environmental factors and other genes. There are also rare monogenic forms of some complex diseases (e.g., Alzheimer's disease and familial combined hyperlipidemia) that may provide important clues about specific biological pathways that are important also for the forms showing complex inheritance (Peltonen et al., 2006).

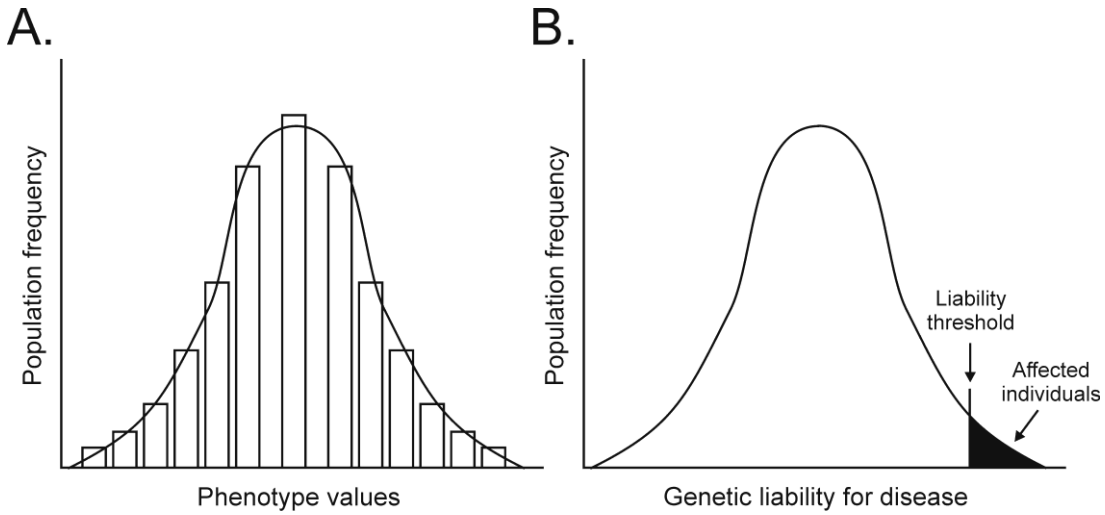


Figure 2 Models explaining quantitative variation in a human trait, and genetic predisposition to a dichotomous phenotype. (A) Fisher's polygenic theory shows that a trait will appear continuosly distributed, when a sufficient number of equally common loci of small and equal effect influence the trait. In reality, alleles at each locus are not equally frequent or of equal effect, e.g. due to dominance or epistasis. (B) Pearson's liability-threshold model suggests that genetic liability to a disease follows a normal distribution in the population. The disease manifests only when an individual carries enough genetic risk variants to exceed the liability threshold.

There is an ongoing debate whether genetic susceptibility to complex diseases is better explained by the additive effects of a large number of common risk alleles (minor allele frequency [MAF] typically > 5%) with modest effects (odds ratios [ORs] mostly = 1.2 - 1.5), or by fewer rarer risk alleles (MAF typically 0.1 – 3%) conferring higher disease risk (OR usually >2) (Bodmer and Bonilla, 2008; Pritchard and Cox, 2002). The former theory is referred to as the common disease – common variant (CD-CV) hypothesis, and the latter one as the common disease – rare variant (CD-CR) hypothesis. Most likely, both theories hold true to some extent and no universal explanation for complex disease predisposition is to be found. For example, common variants in the apolipoprotein E gene increase the risk for Alzheimer's disease (Coon et al., 2007), whereas rare copy number variants are thought to be a major risk factor for autism spectrum disorders (Pinto et al., 2010).

2.2 GENETIC MAPPING

After a genetic basis for a disease has been established by family, twin, and/or adoption studies, attempts to identify or "map" the specific genetic factors involved in determining predisposition are warranted. Two complementary types of methodologies for mapping, each with their specific areas of utility as well as their respective advantages and disadvantages, are commonly used: linkage- and association studies. Both rely on the use of genetic markers to represent the variation of specific chromosomal positions.

2.2.1 GENETIC MARKERS

A genetic marker can be any polymorphic variant showing Mendelian inheritance, and having a known chromosomal location. A genetic marker acts as a flag of its position, and it can be used to follow the inheritance of its surrounding chromosomal segment through a pedigree (Elston and Spence, 2006). Genetic markers can both be examined for co-inheritance with disease phenotypes in pedigrees (linkage studies), or tested for statistical association to disease phenotypes in populations of unrelated individuals (association studies), as described in sections 2.2.2 and 2.2.3.

There are several different types of genetic markers. In general, shifts in contemporary markers of choice have followed advances in molecular technology. The general trend over the past 25 years has been that the number of markers that can be feasibly genotyped has increased, whereas the cost and amount of work per marker has decreased (Elston and Spence, 2006; Schork et al., 2000). The first commonly used markers (prior to the 1980s) were the physiological and biochemical properties of blood group antigens and protein isoforms. These were followed by restriction fragment length polymorphisms (RFLPs), point mutations that altered restriction enzyme digestion sites, in the 1970-1980s (Botstein et al., 1980). After polymerase chain reaction (PCR) technology became commonplace from the 1980s onward, microsatellites became the tools of choice (Weber and May, 1989). Microsatellites are DNA repeat sequences with alleles of varying repeat length. Microsatellites are particularly suitable for linkage studies as they typically are highly polymorphic, easing identification of a specific allele that uniquely co-segregates with a disease phenotype.

Today, the most commonly used genetic markers for gene mapping studies are SNPs (Collins et al., 1997). Their utility is explained by a number of reasons (Schork et al., 2000). They are the most abundant type of genetic polymorphism, dispersed throughout the genome with estimated occurrences of, on average, one SNP every 100 – 300 bp in the human

genome (www.ncbi.nlm.nih.gov/snp). With current technology, up to a million SNPs can be genotyped simultaneously on microarrays, making their high-throughput genotyping a cost-efficient and fairly easy undertaking. They are more stable, i.e. less mutable, than microsatellites. The benefits of SNPs thus compensate for the disadvantage, namely that they are usually only biallelic with two of the four possible alleles A, C, G and T, and therefore less informative than microsatellites.

One further benefit of using SNPs as genetic markers is that they themselves are potential candidates for conferring disease risk (Schork et al., 2000). For instance, exonic SNPs could cause functionally relevant amino acid changes in the encoded protein. SNPs in non-coding regions could alter gene function by either disrupting transcript splice sites or influencing gene expression. SNPs could modulate gene expression by altering transcription factor binding to promoters or regulatory elements, or microRNA (miRNA) binding to target sites in the transcript.

After the human genome sequencing project was completed, interindividual variation in genome sequence has been the focus of major international research ventures. Projects such as the International HapMap Project aim to characterize dense maps of SNP markers across the genome, and determine their allele frequencies and correlation patterns for various populations (The International HapMap Consortium, 2003; www.ornl.gov/sci/techresources/Human_Genome/home.shtml). The information is then made available to researchers to ease the selection of optimal sets of SNPs for use as genetic markers in disease gene mapping studies. Taken together, the Human Genome Project, the SNP consortium, and the HapMap Project have identified close to 10 million common (MAF > 5%) SNPs (International HapMap 3 Consortium, 2010; http://hapmap.ncbi.nlm.nih.gov). Recently, after the advent of next-generation sequencing technologies made it possible, focus has turned to rare SNPs (MAF < 5%). Ventures such as the 1000 Genomes Project aim to catalogue these rarer variants by resequencing of individuals from several populations (1000 Genomes Project Consortium, 2010).

2.2.2 LINKAGE ANALYSIS

Linkage analysis-based methods for positional cloning of disease susceptibility genes are most commonly used for initial genome-wide scans over long chromosomal ranges in pedigrees of affected individuals (Figure 3A). In conventional linkage analysis, the aim is to discover genetic marker alleles that co-segregate with the disease alleles (Terwilliger and Ott, 1994). Markers close to a risk variant will be most strongly “linked”, i.e. inherited together with it, but recombination events between marker and disease alleles within the pedigree further narrow down the chromosomal region containing potential candidate genes.

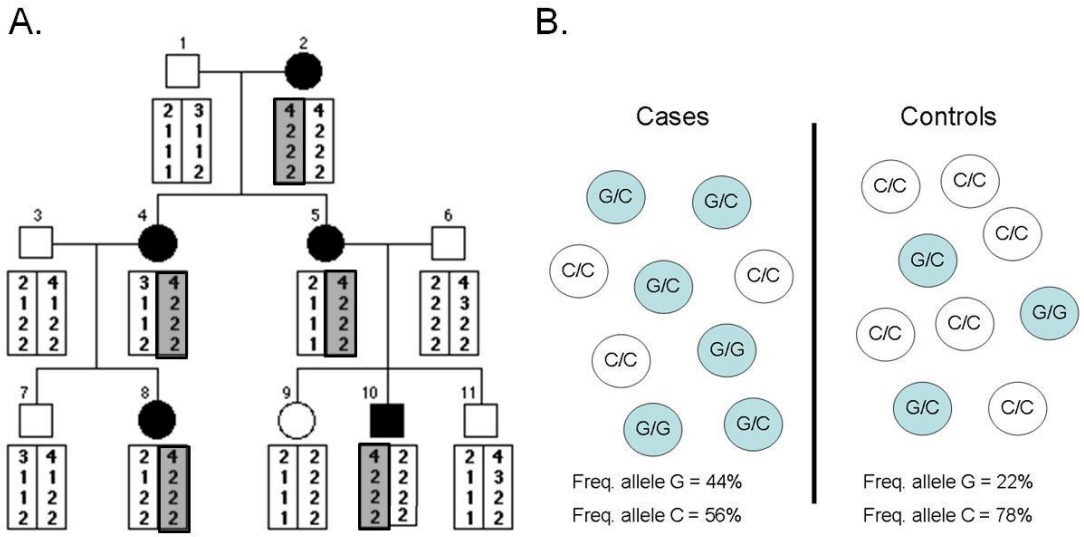


Figure 3 Methods for genetic mapping of disease genes. (A) Linkage analyses examine coinheritance of genetic markers with disease in pedigrees of affected individuals. In this hypothetical example showing two homologous chromosomes with four microsatellite markers, the allele combination 4-2-2-2 is always coinherited with the disease phenotype, suggesting that a disease mutation is located somewhere on that specific haplotype. Further fine mapping is needed to narrow down the interval containing possible candidate genes. (B) Association studies examine the correlation of genetic marker alleles and trait phenotypes in unrelated individuals. In this example, all carriers of at least one copy of a G-allele at one hypothetical SNP have been shaded grey. The G-allele is significantly more frequent among cases than controls, suggesting that it may influence disease onset or be in linkage disequilibrium with a causal variant.

The probability for two loci to be linked is directly related to their genomic distance. The probability for recombination to break up the linkage of two loci is expressed by the recombination fraction (θ), which ranges from 0 (always inherited together) to 0.5 (the loci segregate as being on two independent chromosomes). A test statistic called the LOD-score (logarithm of the odds) is used to evaluate whether a genetic marker co-segregates with a disease phenotype (Morton, 1955). For each marker, it compares the likelihood of the observed data in the pedigree if there is linkage between marker and disease allele, to the null hypothesis that the observations are due to chance. The final LOD-score is given for the most likely recombination fraction between marker and disease allele. A LOD score of ≥ 3 is generally considered as evidence for linkage, as it corresponds to 1000:1 odds in favour that the observed linkage was not due to chance. However, when analyzing a large number of markers in a genome-wide scan, a more appropriate LOD score threshold for genome-wide significance would be 3.3, corresponding to statistical evidence expected to be found by chance with a 5% probability in such an effort (Lander and Kruglyak, 1995).

In more detail, linkage analyses can be classified as either parametric or non-parametric. Parametric analyses require knowledge about the mode of

inheritance, and estimates of disease allele frequencies and penetrance. Such analyses have been successfully applied for identification of genes underlying monogenic disorders that follow simple Mendelian inheritance patterns. In contrast, non-parametric (i.e., model-free) analyses are more suitable for complex diseases that lack a clear model of inheritance, and have more uncertain risk allele frequencies and incomplete penetrance. The non-parametric analyses aim to identify alleles or chromosomal regions that are shared by affected individuals.

Linkage-based approaches have been very successful in identifying the genes responsible for Mendelian disorders, sometimes even in studies including only a few affecteds and families (Risch, 2000). Linkage studies can be useful for the mapping of complex disease genes as well, especially if one has large, well-characterized families to study. However, the track record of linkage in complex diseases is not as convincing. Complex diseases show greater allelic and locus heterogeneity, and one can expect such genetic heterogeneity even within a family. Each individual gene likely contributes only minorly to the phenotype, and environmental factors also influence the trait. The consequence of all of the above is that much larger samples are needed to identify predisposing variants. However, the collection of large family samples is a labor-intensive and time-consuming task. Moreover, the final chromosomal region identified in linkage studies is usually large and containing several potential candidate disease genes, as the resolution of the approach is limited by the number of recombination events between markers and disease alleles (Cardon and Bell, 2001). Therefore, a complementary and refining methodology, association-based mapping, is in use, and it is discussed in the following section.

2.2.3 ASSOCIATION-BASED METHODS

Association analysis

Whereas linkage analyses evaluate the relationship between loci (marker and disease gene), genetic association studies examine the statistical correlation between genetic variants and trait differences, such as disease phenotypes (Cardon and Bell, 2001); Figure 3B. A highly influential paper by Risch and Merikangas suggested that association studies have more power than linkage studies to identify common risk variants with small effect sizes, and that association-based approaches may therefore provide a means of overcoming some of the limitations of linkage analyses in studies of complex diseases (Risch and Merikangas, 1996). This has inspired much of the development during the past 15 years, from small association studies examing specific candidate genes, or fine mapping of linkage regions, to the large scale genome-wide association studies of today. The advantages and disadvantages of linkage and association studies are summarized in Figure 4.

	LINKAGE	ASSOCIATION
+	- no prior knowledge about genomic location or function of causative gene(s) required - not sensitive to population stratification effects - power not influenced by allelic heterogeneity - efficient for identification of rare highly penetrant variants predisposing to monogenic disorders	- recruitment of unrelated subjects relatively easy - hypothesis-free, high-througput genome-wide genotyping efforts with dense marker maps economically and techically feasible - direct testing of potentially causal SNPs possible - data enables testing gene-gene and gene-environment interactions, and pathway analyses
−	- collection of multi-generational family samples is time-consuming and labor-intensive, or impossible in some conditions - identified linkage peak region is typically large and contains several potential candidate genes - less power to detect common risk variants with small effect sizes	- sensitive to population stratification effects - power reduced by allelic heterogeneity - enormous multiple testing burden if testing up to one million markers - enormous study samples required for study of risk variants with small effect sizes

Figure 4 Advantages and disadvantages of linkage- and association-based methods for identification of human disease genes.

The most common association study design is the classical case-control study, in which allele frequencies at genetic marker (usually SNPs) loci are determined in a group of unrelated affected individuals and compared to those in a population of unrelated unaffected control subjects. Likelihood-ratio tests (LRT) or chi-square (χ^2) tests are then used to statistically evaluate whether there are significant differences in allele or genotype distributions between cases and controls. The aim is to identify alleles that are more frequent among cases than controls, and thereby "associated" with the disease. Although one appeal of the association approach is that collection of large samples of unrelated individuals is relatively easy, there is also one major pitfall to it. Any systematic difference in allele frequencies between cases and controls will apperar as a spurious disease association, even though it in reality is the result of evolutionary or migratory history (ethnicity), gender differences, or non-random mating (Cardon and Bell, 2001). Such an effect, the presence of multiple subgroups with different allele frequencies within the study sample, is called population stratification, and it was suggested to be one major reason behind discrepant association findings in the literature (Risch, 2000). Therefore, well characterized and geographically/ethnically matched control samples, or statistical methods to control for ancestry in population controls (Tian et al., 2008) are key elements of a well designed association study.

In addition to case-control studies, there are also family-based association study designs for constellations of parents and affected offspring (Laird and Lange, 2006). The statistical tests used in family-based designs are the transmission disequilibrium test (TDT) and its extension FBAT (family-based association test). The principle of these tests is to consider the alleles not transmitted to affected children as control alleles, and evaluate whether

there is significant overtransmission (deviation from Mendelian transmission) of other alleles to affected children.

Typically, a marker found to be associated in an association study is not necessarily the causal variant itself, but rather a variant in linkage disequilibrium (LD) with the risk variant. This important concept is described next.

Linkage disequilibrium

The concept of LD is the foundation that association studies rely on. It can be defined as non-random association of alleles, or the tendency for specific alleles to be inherited together, coupled as a unit (Ardlie et al., 2002). LD is best understood by considering it from a population-historical perspective. When a mutation increasing the risk for disease first arises and enters a population, it does so on a haplotype background of other pre-existing DNA variants. Subsequently, the mutation will be transmitted together with these other variants as a unit in the population; that is, it will be in LD with other variants nearby. Over time, historical recombination events during meioses in the population will reduce LD by breaking up associations between the mutation and other alleles of the ancestral haplotype. Eventually, only specific variants very close to the mutation will still be co-inherited with it. Thus, combinations of marker alleles represent the recombinatorial history of the chromosomal region they are located in. This is the genetic basis of why alleles of genetic markers provide information about, or capture, other surrounding genetic variation, including potential disease susceptibility mutations. In essence, linkage and association studies are thus both based on the same principle, the co-inheritance of adjacent variants. However, linkage is found and studied in recent pedigrees with known patterns of ancestry, with few recombination events, whereas association reflects long-term, historic recombination in the population that can be inferred to have the properties of an enormous hypothetical pedigree (Cardon and Bell, 2001).

Two different measures are used to quantify the extent of LD between loci: D' and r^2 (Wall and Pritchard, 2003; Ardlie et al., 2002). D' is obtained by dividing D (the difference between the observed frequency of a two locus haplotype and its expected frequency assuming random segregation of alleles), with its maximum possible value given the allele frequencies of the two loci. The value of D' ranges from 0 to 1, with 1 designating complete LD, i.e. no recombination between the two loci. However, low D' values cannot be clearly interpreted in relation to each other, and therefore the variable r^2 is often preferred, as it takes differences in allele frequencies between the loci into account. The r^2 coefficient represents the statistical correlation between two loci, and it is thus a measure of how complete information the loci provide about each other. r^2 values also range from 0 to 1, but a value of 1 is only obtained when there has been no recombination between the loci, and their allele frequencies are equal.

Genome-wide association studies

Traditionally, association studies were focused on specific candidate genes, based on a priori hypotheses justified by previous linkage findings, animal models, and prior knowledge about the function of the gene in biological processes relevant for the disease. Nowadays, hypotheses-free, genome-wide association (GWA) studies examining up to a million SNPs in large samples are technologically and economically feasible and commonplace. Thus, the popularity of the association study approach in recent years is due to a combination of factors, such as development of dense genome-wide SNP maps for use as reference markers, high-throughput genotyping technology on microarrays, and increase in computing power that is necessary for the data analysis. To put GWA studies into the context of genetic theories for explaining disease susceptibility, they were designed to evaluate genetic variation with MAFs > 0.05, and therefore detect any so called common risk variants for complex disease (CD-CV hypothesis).

However, GWA-studies come with an enormous multiple testing penalty, as the number of potential spurious false positive associations increases along with the number of statistical tests performed. It was suggested that an appropriate threshold for a genome-wide significant finding is $P < 5 \times 10^{-8}$ (Risch and Merikangas, 1996), an approximation still considered reasonably accurate today. The need to have sufficient power to reach such thresholds has, together with the very modest effect sizes currently thought to be conferred by individual risk variants, lead to studies with enormous study samples (10 000 – 100 000 individuals). This trend has lead to the formation of numerous international consortia focusing on the study of specific complex disease phenotypes, as these efforts are no longer possible for individual research groups to carry out.

Through the extensive work done across a wide range of phenotypes, the emerging consensus from the GWA studies performed to date is that the CD-CV hypothesis alone is not sufficient to explain the genetic predisposition to complex disorders (Manolio et al., 2009). Rather, the effect sizes of the identified common risk variants are generally small, and they collectively only explain a fraction of the heritability of complex disorders. This seems to be particularly true for psychiatric disorders. Thus, depending on one's point of view, some might call the GWA studies performed to data a success, whereas others remain more hesitant regarding their actual practical utility (Weiss and Terwilliger, 2000). Therefore, the field is currently moving (via exome sequencing) towards performing genome-wide resequencing studies for the detection of any possible rare risk variants for complex disease. Again, this development is fuelled by concurrent development in next-generation sequencing technologies, computing power and statistical and bioinformatical advances.

2.3 ANXIETY DISORDERS

2.3.1 CLINICAL FEATURES

Anxiety as a universal phenomenon has been discussed for ages. Originally, it was not included as a mental illness in disease descriptions made by ancient Greek physicians (Stone, 2009). The focus on anxiety in the medical literature has shifted from treatment of the somatic (objective) manifestations related to activation of the sympathetic nervous system to treatment of the emotional (subjective) manifestations pioneered by psychoanalysts (Table 1). Today, we acknowledge that "normal" anxiety is an adaptive response to potentially harmful situations, but consider it a diagnosable mental disorder when exaggerated. The main criteria for classifying anxiety as pathological are that is excessive and prolonged such that it leads to considerable disability and distress. Disorder subtypes are further identified based on other disorder-specific features regarding the course and onset of the anxiety, and the focus of the anxiety-associated avoidance behavior. The most common classification instrument of mental disorders for research purposes, the Diagnostic and Statistical Manual of Mental Disorders (currently DSM-IV), recognizes the following anxiety disorders: acute stress disorder, agoraphobia (without a history of panic disorder [PD]), generalized anxiety disorder (GAD), obsessive-compulsive disorder (OCD), PD (with or without agoraphobia), phobias (e.g., social phobia and specific phobias), and post-traumatic stress disorder (PTSD) (American Psychiatric Association, 2000). Diagnostic criteria for the major anxiety disorders are summarized in Table 2. Clinicians also commonly use the International Classification of Diseases (currently ICD-10) to classify anxiety disorders (World Health Organization, 1993). It groups anxiety disorders under the neurotic, stress-related, and somatoform disorders.

Table 1. ***Symptoms of anxiety***

Emotional manifestations	*Somatic manifestations*
Uncertainty	Heart palpitations
Despair	Chest pain
Uneasiness	Sweating
Tension	Increased blood pressure
Restlessness	Dizziness
Horror	Dry mouth
Panic	Lump in the throat
Agitation	Tremor
Nightmares	Chills or hot flushes
Flashbacks	Shortness of breath
Obsessive thoughts	Suffocation
Avoidance behavior	Nausea
Fear of losing control of life	Diarrhea

Table 2. ***Diagnostic criteria for selected anxiety disorders adapted from the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV-TR)***

Panic disorder
A. Both (1) and (2) (1) Recurrent unexpected panic attacks. (2) At least one of the attacks has been followed by at least 1 month of one or more of the following: Persistent concern about having additional panic attacks Worry about the implications of the attack or its consequences A significant change in behavior related to the attacks
B. Presence or absence of agoraphobia.
C. The panic attacks are not due to the direct physiologic effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hyperthyroidism).
D. The panic attacks are not better accounted for by another mental disorder.
Generalized anxiety disorder
A. Excessive anxiety about a number of events or activities, occurring more days than not, for at least 6 months.
B. The person finds it difficult to control the worry.
C. The anxiety and worry are associated with at least three of the following six symptoms (with at least some symptoms present for more days than not, for the past 6 months): Restlessness or feeling keyed up or on edge Being easily fatigued Difficulty concentrating or mind going blank Irritability Muscle tension Sleep disturbance
D. The focus of the anxiety and worry is not confined to features of an Axis I disorder, being embarrassed in public (as in social phobia), being contaminated (as in obsessive-compulsive disorder), being away from home or close relatives (as in separation anxiety disorder), gaining weight (as in anorexia nervosa), having multiple physical complaints (as in somatization disorder), or having a serious illness (as in hypochondriasis), and the anxiety and worry do not occur exclusively during posttraumatic stress disorder.
E. The anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social or occupational functioning.
F. The disturbance does not occur exclusively during a mood disorder, a psychotic disorder, pervasive developmental disorder, substance use, or general medical condition.
Agoraphobia
A. Fear of being in places or situations from which escape might be difficult (or embarrassing) or in which help might not be available in the event of having unexpected panic-like symptoms.
B. The situations are typically avoided or require the presence of a companion.
C. The condition is not better accounted for by another mental disorder.
Social phobia
A. A fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others and feels he or she will act in an embarrassing manner.
B. Exposure to the feared social situation provokes anxiety, which can take the form of a panic attack.
C. The person recognizes that the fear is excessive or unreasonable.
D. The feared social or performance situations are avoided or are endured with distress.
E. The avoidance, anxious anticipation, or distress in the feared situation interferes significantly with the person's normal routine, occupational functioning, or social activities or relationships.
F. The condition is not better accounted for by another mental disorder, substance use, or general medical condition.
G. If a general medical condition or another mental disorder is present, the fear is unrelated to it.
H. The phobia may be considered generalized if fears include most social situations.

Specific phobia

A. Persistent fear that is excessive or unreasonable, cued by the presence or anticipation of a specific object or situation.

B. Exposure provokes immediate anxiety, which can take the form of a situationally predisposed panic attack.

C. Patients recognize that the fear is excessive or unreasonable.

D. Patients avoid the phobic situation or else endure it with intense anxiety or distress.

E. The distress in the feared situation interferes significantly with the person's normal routine, occupational functioning, or social activities or relationships.

F. In persons younger than 18 years, the duration is at least 6 months.

G. The fear is not better accounted for by another mental disorder.

Post-traumatic stress disorder

A. The person has been exposed to a traumatic event in which both of the following were present:
(1) The person experienced, witnessed, or was confronted with an event that involved actual or threatened death or serious injury or a threat to the physical integrity of others.
(2) The person's response involved intense fear, helplessness, or horror.

B. The traumatic event is persistently re-experienced in at least one of the following ways:
Recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions.
Recurrent distressing dreams of the event.
Acting or feeling as if the traumatic event were recurring, including a sense of reliving the experience, illusions, hallucinations, and flashback episodes.
Intense psychological distress at exposure to cues that symbolize an aspect of the traumatic event.
Physiologic reactivity on exposure to cues that symbolize or resemble an aspect of the traumatic event.

C. The person persistently avoids stimuli associated with the trauma and has numbing of general responsiveness including at least three of the following:
Efforts to avoid thoughts, feelings, or conversations associated with the trauma
Efforts to avoid activities, places, or people that arouse recollections of the trauma
Inability to recall an important aspect of the trauma
Markedly diminished interest or participation in significant activities
Feeling of detachment or estrangement from others
Restricted range of affect

D. Persistent symptoms of increased arousal are indicated by at least two of the following:
Difficulty falling or staying asleep
Irritability or outbursts of anger
Difficulty concentrating
Hypervigilance
Exaggerated startle response

E. Duration of the disturbance is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Obsessive-Compulsive Disorder

A. Either obsessions or compulsions (or both) are present on most days for a period of at least 2 weeks.
(1) Obsessions
Recurrent and persistent thoughts, impulses, or images that are experienced as intrusive and inappropriate, causing anxiety or distress.
The thoughts, impulses, or images are not simply excessive worries about real-life problems.
The person attempts to ignore or suppress such thoughts, impulses, or images or to neutralize them with some other thought or action.
The person recognizes that the obsessional thoughts, impulses, or images are a product of his or her own
(2) Compulsions
Repetitive behaviors or mental acts that the person feels driven to perform in response to an obsession or according to rules that must be applied rigidly.
The behaviors or mental acts are aimed at preventing or reducing distress or preventing some dreaded event or situation.
These behaviors or mental acts either are not connected in a realistic way with what they are designed to neutralize or prevent, or they are clearly excessive.

B. At some point during the course of the disorder, the person has recognized that the obsessions or compulsions are excessive or unreasonable.

C. The obsessions or compulsions cause marked distress, take up more than 1 hour a day, or significantly interfere with the person's normal routine, occupation, or usual social activities.

D. If another Axis I disorder, substance use, or general medical condition is present, the content of the obsessions or compulsions is not restricted to it.

2.3.2 ANXIETY-RELATED PERSONALITY TRAITS

In one of the fundamental principles of the integrative science of personality, human personality is defined as an individual's unique variation on the general evolutionary design of human nature (McAdams and Pals, 2006). In addition to defining anxiety by the use of clinical anxiety disorder diagnoses, human anxiety is commonly assessed by quantitative measurement of a number of human personality traits. These are assessed with psychological personality inventories, such as the NEO-PI-R (Neuroticism-Extraversion-Openness Personality Inventory Revised; Costa and McCrae, 1992), EPQ (Eysenck Personality Questionnaire; Eysenck and Eysenck, 1975), or the TPQ/TCI (Tridimensional Personality Questionnaire/Temperament and Character Inventory; Cloninger, 1994). From a research point of view, personality traits offer the benefit that they can relatively easily be assessed in large samples using self-report questionnaires. Moreover, they are measured on a continuous quantitative scale, offering more power for statistical analyses than dichotomous clinical diagnoses.

In particular, personality traits such as high neuroticism, low extraversion, high harm avoidance, low novelty seeking, or high behavioral inhibition are related to anxiety disorders. They are heritable characteristics, and aggregate in families with anxiety disorders (Smoller et al., 2008a). Personality traits may relate to clinical anxiety disorders either by being susceptibility factors themselves or by being consequences of the disease (Brandes and Bienvenu, 2006). Personality traits may also share etiology with anxiety disorders, or shape the course of the disease. It is thought that some personality traits, such as the ones listed above, are risk markers for anxiety disorders, but also that remission may result in at least partial "improvement" in personality measures. It is also known that anxiety disorders in early life shape personality (Brandes and Bienvenu, 2006). Taken together, anxiety disorders and personality traits likely represent an overlapping spectrum of behavior. Moreover, also from a genetic perspective, anxiety disorders and personality traits represent overlapping entities, as correlation models based on twin studies suggest that they share genetic risk factors to a large extent. For instance, the genetic risk factors influencing variation in neuroticism and extraversion entirely account for genetic liability to social phobia and agoraphobia (Bienvenu et al., 2007). The factors modulating variation in neuroticism further show substantial overlap with those influencing PD and GAD (Hettema et al., 2006).

2.3.3 EPIDEMIOLOGY

Prevalence

The prevalence of anxiety disorders shows large variability between populations. Rather than being mostly due to true underlying reasons, the large variations in estimates across studies are accounted for by factors such as diagnostic criteria, diagnostic instrument, sample size, study country, and response rate (Somers et al., 2006). A recent large cross-European study reported that 14.0% of Europeans annually suffer from anxiety disorders (Wittchen et al., 2011). The corresponding figure from an American nationwide study was 18.1% (Kessler et al., 2005b). A systematic review of 41 anxiety disorder prevalence studies estimated pooled rates of 11% for 12-month prevalence, and 17% for lifetime prevalence (Somers et al., 2006). In Finland, the 12-month prevalence of anxiety disorders was 4.1% in the general adult (age ≥ 30 years) population (Pirkola et al., 2005b). However, this is an underestimate, as OCD and PTSD were not assessed. A supplementing examination of Finnish young adults (aged 19-34) that included those diagnoses reported a lifetime prevalence of 12.6% for anxiety disorders (Suvisaari et al., 2009).

A general pattern that emerges from the lifetime prevalence studies performed to date is that as a group anxiety disorders are always the most common type of mental disorders (Kessler et al., 2009). Among the anxiety disorders, specific phobias are typically the most common disorders (prevalence usually 6-12%), followed by social phobia (up to 10%), and PTSD (1% - more than 10%, depending on the population); Table 3. The other anxiety disorders usually have lower lifetime prevalences around 2-4%.

Table 3. ***Summary of epidemiological measures of the major anxiety disorders.*** *Estimates based on* (Kessler et al., 2009; Smoller et al., 2008a; Somers et al., 2006; Kessler et al., 2005a; Kessler et al., 2005b; Pirkola et al., 2005b; Hettema et al., 2001; Kendler et al., 1999) *are presented to give a general, albeit not all-encompassing, idea.*

	12-month prevalence (%)	Lifetime prevalence (%)	Median age-of-onset (yrs)	Odds ratio for 1st degree relatives of probands	Heritability (h^2)
Panic disorder	1-3	2-5	24	5	48
Generalized anxiety disorder	1-3	3-6	31	6	32
Obsessive-compulsive disorder	0.5-1	1-3	19	4	30–45
Post-traumatic stress disorder	1-4	1-10	23	-	30
Social phobia	1-7	4-10	13	3–10	51
Specific phobia	3-9	5-12	7	3–4	30
Agoraphobia	1-2	2-4	20	3-4	61

Comorbidities

Anxiety disorders frequently co-occur with each other, and with other psychiatric and physical disorders. In fact, the majority of anxiety disorder subjects will meet the criteria for another psychiatric disorder during their lifetime (Kessler et al., 2005b). It is not uncommon to meet criteria for two or more anxiety disorders. In the Finnish nationally representative Health 2000 sample, the most common comorbidity among the anxiety disorders was between PD and social phobia (6.4% of all anxiety disorder subjects), and 14.5% met the criteria for more than one anxiety disorder (Pirkola et al., 2005b).

Two major classes of mental disorders are often comorbid with anxiety disorders: mood and substance use disorders. In the Finnish Health 2000 sample, the annual prevalences of anxiety, depressive, and alcohol use disorders were 4.1%, 6.5%, 4.5%, respectively (Pirkola et al., 2005b). Of subjects with anxiety disorders in that study, 35.9% met criteria for a comorbid depressive disorder (major depressive disorder [MDD] and/or dysthymia), and 22.4% for a comorbid alcohol use disorder (alcohol abuse and/or dependence). In the American National Comorbidity Survey Replication, estimates of ORs for concomitant co-occurrence of anxiety disorders with MDD, dysthymia, and bipolar disorder ranged from 2.3–12.3, with lifetime estimates being even higher (Merikangas and Swanson, 2009). The corresponding figures for any alcohol use disorder were 1.5–4.7, and 1.1–3.5 for any drug abuse disorder. There is a general trend that substance dependence is more highly comorbid with anxiety disorders than substance abuse (Merikangas and Swanson, 2009).

A variety of physical conditions also co-occur with anxiety disorders in epidemiological studies, either in general or with specific anxiety disorder subdiagnoses. Examples include respiratory conditions like asthma with PD and phobias (Goodwin et al., 2003), gastrointestinal diseases like ulcer with PD/GAD (Sareen et al., 2005), brain injuries with any anxiety disorder (Luis and Mittenberg, 2002), and cardiovascular diseases and migraine with any anxiety disorders (Harter et al., 2003).

While the epidemiological evidence for comorbidity between anxiety disorders and the other conditions described is clear, the underlying reasons and causal relationships between the observations are not. There might be shared biological and genetic mechanisms that account for some of the co-occurences. Family and twin studies suggest that anxiety and depression are distinct disorders, but that they partially share underlying risk factors, including genetic ones (Merikangas and Swanson, 2009; Middeldorp et al., 2005). On the other hand, a consensus from family studies seems to be that substance use disorders are transmitted independently of anxiety disorders, and are not caused by the same familial risk factors. Use of alcohol for self-medication purposes of anxiety disorders is known to directly increase the risk for subsequent substance use disorders, and may represent one

explanation for the high comorbodity (Robinson et al., 2011). The causal relationships between physical- and anxiety disorders remain unclear, but they likely provide clues about biological processes that are relevant for anxiety.

Risk factors

Acknowledged risk factors for anxiety disorder onset include gender, age, family history and genetic factors, traumatic or stressful life events (particularly in childhood), socioeconomical factors, and certain medical conditions. The main risk factors are discussed in the following sections.

Gender

In general, anxiety disorders are about 1.7-2.0 times more common in women than men (McLean et al., 2011). However, this is not true for all anxiety disorder subtypes, as for instance social phobia is equally common in men and women. Anxiety disorders may also be more debilitating in women than men, as women with a lifetime diagnosis of anxiety disorder have greater illness burden, and are more likely to have psychiatric comorbidities, such as another anxiety disorder, MDD, or bulimia nervosa (McLean et al., 2011). However, epidemiological models suggest that the environmental and genetic factors that underlie anxiety disorders are similar between men and women (Hettema et al., 2005).

Age

A general pattern from age-of-onset studies of anxiety disorders is that they usually have much earlier onset than other common mental disorders like mood disorders, disruptive behavior disorders, nonaffective psychoses, and substance use disorders (Kessler et al., 2009). Both across Europe and in the USA, the onset of anxiety disorders is typically in childhood, adolescence, or early adulthood, with one estimate of the median age of onset being 11 years (Goodwin et al., 2005; Kessler et al., 2005a). However, different anxiety disorder subtypes show considerable variability. For instance, specific phobias and separation anxiety have very early median onset (7 years), social phobia intermediate onset (13 years), and other anxiety disorders (in ascending order of median age of onset: OCD, agoraphobia, PTSD, PD and GAD) relatively late onset (19-31 years). Despite the early age of onset, most subjects receive their first treatment in adulthood, even more than a decade later (Christiana et al., 2000). Anxiety disorders are typically persistent throughout life, often with a recurrent-intermittent course featuring episodes of different comorbid anxiety disorders (Kessler et al., 2009).

Family history

Family studies assess to what extent a disorder aggregates in a family (i.e., is familial). This is an indice of the total contribution of both genetic and environmental factors that the family shares. Anxiety disorders clearly aggregate in families. Odds ratios predicting disease risk for first-degree relatives of affected individuals typically range from 4-6 in family studies, and are similar across PD, GAD, OCD, and phobias (Smoller et al., 2008a; Hettema et al., 2001); Table 3, page 31. The contribution of genetics to anxiety disorder susceptibility is discussed in more detail in section 2.3.4.

Stressful life events and childhood adversities

One view explaining anxiety disorder onset is that environmental factors, such as traumatic life events, trigger them in individuals that are susceptible due to genetic, biochemical, or psychological factors. The clearest example of this is PTSD, where a single major traumatic life event (such as experience of combat, natural disasters, or sexual abuse) triggers an anxiety disorder in specific individuals, whereas others remain stress-resilient (Koenen et al., 2009). In general, even any milder life events that require adaptation, and involve change and uncertainty, can be emotional triggers of clinical anxiety disorders. Examples include financial difficulties, illness and lack of a social network.

Childhood is a particularly sensitive developmental period in life, and early experiences can have profound and persistent biobehavioral effects (Bale et al., 2010). Childhood adversities, in particular ones related to maladaptive family functioning (parental mental illness, substance abuse disorder, criminality, violence, physical/sexual abuse, and neglect) represent some of the strongest known risk factors for anxiety disorder onset and persistence (Green et al., 2010; McLaughlin et al., 2010). It is thought that traumatic events during childhood can influence later behaviour in interaction with genetic variants during development of the neurocircuitry that regulates emotional states (Gillespie et al., 2009). Therefore, analyses of predictors of anxiety disorder onset that have the possibility to take both genetic variation and childhood adversities into account might be advantageous.

2.3.4 GENETIC EPIDEMIOLOGY

Heritability

As noted above, family studies indicate that anxiety disorders "run in the family". However, family studies do not provide an answer to whether this is due to the genes, or the environment that relatives share. Twin studies that compare the concordance rate of a disorder in monozygous twins to that in dizygous twins make it possible to estimate the relative contribution of genetic and environmental factors to a phenotype. The proportion of total phenotypic variability in a trait that can be explained by additive genetic variation is called heritability. Twin studies suggest that anxiety disorders typically have moderate (30-40%) heritability (Hettema et al., 2001); Table 3, page 31. Although the major source of familiar risk is thought to be due to genetic factors, the major part of the overall variability in susceptibility to anxiety disorders seems to be explained by individual-specific environmental factors.

The heritabilities estimated for anxiety disorders are lower than for most other psychiatric disorders. However, they are likely no less genetically complex than other psychiatric disorders. Rather, the environment may play a relatively larger role in triggering onset of anxiety disorders than in other psychiatric disorders. Moreover, heritability is informative in that it tells us that genetics are involved, but not about how many variants influence a trait, or what their frequencies and relative effect sizes are. One should also remember that heritability estimates only apply to the specific population they were measured in, in its specific environment at that time.

Genetic architecture

The genetic architecture, or the structure of genetic determinants underlying of anxiety disorders, is unknown. Family studies suggest that the clinical disorders are not inherited as distinct entities, as relatives of affected individuals are at increased risk not only for the anxiety disorder of the proband, but also for other anxiety disorders (Smoller et al., 2008a). Multivariate models suggest that the genetic risk factors for different anxiety disorders overlap, and that they further cluster together with those of other major internalizing mental disorders of the DSM-IV axis I (MDD, eating disorders and somatoform disorders), forming a group distinct from the axis I externalizing disorders (e.g., substance abuse/dependence and conduct disorder) and the axis II personality disorders (Kendler et al., 2011). More specifically, within the anxiety disorders, PD, agoraphobia, and GAD seem to be influenced by one genetic risk factor, whereas another one mainly influenced specific phobias (Hettema et al., 2005). Social phobia was

influenced by both genetic risk factors. The consensus seems to be that genes do not know DSM-IV boundaries, and what is inherited might for instance be a type of quantitative anxiety proneness, or anxiety-predisposing changes in brain function and neurocircuitry (Smoller et al., 2008a). As noted above, genetic risk factors for anxiety disorders overlap with those influencing anxiety-related personality traits (Bienvenu et al., 2007; Hettema et al., 2006). One plausible consensus is that there are genes that are specific for some anxiety disorder-subtypes, some that increase anxiety susceptibility in general, and yet others that are shared risk genes with other psychiatric disorders. This hypothetical view of the genetic architecture of anxiety disorders in illustrated in Figure 5.

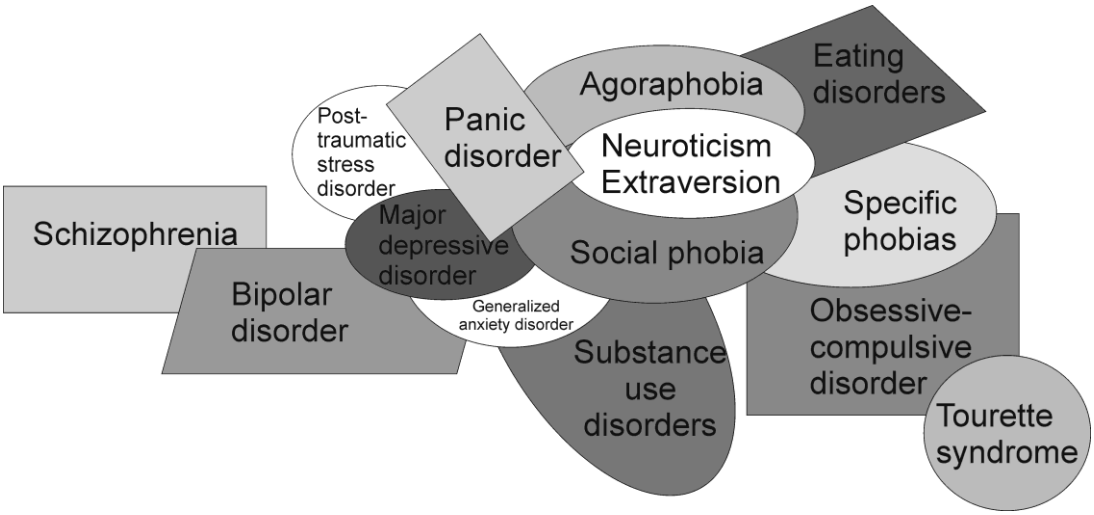


Figure 5 A hypothetical view of the genetic architecture of anxiety disorders. Twin- and family studies suggest that some genes increase susceptibility to anxiety in general, that other genes are shared susceptibility factors with other anxiety disorders or psychiatric disorders and that some may be specific for certain subtypes of anxiety. There is also considerable overlap between the genes influencing anxiety-related personality traits, and those influencing clinical anxiety disorders. Figure adapted based on an idea from (Smoller et al., 2008a).

2.3.5 NEUROBIOLOGY

Brain regions regulating anxiety

A vast body of research from both animal models and humans has aimed at identifying brain circuitry involved in anxiety regulation. This research is rooted in animal models of or human observations of the consequences of specific brain lesions, electrical stimulation work, animal paradigms of conditioned fear responses, and in neuroimaging studies of brain responses to emotional stimuli in humans (Shin and Liberzon, 2010). Imaging studies of subjects with specific anxiety disorders have also been carried out.

Ivan Pavlov's studies on conditioned responses in dogs in the 1920s laid the foundation for later research into mechanisms of fear conditioning, and coined the terminology still used today (Pavlov, 1927). Classical Pavlovian fear conditioning is based on repeatedly presenting a neutral stimulus like a tone together with an unpleasant stimulus (the unconditioned stimulus, US) such as an electrical shock that elicits an unconditioned response (UR) such as increased freezing. After repeating this for a number of times, presenting the originally neutral stimulus alone is sufficient to elicit a fear response. Once this has occurred, the neutral stimulus is called a conditioned stimulus (CS), as it has been coupled to a conditioned response (CR; increased fear response in this case). As anxiety is an evolutionarily conserved response, animal models of conditioned fear responses offer important insight into biology relevant also for human anxiety. In addition, distorted or overinterpreted conditioned fear reactions are directly thought to be involved in the pathogenesis of PD and other anxiety disorders (Gorman et al., 2000). Animal models aiming to identify neural circuitry relevant for anxiety offer the additional advantage that specific lesions or transgenic and pharmacological manipulations can be combined with neuroimaging, in vivo electrophysiological recordings, and behavioral testing. The effects of stimulating specific circuitry can also be evaluated.

Rodent behavioral paradigms of Pavlovian fear conditioning, inhibitory avoidance, and fear-potentiated startle have identified key components of the neurocircuitry of fear. These include the amygdala, nucleus accumbens including the bed nucleus of the stria terminalis, hippocampus, hypothalamus, periaqueductal grey, insular cortex, parts of the prefrontal cortex, and nuclei from the thalamus and brain stem (Maren, 2008; Quirk and Mueller, 2008; Davis, 2006). These regions appear to have their own specific roles in fear processing, such as threat perception, coupling of a neutral stimulus to a conditioned response, execution of the fear response, and modulation of the fear response (Shin and Liberzon, 2010). However, as a whole, anxiety is likely an emerging property of interacting brain regions (Morgane et al., 2005). An overview of the most relevant neuroanatomical pathways for fear processing is shown in Figure 6.

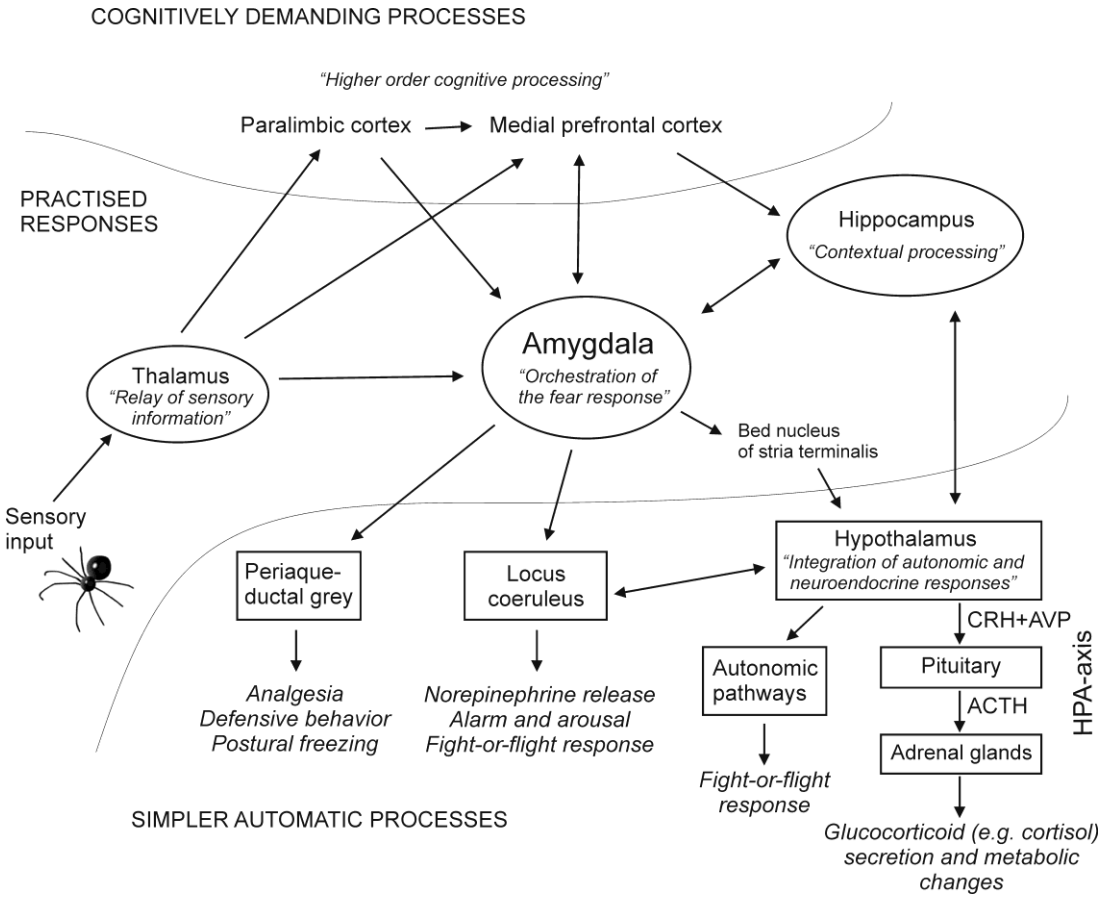


Figure 6 Simplified view of major neural circuits relevant for anxiety and fear response. The central co-ordinator of the fear response is the amygdala. Its lateral or basal nucleus receives input from three major sources: the thalamus, cortical pathways, and the hippocampus. The direct link between the thalamus and the amygdala enables fast, reflex-like response to stimuli predicting threat. The amygdala also receives processed and modulated neurocognitive information from the cortex, enabling more complex and appropriate reaction to the threat, depending on its extent and type. The hippocampus is thought to be responsible for fear memory, allowing for contextual processing of threat based on previous experiences. There are three major efferent pathways from the central nucleus of the amygdala that execute the fear response via the hypothalamus, locus coeruleus and periaqueductal grey. The hypothalamus activates the HPA (hypothalamus-pituitary-adrenal)-axis that leads to metabolic and behavioral changes crucial for the stress response. The key players of this pathway are the neuropeptides CRH (corticotropin-releasing hormone), AVP (arginine vasopressin) and ACTH (adrenocorticotropic hormone). The hypothalamus is also critical for inducing the symphatetic nervous system-mediated physiological changes that accompany the fight-or-flight response. Similarly, the locus coeruleus is responsible for stong physiological arousal induced by norepinephrine release. The periaqueductal grey dampens the conscious perception of pain. Figure based on (Finn et al., 2003; Gorman et al., 2000).

One of the most solid rodent findings is the involvement of the amygdala in fear acquisition and expression of the fear response. This is also supported by studies of Pavlovian fear conditioning and pharmacologically induced fear in non-human primates (LeDoux, 2000), and functional neuroimaging of responses to emotional stimuli and facial expressions in humans (Alvarez et al., 2008). Taken together, these studies suggest that the amygdala responds to threat-predicting stimuli, and mediates states of fear and anxiety. This has

formed the hypothesis that amygdalar responses are hyperreactive in anxiety disorder patients, which is supported by studies of subjects with e.g. PTSD, social phobia and specific phobias (Dilger et al., 2003; Tillfors et al., 2001; Shin et al., 1997).

Other key brain structures important for fear processing are the hippocampus and the ventromedial prefrontal cortex (vmPFC). The hippocampus has been implicated in contextual processing of fear, and the infralimbic cortex in extinction recall, which means recall of an extincted fear (Shin and Liberzon, 2010). These structures appear important for fear memory, or learning and remembering that a stimulus that used to predict threat no longer does so. This has lead to the hypothesis that exaggerated fear and worry in anxiety disorder patients is due to impaired ability to context-dependently quench anxiety responses when they are unnecessary, or recall that a specific stimulus no longer predicts threat. Such impaired fear extinction was observed e.g. in PTSD patients (Blechert et al., 2007b).

Neurochemistry of anxiety: Neurotransmitter systems and neuropeptides

Specific neurotransmitter- and neuropeptide signaling systems mediate the anxiety-regulating actions of the brain regions described above. Their release and signaling during stress has both central and peripheral functions in preparing the body for threat and coping with it. The central functions include increased attention and vigilance, modulation of memory to make use of prior experience and planning and preparation for action (Garakani et al., 2009). The peripheral effects include increased heart rate and blood pressure, and the modulation of the organism's allocation of energy. Neurotransmitters and neuropeptides thus have evolutionarily important adaptive functions with regard to fear responses, but their dysfunction could be an important factor underlying pathological anxiety. Therefore, current pharmacological treatment of anxiety disorders is based on targeting specific components of relevant neurotransmitter signaling systems, which has behavioral consequences. Pharmacological or genetic manipulation of neurotransmitters or their receptors in animals create models of anxiety-like behavioral, enabling functional studies of the underlying neural circuits. In humans, methods combining use of pharmacological agonists and antagonists of specific neurotransmitters with behavioral challenge tests and functional neuroimaging help to understand the function of neurotransmitter systems relevant for anxiety.

The four main neurotransmitter systems implicated and studied in anxiety disorders are the GABA (γ-aminobutyric acid)ergic, serotonergic, noradrenergic and dopaminergic systems (Durant et al., 2010).

γ-aminobutyric acid (GABA)

GABA is the major inhibitory neurotransmitter in the central nervous system (CNS), and thereby the principal regulator of the excitability of neurons and ongoing neural activity (Nutt, 2006). It is synthesized from glutamate by the glutamate decarboxylase enzymes. GABAergic neurons are widespread throughout the brain, and GABA has at least three main receptor types (Bormann, 2000). The most widely studied GABA receptor in the context of anxiety is the most common one, $GABA_A$, as subtypes of it are targeted by the benzodiazepine class of anxiolytics. Enhancing GABA signaling with $GABA_A$ –agonists has anxiolytic and sedative effects, while attenuating it with inverse agonists increases anxiety, arousal and restlessness (Durant et al., 2010). Consistently, there is clinical evidence supporting that subjects with PD and social phobia have lower brain GABA levels (Pollack et al., 2008; Chang et al., 2003).

Serotonin (5HT)

Serotonergic neurons arise from the so called raphe nuclei in the brainstem, and project throughout the forebrain (Durant et al., 2010). Serotonin (5HT; 5-hydroxytryptamine) is synthesized from the amino acid tryptophan. In general, serotonin influences central processing of emotional information (Harmer, 2008). Its release can have both anxiolytic and anxiogenic effects, depending on which region of the forebrain is involved, and which receptor subtype is activated (Garakani et al., 2009). One theory proposes that 5HT has dual roles in anxiety regulation: on one hand, it increases defensive responses and anticipatory anxiety by activating the amygdala and PFC, on the other hand, it inhibits the fight-or-flight response by activating the periaqueductal grey. This may explain why no simple relationships between the serotonergic system and anxiety have been established, and there is evidence supporting both a 5HT excess, and 5HT deficiency theory in anxiety predisposition (Durant et al., 2010). The serotonergic system components most relevant for anxiety are the 5HT1A receptor (one of 13 identified receptors) and the serotonin transporter (5HTT). In particular, selective serotonin reuptake inhibitors (SSRIs) are the most common group of anxiolytics used today, and they block the uptake of 5HT from the synaptic cleft by the 5HTT. Particularly relevant from a genetic perspective is the existence of a length repeat in the promoter region of the 5HTT encoding gene *SLC6A4*, which influences its expression level and thereby 5HT uptake. This repeat has been extensively evaluated for association to mood and anxiety disorder phenotypes since it was first shown to interact with stressful life events in influencing depression (Caspi et al., 2003). Although some smaller negative meta-analyses of the interaction exist (Munafo et al., 2009a; Risch et al., 2009), the largest one to date supports that the functional repeat

sequence in 5HTT moderates the relationship between childhood/life stress and depression (Karg et al., 2011).

Norepinephrine (NE)

Norepinephrine, also called noradrenaline, levels are increased in the brain in response to stress and modulate behavioral components of the stress response (Garakani et al., 2009). Most noradrenergic neurons arise in the locus coeruleus, and project from there throughout the forebrain (Durant et al., 2010). There are two main types of NE adrenoreceptors, α and β, with the α_2-receptor subtype appearing to be the most relevant one for anxiety disorders. Generally, agonists of adrenoreceptors have anxiolytic effects, whereas antagonists are anxiogens that may e.g. induce panic attacks. Activation of adrenoreceptors mediates many of the autonomous nervous system-dependent effects of anxiety that underlie the fight-or-flight response, such as increased heart rate, blood pressure and sweating.

Dopamine (DA)

Dopamine is synthesized from the amino acid tyrosine, and it is used as a neurotransmitter in several brain regions important for anxiety behavior. The main dopaminergic pathways include the nigrostriatal, mesolimbic and mesocortical ones (Durant et al., 2010). These are important for, among other things, stress responsiveness and reward behavior in brain regions such as the ventral tegmental area. There are clinical observations, and imaging studies suggesting that there might be a link between dopamine deficiency and social phobia (Tiihonen et al., 1997; Berrios et al., 1995).

Neuropeptides

Neuropeptides are small polypeptide signaling molecules that act as neurotransmitters or hormones and modulate neuronal function by binding to their specific receptors. They are involved in a wide variety of functions, such as regulation of feeding behavior, arousal and wakefulness, anxiety, learning and memory, lactation, pain and inflammatory responses (Hokfelt et al., 2000). The behavioral effect of some neuropeptides is to increase anxiety, whereas others are anxiolytic. Examples of neuropeptides that are particularly relevant for the regulation of anxiety are corticotropin-releasing hormone, neuropeptide Y, neuropeptide S, oxytocin, cholecystokinin, galanin and arginine vasopressin (Garakani et al., 2009; Madaan and Wilson, 2009; Thorsell, 2008; Xu et al., 2004; Bradwejn et al., 1991). Whereas NPY is an example of a ubiquous anxiolytic neuropeptide (Wu et al., 2011), injections of cholecystokinin tetrapeptide (CCK-4) can be used to induce panic attacks for experimental purposes (Bradwejn et al., 1991). In general, neuropeptides thus represent promising, and as of yet underexplored, targets for novel

anxiolytics (Madaan and Wilson, 2009). However, given that they usually have widespread behavioral and physiological effects, questions regarding their specificity and safety still remain to be resolved.

Many neuropeptides participate in the function of, or interact with, the hypothalamic-pituitary-adrenal (HPA) axis that controls stress responses and homeostatic processes related to digestion, energy allocation, sexuality, and mood or emotions. The relevance of this axis for anxiety is described next.

Hypothalamic-pituitary-adrenal (HPA) -axis

The neurocircuitry of anxiety overlaps and interacts with that of the stress response, of which the HPA-axis is an important component. The HPA-axis is a neuroendocrine system that consists of a set of interactions and feedback regulation between mainly the paraventricular nucleus of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal cortices (Figure 6, page 38). Upon a stressful stimulus, the neuropeptides corticorticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are synthesized and secreted from the paraventricular nucleus (Pego et al., 2010). They in turn stimulate the release of adrenocorticotropic hormone (ACTH) from the pituitary. It is transported by the blood to the adrenal cortex, where it stimulates the production of glucocorticoids such as the stress hormone cortisol. The glucocorticoids are important for the adaptive response to stress, as they dampen the immune and inflammatory responses, and influence energy metabolism by stimulating gluconeogenesis and release of substrates for energy production (Tsigos and Chrousos, 2002). Cortisol also has important central effects relevant for anxiety behavior, as it modulates the function of the amygdala, hippocampus, and PFC (Garakani et al., 2009). These actions are mainly mediated by binding of glucorticoids to the ubiquously distributed glucocorticoid receptor (Tsigos and Chrousos, 2002).

Dysregulation of the HPA-axis has been observed in several mood- and anxiety disorders. One consensus interpretation suggests that HPA-hyperreactivity is consistenly seen in depression, while the more heterogeneous anxiety disorder classes show a wider spectrum of HPA-alterations (Pego et al., 2010). As examples, PD subjects may have exaggerated and hypersensitive HPA-activity in response to novelty or contextual cues (Abelson et al., 2007), whereas patients with PTSD may have hypoactive HPA-axis responses (Yehuda et al., 1995). Of particular relevance for the development of anxiety disorders later in life may be early life traumatic events occuring during a developmental period of neuronal plasticity. These may cause persistent neuroendocrine changes that sensitize the HPA-axis, with predisposition for increased anxiety in adolescence and adulthood as a consequence (Bale et al., 2010; Gillespie et al., 2009).

2.3.6 TREATMENT

Anxiety disorders are usually treated with anxiolytic medication, psychotherapy, or a combination of both. Medication is important for keeping many of the physiological and psychological symptoms under control, while underlying reasons for the disease are explored in therapy where behavioral change is supported.

Pharmacological treatment

The first drugs developed specifically to target symptoms of anxiety were barbiturates in 1903 (Durant et al., 2010). They were widely used until the 1950s and were highly effective, but caused many accidental deaths due to respiratory arrest. They were therefore replaced by benzodiazepines in the 1950s, which are still among the most prescribed drugs for anxiety disorders. Both barbiturates and benzodiazepines are now known to potentiate GABA receptor function. Examples of commonly prescribed benzodiazepines include diazepam, lorazepam, clonazepam, and alprazolam (Pillay and Stein, 2007). Although benzodiazepines are effective in short term, they are usually prescribed only for short periods of time, as they are associated with physical and psychological dependence, and tolerance (Atack, 2010). They also have side effects such as sedation and cognitive impairment.

In the 1960s, the anxiolytic effects of antidepressants such as the tricyclics imipramine and clomipramine, and monoamine oxidase inhibitors (MAOIs) such as phenelzine received attention (Bespalov et al., 2010). These drugs increase available levels of both serotonin and norepinephrine (by either inhibiting their re-uptake from the synapse or inhibiting the monoamine oxidase that degrades them, respectively), but their anxiolytic effects are thought to be due to their serotonergic component. However, also tricyclics and MAOIs have side effects by influencing physiological processes mediated by the autonomic nervous system, interaction with the dietary monoamine tyramine (MAOIs) and antihistamine effects (tricyclics).

The selective serotonin re-uptake inhibitors (SSRI) class of antidepressants, such as fluoxetine, sertraline, escitalopram and paroxetine (Pillay and Stein, 2007) are considered a safer, more tolerable and efficient alternative than tricyclics and MAOIs (Bespalov et al., 2010). They are therefore the current recommended first line of treatment for anxiety disorder according to US and European treatment guidelines (Bandelow et al., 2008). SSRIs are believed to function by increasing levels of serotonin in the synaptic cleft by inhibiting its reuptake into the presynaptic cell. A disadvantage of SSRIs is that it takes up to several weeks for them to reach their full working potential. They also have a range of side effects, such as nausea, headache, and disturbances of sexual functioning. In addition to

SSRIs, also inhibitors of both serotonin and norepinephrine reuptake (SNRIs), such as venlafaxine and duloxetine, are in use.

Cognitive-behavioral therapy

Different types of cognitive-behavioral therapy are the most common type of psychotherapy for anxiety disorders. Methods used include exposure programs, cognitive restructuring procedures, anxiety management techniques, and their combinations (Choi et al., 2010). Cognitive-behavioral therapy aims to help people change the thinking patterns that support their fears, and to change the way they react to anxiety-provoking situations. For instance, PD patients are taught to think that panic attacks do not indicate a heart attack, and social phobia patients that they are not constantly judged by others. Exposure therapy, where the patient gradually encounters the object or situation that is the focus of his/her fear more strongly, is used in treatment of phobias.

Combining psychotherapy with medication

In general, it is thought that best results for the treatment of anxiety disorders are achieved by a combination of psychotherapy and drug treatment. This view is supported by meta-analytic comparisons of pharmacotherapy combined with psychotherapy in depressive disorders (Cuijpers et al., 2009). However, with regard to anxiety disorders, there are also several disappointing large trials that suggest that combined treatment provides little benefit compared to either type alone, and that responses to combined treatment may vary across the disorder subtypes (Otto et al., 2007; Foa et al., 2002). Pharmacological targeting of symptoms alone without treating the root source of the disorder may be therefore not be the optimal treatment strategy. The possibility to pharmacologically enhance the new learning that occurs in psychotherapy has emerged as a promising and more efficient strategy (Choi et al., 2010). One notable example of this comes from treatment of fear of heights, where D-cycloserine (a partial agonist of the N-methyl-d-aspartate receptor) treatment significantly accelerated the associative learning processes that are a part of fear extinction in patients undergoing behavioral exposure therapy (Ressler et al., 2004)

To summarize, many anxiolytics are available but a large percentage of anxiety disorder patients show only partial response or treatment resistance (Trivedi et al., 2006). All anxiolytics have complicating side effects. Therefore, there is a need to develop safer, more specific and more efficient anxiolytics.

2.3.7 LINKAGE STUDIES

The evidence suggesting that genetic factors are involved in determining anxiety predisposition has spurred numerous attempts at identifying specific susceptibility genes. Both studies using clinical dichotomous anxiety disorders, as well as quantitative measures of anxiety-related personality traits as phenotypes, have been carried out. In addition, both genome-wide linkage scans in families, and candidate gene analyses in case-control cohorts have been performed. Most recently, following the general trend in genetic mapping research, GWA studies have been carried out. The most promising findings from gene mapping attempts in anxiety disorders are described in the following sections. They are graphically summarized in Figure 7, page 52.

Few linkage analysis findings in anxiety disorders meet the criteria for genome-wide significance, LOD ≥ 3.3. Therefore, both these loci, and loci showing suggestive linkage (as defined by Lander and Kruglyak, 1995; LOD ≥ 1.9) in at least two independent samples are summarized in Table 4. Notably, due to the limited number of studies that have been carried out with each specific phenotype, solid susceptibility loci are scarce when requiring findings to the same phenotype and chromosomal position. Some potentially interesting overlaps are observed when anxiety disorders and anxiety-related personality traits are considered together. Of particular interest are loci on 7p (implicated in PD and neuroticism), 12q (neuroticism and shared genetic risk for PD and bipolar disorder), 13q (broadly defined PD syndrome), 14q (OCD, neuroticism, trait anxiety and phobias), and 22q (neuroticism and PD syndrome). Overall, there has been disappointingly little success in identifying specific susceptibility genes under the linkage peaks, and they typically harbor a large number of potential candidates. Fine mapping attempts also suffer from many of the same limitations as candidate gene association studies, described below.

Table 4. ***Genome-wide linkage scans in anxiety disorders and related personality traits.*** *Peaks were included if showing significant evidence for linkage (LOD ≥ 3.3) in at least one study, or if there was suggestive evidence (LOD ≥ 1.9) in more than one study.*

Linkage peak position	Maximum LOD score/ P-value	Phenotype	Subjects (N subjects/families)	Population	Reference
1p21	2.16	OCD	35/3	Costa Rica	Ross et al., 2011
1p22	3.25	EPQ neuroticism	561 sibpairs	England	Fullerton et al., 2003
2p22	3.3	PD	992/120[c]	USA	Logue et al., 2009
2q37	4.6	PD + bipolar disorder risk	992/120[c]	USA	Logue et al., 2009
3q27.3	2.9	OCD/compulsive hoarding	919/219[a]	USA	Samuels et al., 2007
3q27-28	2.67	OCD	649/219[a]	USA	Shugart et al., 2006
4q31	3.15	EPQ neuroticism subscale	561 sibpairs	England	Fullerton et al., 2003
4q31-4q34	$4.3x10^{-4}$	Anxiety disorders	219/19[b]	Connecticut	Kaabi et al., 2006
7p14	3.18	EPQ neuroticism	561 sibpairs	England	Fullerton et al., 2003
7p14.1	2.23	PD or panic attacks	113/23	US Midwest	Crowe et al., 2001
7p15.1	2.45	PD	368/23[c]	USA	Knowles et al., 1998
9q31.1	4.18	PD	67/25	Iceland	Thorgeirsson et al., 2003
10p14-15.3	2.0	EPQ/NEO neuroticism	5069 sibpairs	Australia and Netherlands	Wray et al, 2008
10p15	2.43[d]	OCD	121/26	USA	Hanna et al., 2007
12q23	3.6	PD + bipolar disorder risk	992/120[c]	USA	Logue et al., 2009
12q23.1	3.95	EPQ neuroticism	561 sibpairs	England	Fullerton et al., 2003
12q24.3	2.13	EPQ neuroticism	714 sibpairs	Ireland	Kuo et al., 2007
13q32	4.2	PD syndrome	476/34[c]	USA	Weissman et al., 2000
13q32.1-q32.3	3.57	PD syndrome	587/60[c]	USA	Hamilton et al., 2003
14q13	3.7	Specific phobia	129/14	Connecticut	Gelernter et al., 2003
14q31.3	3.66	OCD/compulsive hoarding	919/219[a]	USA	Samuels et al., 2007
14q32.1	2.6	EPQ/NEO neuroticism	5069 sibpairs	Australia and Netherlands	Wray et al, 2008
14q32.2	3.4	State Trait Anxiety Inventory	2188/566	Netherlands	Middeldorp et al., 2008
16p12.1	1.9	PD or panic attacks	113/23	US Midwest	Crowe et al., 2001
16p13.3-p13.2	3.13	PD syndrome	587/60[c]	USA	Hamilton et al., 2003
21q22	3.42[d]	NEO neuroticism	221 descendants of 20 related couples	Netherlands	Amin et al,. 2011
22q11	3.07	NEO neuroticism	221 descendants of 20 related couples	Netherlands	Amin et al,. 2011
22q12.3-q13.1	4.11	PD syndrome	587/60[c]	USA	Hamilton et al., 2003

Chr = chromosome; EPQ = Eysenck Personality Questionnaire; LOD = logarithm of the odds; NEO = Neuroticism-Extraversion-Openness Personality Inventory; OCD = obsessive-compulsive disorder; PD = panic disorder; PD syndrome = PD with bladder/renal conditions, serious headaches, thyroid problems and/or mitral valve

[a, b, c] Partially overlapping families

[d] Non-parametric LOD

2.3.8 CANDIDATE GENE STUDIES

Selection of genes for examination in candidate gene association studies of anxiety disorders has primarily been based on prior assumptions about the underlying biology. The most studied candidates are genes encoding proteins involved in neurotransmitter metabolism and signaling, proteins targeted by anxiolytics, neuropeptides and genes of the stress response. Among the single most studied genes are *COMT* (catechol-*O*-methyltransferase) and *SLC6A4* (serotonin transporter). COMT degrades dopamine, epinephrine and norepinephrine and thereby influences neurotransmitter levels, whereas SLC6A4 is responsible for the uptake of serotonin from the synaptic cleft.

Due to the large number of association studies carried out and the high probability that some published associations are spurious, strict criteria are needed to identify the most likely true susceptibility genes. Genes showing evidence for association with $P \leq 0.01$ in at least two independent studies of anxiety disorders or anxiety-related personality traits are summarized in Table 5. Yet stricter criteria would be requiring that the same allele of the same variant has been associated with the same phenotype in at least two studies. Such level of evidence has been obtained for *COMT* (in PD and OCD), *HTR2A* (5-hydroxytryptamine [serotonin] receptor 2A; in PD), *DRD2* (dopamine receptor D2; in PTSD) and *FKBP5* (FK506-binding protein 5; in PTSD). However, it is important to note that the majority of published studies for the listed genes are in fact negative (Smoller et al., 2009).

Meta-analyses of the three most studied individual variants, Val158Met in *COMT*, the promoter length repeat polymorphism (5-HTTLPR) in *SLC6A4* and Val66Met in *BDNF* (brain-derived neurotrophic factor) exist. They suggest that *COMT* Val158Met is a susceptibility factor for PD, but the risk allele shows ethnic heterogeneity and effects female-specificity (Domschke et al., 2007). Meta-analyses of 5-HTTLPR and neuroticism/harm avoidance show conflicting results, suggesting that its effect, if any, is minor (Munafo et al., 2009b; Sen et al., 2004). Overall, Met-carriers of *BDNF* Val66Met have lower neuroticism scores, but there was no association between the variant and anxiety disorders (Frustaci et al., 2008). Taken together, it is difficult to make solid claims regarding links between the most popular candidate genes for neuropsychiatric disorders and susceptibility to anxiety. However, animal evidence supporting their involvement in regulation of anxiety-like behavior should not be forgotten (e.g., Papaleo et al., 2008; Holmes et al., 2003).

Lately, it has been increasingly acknowledged that gene x environment (GxE) interactions play an important role in determining individual variation in stress resilience and vulnerability to mental disorders (Wermter et al., 2010). Several studies have thus examined genetic variation for effects in modulating disease predisposition in interaction with the environment. The environmental factors most studied in the context of anxiety are childhood

adverse life events, which are known strong risk factors for anxiety disorders later in life (Green et al., 2010). Again, the length polymorphism of *SLC6A4* has received the most attention after the initial finding that it modulates the effect of stressful life events on depression, depressive symptoms and suicidality (Caspi et al., 2003). This finding is supported by the largest meta-analysis of the interaction to date, in particular with childhood maltreatment as the stressor (Karg et al., 2011). Interactions between *SLC6A4* and early life stress in influencing anxiety sensitivity and PTSD diagnosis were also seen (Xie et al., 2009; Stein et al., 2008). Recent GxE findings in anxiety further include interaction of FK506-binding protein 5 (*FKBP5*), a regulator of glucocorticoid receptor sensitivity, with early life stress in modulating risk for PTSD symptoms and diagnosis (Xie et al., 2010; Binder et al., 2008).

A likely reason for the many discrepancies in the candidate gene literature of anxiety- and other neuropsychiatric disorders is that many studies, especially in the early days, were performed with small sample sizes. They were underpowered to detect the modest effects now thought to be conferred by individual risk variants. The genetic and phenotypic heterogeneity that underlie complex mental disorders have further complicated their study, increasing the sample size required to observe statistically significant evidence for association that survives correction for multiple testing.

Table 5. ***Putative susceptibility genes for anxiety disorders and related personality traits.*** *Only genes showing evidence for association with P ≤ 0.01 in at least two independent human study samples were included in this summary.*

Gene symbol	Gene name	Phenotype	References
ADORA2A	adenosine A2a receptor	PD, TPQ harm avoidance	Hohoff et al., 2010; Deckert et al., 1998
BDNF	brain-derived neurotrophic factor	OCD, phobias, TCI harm avoidance	Xie et al., 2011; Montag et al., 2010; Hall et al., 2003
CCKBR	cholecystokinin B receptor	PD	Hösing et al., 2004; Kennedy et al., 1999
COMT	catechol-*O*-methyltransferase	PD, OCD, phobic anxiety, TCI harm avoidance, genetic susceptibility shared by anxiety spectrum phenotypes	Hettema et al., 2008; Pooley et al., 2007; Kim et al., 2006; Rothe et al., 2006; McGrath et al., 2004
DRD2	dopamine receptor D2	PTSD, GAD, social phobia	Sipilä et al., 2010; Lawford et al., 2006; Young et al., 2002
FKBP5	FK506-binding protein 5	PTSD	Xie et al., 2010; Binder et al., 2008
HTR2A	5-hydroxytryptamine receptor 2A	PD, OCD	Maron et al., 2005; Meira-Lima et al., 2004; Inada et al., 2003
MAOA	monoamine oxidase A	PD, GAD, phobias	Samochowiek et al., 2004; Tadic et al., 2003; Deckert et al., 1999
RGS2	regulator of G-protein signaling 2, 24kDa	PD, PTSD symptoms, behavioral inhibition	Otowa et al., 2011; Amstadter et al., 2009;Smoller et al., 2008
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	PD, OCD, STAI, anxiety and/or MD, neuroticism	Costas et al., 2010; Wray et al., 2009; Strug et al., 2010; Bloch et al., 2008

GAD = generalized anxiety disorder; MD = major depression; OCD = obsessive-compulsive disorder; PD = panic disorder; PTSD = post-traumatic stress disorder; STAI = State Trait Anxiety Inventory; TCI = Temperament and Character Inventory; TPQ = Tridimensional Personality Questionnaire

2.3.9 GENOME-WIDE ASSOCIATION STUDIES

As previously noted, hypothesis-free GWA studies are now commonplace in the study of complex disorders. A limited number of such studies have examined anxiety-related phenotypes, and only one has reported genome-wide significant results ($P < 5 \times 10^{-8}$; defined by Risch and Merikangas, 1996). All studies performed to date that have reported at least suggestive associations ($P < 10^{-4}$) are summarized in Table 6.

Table 6. ***Genome-wide association studies in anxiety disorders and related personality traits.*** *SNP findings are ranked from most to least significant evidence for association.*

Implicated chromosomal position	Implicated gene	Most significant P-value[a]	Phenotype	Subjects (cases/ controls)[a]	Population	Reference
12p13	*ANO2* (anoctamin 2)	3.7×10^{-9}	PD	200/200	Japan	Otowa et al., 2009
1q32	*PKP1* (plakophilin 1)	4.6×10^{-8}	PD	200/200	Japan	Otowa et al., 2009
13q32	*GPC6* (glypican 6)	1.0×10^{-7} [b]	EPQ neuroticism	2235 subjects	Switzerland	Calboli et al., 2010
12q24.33	*TMEM132D* (transmembrane protein 132D)	1.2×10^{-7}	PD	909/915	Germany	Erhardt et al., 2011
17q25	*SDK2* (sidekick homolog 2)	2.1×10^{-7}	PD	200/200	Japan	Otowa et al., 2009
13q32	-	3.1×10^{-7}	PD	200/200	Japan	Otowa et al., 2009
12q13	*CALCOCO1* (calcium binding and coiled-coil domain 1)	3.3×10^{-7}	PD	200/200	Japan	Otowa et al., 2009
6q21	*NKAIN2* (Na+/K+ transporting ATPase interacting 2)	3.4×10^{-7}	EPQ neuroticism	2235 subjects	Switzerland	Calboli et al., 2010
6q25	*PLEKHG1* (pleckstrin homology domain containing, family G)	4.9×10^{-7}	PD	200/200	Japan	Otowa et al., 2009
8p21	*CLU* (clusterin)	6.8×10^{-7}	PD	200/200	Japan	Otowa et al., 2009
14q21.3	*MDGA2* (MAM domain containing glycosylphosphatidylinositol anchor 2)	6.9×10^{-7}	EPQ neuroticism	3107 subjects	USA	van den Oord et al., 2008
6q14.1	*LCA5* (Leber congenital amaurosis 5)	7×10^{-7}	Hoarding	3410 subjects	Caucasian	Perroud et al., 2011
15q26.3	*ARRDC4* (arrestin domain containing 4)	1.5×10^{-6}	EPQ neuroticism	2235 subjects	Switzerland	Calboli et al., 2010
5q11.2	-	2×10^{-6}	Hoarding	3410 subjects	Caucasian	Perroud et al., 2011
5q12	*PDE4D* (phosphodiesterase 4D, cAMP-specific)	2×10^{-6}	EPQ neuroticism	3500 subjects	England	Shifman et al., 2008
20p12-p11.2	*SNAP25* (synaptosomal-associated protein, 25kDa)	5×10^{-5}	NEO neuroticism	7012 subjects	Sardinia, USA and Netherlands	Terracciano et al., 2010

EPQ = Eysenck Personality Questionnaire; Hoarding = acquisition of items in pathological excess, and failure to discard them - a manifestation of obsessive-compulsive disorder; NEO = Neuroticism-Extraversion-Openness Personality Inventory; PD = panic disorder

[a] Based on inclusion of replication samples, where applicable

[b] P-value for interaction with age

One general implication from GWA studies in anxiety-related phenotypes has been that the effect sizes of individual common risk variants on the phenotype are small, as in most other complex disorders (Bodmer and Bonilla, 2008). For instance, SNPs reported to influence neuroticism explain less than 1% of the total genetic variation in the trait (Shifman et al., 2008; van den Oord et al., 2008). Therefore, there is a call for studies performed by international collaborative networks with the larger sample sizes that are likely required to identify any putative common variants with small sample sizes. Such studies have already been performed in other psychiatric disorders, and are currently ongoing in anxiety disorders.

Another general implication from the GWA studies performed to date is that there is little specific overlap with the linkage studies performed with the corresponding phenotypes. Notable and potentially interesting overlaps when considering clinical anxiety disorders and anxiety-related personality traits together include the 13q32 region, for which strong genetic linkage (LOD = 4.2) with a broadly defined PD syndrome was reported (Weissman et al., 2000), along with GWA associations with PD and neuroticism (Calboli et al., 2010; Otowa et al., 2009). However, no specific candidate genes have been conclusively identified in the region so far.

Another interesting position may be the 12q23-24 reqion, which was implicated in linkage studies of neuroticism and shared genetic risk factors for PD/bipolar disorder (Logue et al., 2009; Kuo et al., 2007; Fullerton et al., 2003). This region also showed significant association in a GWA study of PD (Erhardt et al., 2011). Extension of the study with two replication samples confirmed the observed associations with PD in the *TMEM132D* (transmembrane protein 132D) gene, but also showed that it associated with anxiety symptoms in a broader sense. The authors further demonstrated that anterior cingulate cortex expression levels of *Tmem132d* were positively correlated with anxiety-like behavior in a mouse models of extremes of trait anxiety, and a that a SNP from the gene associated with anxiety-related behavior. Taken together, *TMEM132D* is one of the more promising candidate genes to have been identified by the GWA approach, but its function in general, and in anxiety, is not well characterized yet. It is a membrane protein that could be involved in neural interconnection and signaling (Erhardt et al., 2011).

Two other candidate genes first identified by GWA approaches have subsequently shown evidence for association in other independent studies, *MDGA2* (MAM domain containing glycosylphosphatidylinositol anchor 2) and *PDE4D* (phosphodiesterase 4D). *MDGA2* was first identified in a GWA of neuroticism in an American sample, and the findings were supported by replication in a German sample (van den Oord et al., 2008). A subsequent candidate gene study replicated the finding with neuroticism, and extended them by also reporting associations between the gene and harm avoidance (Heck et al., 2011). Although the function of *MDGA2* is not well understood, it is expressed in the brain and it encodes an immunoglobulin domain cell

adhesion molecule that could regulate neuronal migration and axon guidance. *PDE4D* was identified in the first GWA of neuroticism that was published (Shifman et al., 2008), and some nominally significant support was obtained for the association in the largest neuroticism GWA to date (Calboli et al., 2010). *PDE4D* may well have behavioural effects relevant for anxiety, as it encodes an enzyme that degrades the important second messenger molecule cAMP (cyclic adenosine monophosphate). Furthermore, mice deficient of *Pde4d* show reduced depression-like behavior.

The only two GWA findings meeting strict criteria for genomewide significance ($P < 5 \times 10^{-8}$) are from a study of PD in the Japanese population (Otowa et al., 2009). The two implicated genes were *PKP1* (plakophilin 1) and *ANO2* (anoctamin 2). Of these, PKP1 participates in desmosome formation, and ANO2 is a calcium-activated chloride channel. However, these findings have so far not been replicated in independent samples.

Taken together, the GWA studies performed in anxiety disorders and related personality traits so far have identified a few new promising candidate genes. The hypothesis-free genome-wide scans have an important role in that they may lead to discoveries beyond the "usual suspect" candidate genes heavily studied in anxiety disorders. However, additional replication and functional studies are essential for confirming their involvement in susceptibility to anxiety. Figure 7 graphically summarizes the most promising results from human linkage studies, and candidate gene and genome-wide association studies, to date.

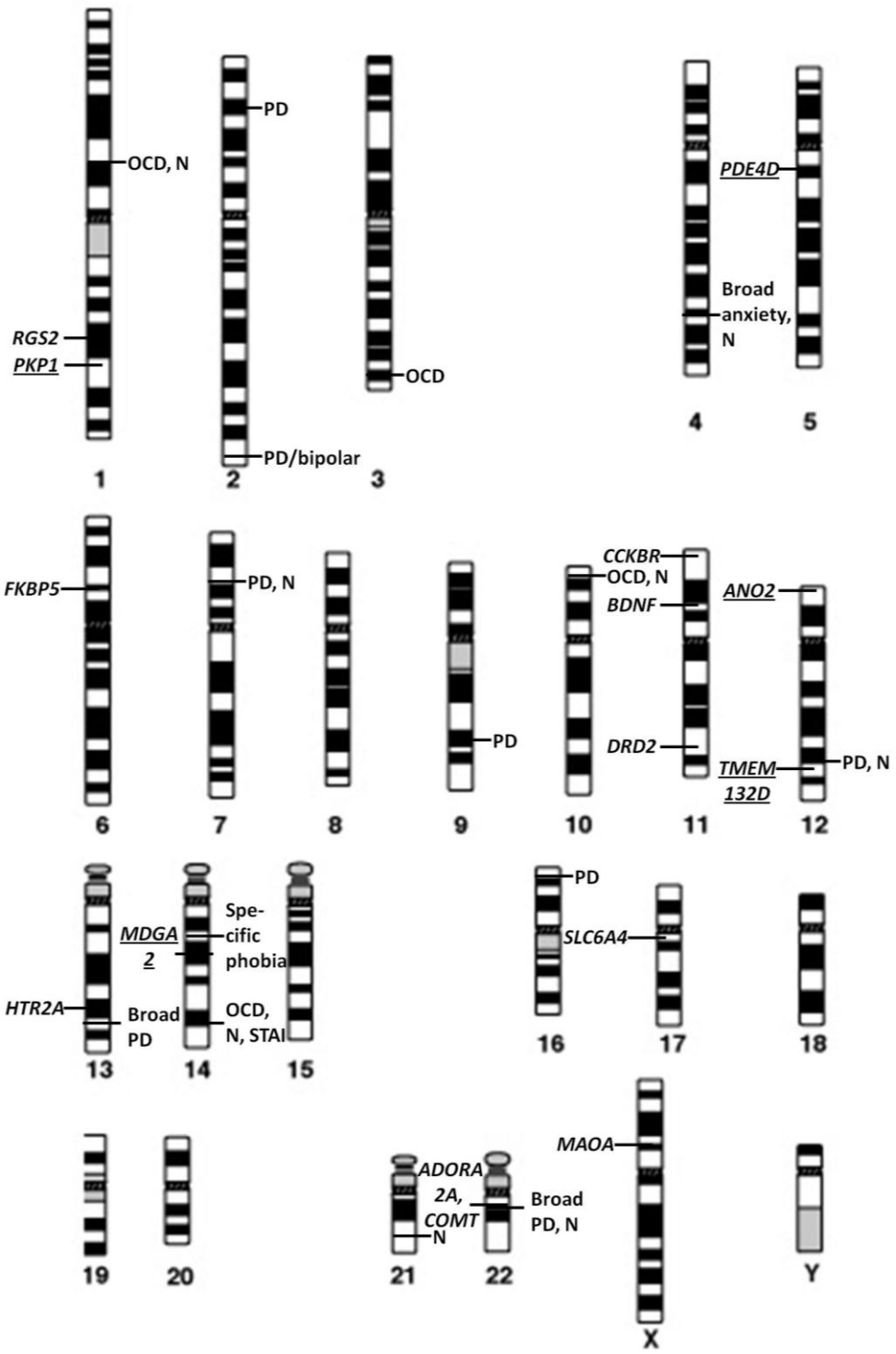


Figure 7 The genomic landscape of anxiety disorders and anxiety-related personality traits. Figure based on Tables 4, 5 and 6. Please refer to them for specifc references and full gene names. Linkage findings with either LOD ≥ 3.3 in one study, or LOD ≥ 1.9 in at least two independent studies, are shown on the right side of each chromosome. Candidate genes showing evidence for association with P ≤ 0.01 in at least two studies are shown in italics on the left side of each chromosome. The most promising gene findings from genome-wide association studies are shown in underlined italics on the left side of each chromosome. Cytogenetic map by courtesy of David Adler (www.pathology.washington.edu/ research/cytopages/idiograms/human/).

2.3.10 GENE EXPRESSION PROFILING STUDIES

In addition to evaluations of differences in DNA-level variations between anxiety disorder patients and controls, gene expression profiling studies have also been carried out. Such studies aim to identify transcripts that are either up- or downregulated in high anxiety conditions. As expression levels are functional mediators of gene function, global gene expression profiling can lead to identification of regulatory networks and biological pathways that are dysregulated in disease states. Such studies have the additional advantage that they can be carried out on samples from the tissue most relevant for the investigated disease, such as post-mortem samples of specific brain regions. However, for practical reasons, most profiling studies done in human anxiety disorders so far were done using peripheral lymphoblasts as source material. The total number of performed gene expression studies is also limited.

Two studies have examined gene expression profiles in panic-related phenotypes. The first compared lymphoblastoid transcriptomes of 16 PD patients and 17 controls, and identified ~3000 transcripts with either up- or downregulation in all patient cell lines (Philibert et al., 2007). Disappointingly, no enriched functional pathways were found among the differentially regulated transcripts. The second study used panic attacks induced in 31 healthy subjects by CCK-4 injections as an experimental model (Maron et al., 2010). Sixty-one transcripts were differentially expressed between subjects who responded to the CCK-4 challenge with a panic attack, and those who did not panic. Many of them were involved in immune response or related to the clinical phenotypes of disorders comorbid with PD, such as asthma, diabetes or coronary artery disease. Three transcripts were dysregulated in both of the aforementioned studies, namely *SREBF2* (sterol regulatory element binding transcription factor 2), *ARHGEF1* (Rho guanine nucleotide exchange factor 1) and *EPSTI1* (epithelial stromal interaction 1), and in particular these should be further examined in PD susceptibility.

All other gene expression profiling studies in anxiety have examined subjects with PTSD following trauma exposure. They were carried out with limited sample sizes and show little overlapping results (Sarapas et al., 2011; Zieker et al., 2007; Segman et al., 2005). The largest study to date (40 subjects) examined survivors of the 9/11 World Trade Center attacks, and found distinct expression profiles associated with PTSD risk, resilience and symptom recovery (Sarapas et al., 2011). More specifically, the study identified MHC class II and *FKBP5* (also showing GxE interactions in modulating PTSD symptoms; Xie et al., 2010) as state markers of PTSD with lower expression in subjects with active or remittent disease.

To summarize, gene expression studies may result in identification of biomarkers or risk profiles for anxiety, but larger studies are needed. Moreover, the relevance of gene expression findings in peripheral blood for CNS pathology remains poorly examined in anxiety-related phenotypes.

2.3.11 ANIMAL MODELS

In general, animal models offer advantages such as access to biomaterial from specific brain regions, minimized genetic heterogeneity in inbred strains and possibilities to control environmental effects and administer specific compounds. As discussed earlier, anxiety is an evolutionarily conserved response, and many of the biological mechanisms identified in for instance rodents are likely to be relevant also for human anxiety. A large variety of behavioral paradigms have been developed for the measurement of anxiety-like behavior in rodents, and pharmacologically validated with anxiolytic drugs. The most commonly used ones include the elevated plus maze (Lister, 1987), light/dark box (Crawley and Goodwin, 1980), and open field tests (Hall and Ballachey, 1932), which are all based on the approach-avoidance conflict, or the choice a rodent has when exposed to a novel environment: to either explore the potentially threatening but also interesting surroundings, or stay in a more sheltered area. Other paradigms are based on social interaction or social defeat (Björkqvist, 2001; File and Hyde, 1978). Rodent paradigms likely model aspects of human anxiety, but no single optimal model exists for particular human anxiety disorders.

There are a number of different experimental approaches that make use of animal models to either identify genes that influence anxiety, or to study the function of specific candidate genes. Examples of animal model-based approaches that have lead to important insight into mechanisms underlying anxiety, and identification of novel potential candidate genes, are given in the following sections.

Mapping of quantitative trait loci (QTL) for anxiety

More than a hundred different inbred mouse strains have been produced for laboratory use, such as C57BL/6, DBA/2, and BALB/c. They have different behavioral characteristics, including differential levels of innate anxiety, as a consequence of the genetic background. Quantitative trait locus (QTL)-mapping makes use of this to identify loci that influence a trait. The principle is to cross two inbred strains with extreme behavior (e.g., high and low anxiety). The F1 (filial 1) offspring will all have the same heterozygous genome, but when they are crossed with each other to produce the F2 generation the result is genetically and phenotypically unique offspring. Finally, behavioral phenotyping of the F2 individuals is combined with genotyping of markers spread throughout the genome. The final analysis is a genome-wide linkage scan for loci influencing the trait of interest, followed by fine mapping efforts. Nowadays, panels of recombinant inbred mice with genomes that are random mosaics of founder haplotypes from parental inbred strains (typically two strains) are available, and offer an easier start for QTL-mapping studies (Flint et al., 2005). Efforts like the Collaborative Cross that aim to create recombinant inbred strains that are mosaics of up to

eight different inbred mouse strains are ongoing. Such efforts would allow for much greater mapping resolution, which would be important as the main disadvantage of QTL-mapping is that the identified QTLs are typically large (10-20 cM) and contain many polymorphisms in several potential candidate genes.

Multiple QTL-mapping efforts focusing on murine anxiety have been published (Henderson et al., 2004; Turri et al., 2001). The corresponding chromosomal regions have subsequently been tested for linkage or association to PD, anxiety proneness and behavioral inhibition in human study samples (Smoller et al., 2001; Smoller et al., 2001). The only true success story in QTL-mapping of anxiety-related phenotypes is identification of the *Rgs2* (regulator of G-protein signaling 2) gene. A QTL for emotionality was first identified on chromosome 1 (Flint et al., 1995), and subsequently finemapped to a narrow 0.8 cM interval in the 58th generation of mice originally descending from 8 inbred mouse strains (Talbot et al., 1999). A quantitative complementation approach finally identified the gene responsible for the signal as *Rgs2* (Yalcin et al., 2004). Today, genetic variation in *RGS2* has been associated with human PD, PTSD symptoms and behavioral inhibition (Otowa et al., 2011; Amstadter et al., 2009; Smoller et al., 2008b). Remarkably, a SNP in *RGS2* was estimated to explain 10-15% of the variation in amygdala and insular cortex activation in response to emotional faces (Smoller et al., 2008b).

Gene expression and proteomics-based approaches for identification of genes influencing anxiety

Global gene expression and proteomic profiling approaches have also been used in animal models to identify genes and biochemical pathways important for anxiety. In one study particularly relevant for the work presented in this thesis, Hovatta et al. used inbred mouse strains that differ in their innate levels of anxiety-like behavior as a model, and performed gene expression profiling of seven brain regions involved in the regulation of anxiety (Hovatta et al., 2005). They found 17 genes with an expression pattern that correlates with anxiety, suggesting that they may modulate the trait. Due to its relevance for the candidate gene selection of this thesis, the work of Hovatta et al. is described in more detail in the Materials and Methods (section 4.2).

Other laboratories have used selective breeding schemes to produce mouse strains with high or low anxiety-like behavior (HAB and LAB, respectively) from outbred strains. Recently, two independent studies comparing brain region transcription profiles of such HAB and LAB strains were published (Czibere et al., 2011; Virok et al., 2011). Czibere et al. identified four genes with large (> 500%) expression changes between the strains in all examined brain regions, namely *Abca2* (ATP-binding cassette, sub-family A member 2), *Ctsb* (cathepsin B), *Enpp5* (ectonucleotide pyrophosphatase /phosphodiesterase 5) and *Ttbk1* (tau tubulin kinase 1). Of

these, *Ctsb* emerged as the most promising novel candidate for influencing emotionality, as *Ctsb*-deficient mice showed increased anxiety-like behavior. Virok et al. identified both previously known and novel functional gene networks among the gene sets differentially regulated between HAB and LAB mice. One of the most prominent findings was alterations of neuropeptide-encoding genes and members of neuropeptidergic signaling, such as NPY, neurotrophin 3, neurotensin and angiotensinogen.

Similarly, proteomic comparisons of brain tissues from HAB and LAB mice have been made to identify changes in protein levels potentially relevant for anxiety. Enolase-phosphatase 1 (Enoph1) and glyoxalase 1 (Glo1) were identified by 2D polyacrylamide gel electrophoresis combined with mass spectrometry as protein markers of anxiety (Ditzen et al., 2010; Krömer et al., 2005). HAB and LAB mice have different isoforms of Enoph1, a member of the methionine salvage biochemical pathway, with the HAB strain isoform having relatively lower enzyme activity (Ditzen et al., 2010). HAB mice also have lower levels of Glo1, an antioxidative and detoxification enzyme that protects agains oxidative stress (Krömer et al., 2005). Downregulation of Glo1 in the brain of anxious mice was also observed among the 82 differentially expressed proteins discovered by a separate laboratory using a similar selective breeding approach (Szego et al., 2010). In contrast, upregulation of *Glo*1 in more anxious inbred mouse strains was observed in the gene expression profiling work mentioned above (Hovatta et al., 2005). Moreover, local overexpression of *Glo1* in the cingulate cortex increased anxiety-like behavior, whereas silencing decreased it, further supporting a causal role between Glo1 levels and anxiety. It has now become evident that the difference in *Glo1* expression between inbred mouse strains is due to a copy number variant, the presence of which correlates positively with anxiety-like behavior (Williams et al., 2009). These findings, combined with observations of reduced Glo1 expression in human patients with active depression or bipolar disorder (Fujimoto et al., 2008), underscore the need for further studies of the gene as a potential susceptibility factor for psychiatric disorders.

In recent years, the importance of miRNAs as functionally important post-transcriptional regulators of gene expression has been realized. miRNAs bind to complementary sequences on target mRNA transcripts, typically conferring translational repression or target degradation and thereby gene silencing. Attempts to identify specific disease-associated miRNAs have been made. So far, no large scale studies have been made in anxiety-related phenotypes. One study looked for hippocampal miRNA expression differences between four inbred mouse strains, and reported 11 miRNAs with expression patterns that correlated significantly with different behavioral measures (Parsons et al., 2008). Of these, the most relevant for anxiety were miR-34c and miR-323. Later on, it was shown that lentiviral-mediated overexpression of miR-34c in the central amygdala induces anxiolytic behavior (Haramati et al., 2011).

To summarize, some promising candidate genes for influencing anxiety have first been identified by animal-model based identification strategies such as QTL-mapping and global expression/proteomic approaches. For some genes (e.g., *Rgs2* and *Glo1*), there has been some subsequent evidence demonstrating also potential relevance for human psychiatric disorders.

Genetically modified animal models

Transgenic models are an important tool for understanding how a specific gene functions and influences behavior. A large number of knockout mouse strains have been assessed for differences in anxiety-like behavior. It is also possible to make conditional models, with the gene deficiency or overexpression specific to a certain tissue or developmental time point. A general feature of studies of anxiety-related phenotypes in genetically modified animals has been that results are not always consistent between studies, or different behavioral paradigms within the same study. Potential reasons for this include different genetic background of the animals, slightly different protocols for behavioral testing between laboratories and that different paradigms may measure different aspects of anxiety-like behavior. There are also commonly other mechanisms that are able to compensate for the introduced gene deficiency, and thereby rescuing the phenotype of the transgenic animal.

The most studied models are mice that lack genes functioning in neurotransmitter systems regulating anxiety, such as norepinergic, serotonergic and GABAergic genes, or genes involved in function of the HPA-axis (reviewed in Finn et al., 2003). Some of the most solid (in more than one behavioral test) reported anxiety-related phenotypes are from mice lacking *Slc6a4*, *Htr1a*, *Gabrg2* ($GABA_A$ receptor, subunit γ2) or *Gad65* (glutamic acid decarboxylase, 65 kDa isoform). All of the above show increased anxiety-like behavior. Examples of knockout mice showing decreased anxiety-related behaviour include mice deficient for the glucocorticoid receptor (*N3rc1*), *Htr1b* or *Crhr1* (corticotropin releasing hormone receptor 1).

In addition, transgenic mouse models that overexpress desired genes of interest have been made. Examples include mice that overexpress human variants of *COMT* and show alterations in cognitive and affective functions relevant for anxiety (Papaleo et al., 2008), and mice that overexpress the neurotrophin-3 receptor TrkC in the brain and show increased anxiety-like behavior and enhanced panic reaction as a consequence (Dierssen et al., 2006).

3 AIMS OF THE STUDY

The aim of the present study was to extend knowledge on the genetic basis of human anxiety disorders by examining whether there is predisposing genetic variation in selected novel and previously implicated candidate genes.

The following specific aims were addressed in the studies included in this thesis:

1. To investigate whether any of the human homologues of 13 murine anxiety candidate genes, selected based on up- or downregulated expression in inbred mouse strains with higher innate anxiety, predisposes to human anxiety disorders in the Finnish population-based Health 2000 anxiety disorder study sample (Study I).

2. To investigate whether the genes encoding the asthma susceptibility factor neuropeptide S receptor 1 (NPSR1) and its ligand neuropeptide S (NPS) are involved in anxiety susceptibility in three independent samples from Finland, Spain and Sweden (Study II).

3. To investigate whether genetic variation in any of 16 putative anxiety susceptibility genes, selected based on previously reported associations with human anxiety disorders or anxiety-related personality traits, predisposes to anxiety disorders in the Health 2000 anxiety disorder study sample (Study III). In addition, we aimed to test whether any of the studied candidate polymorphisms interacted with childhood adverse life events in modulating the risk for anxiety disorders.

4 MATERIALS AND METHODS

All methods used in this study have been described in detail in the original publications (I-III). An overview of the methods is presented here (Table 7) and their use is explained in the following sections.

Table 7. ***Overview of the methods used in the present study***

Method	Reference	Publication(s)
Experimental procedures		
5'-nuclease cleavage assay (TaqMan) genotyping	Applied Biosystems, Foster City, CA, USA	III
DNA extraction	Gentra Systems, Minneapolis, MN, USA	I, II, III
Electrophoretic mobility shift assay (EMSA)	Cold Spring Harbor Laboratory Press, 2005	II
Immobilization stress	Buynitsky and Mostofsky, 2009	II
Polymerase Chain Reaction (PCR)		I, II, III
RNA extraction	Molecular Research Center, Cincinnati, OH, USA	II
Sequenom MassARRAY iPLEX and iPLEX Gold SNP genotyping	Sequenom, San Diego, CA, USA	I, II, III
SNPlex multiplex genotyping	Applied Biosystems, Foster City, CA, USA	II
Quantitative Real-Time PCR with SYBR Green chemistry	Applied Biosystems, Foster City, CA, USA	II
Statistical methods and analysis software		
Genetic Power Calculator	Purcell et al., 2003	I, II
Haploview	Barret et al., 2005	I, II, III
Inverse-variance meta-analysis	Borenstein et al., 2009	III
Likelihood-Ratio Test for Case-Control Material	Terwilliger and Hiekkalinna, unpublished software	I, II, III
Locusview	Petryshen, Kirby and Ainscow, unpublished software	I, II, III
Logistic regression modelling of gene x environment interactions	Caspi et al., 2003	III
MEGA	Tamura et al., 2007	II, III
Pedcheck	O'Connell and Weeks, 1998	I, II, III
SNPInspector and MatBase	Genomatics, Munich, Germany	II
Tagger	deBakker et al., 2005	I, II, III
Typer Analyzer	Sequenom, San Diego, CA, USA	I, II, III
Unphased	Dudbridge et al., 2003	I, II, III
Databases and online resources		
dbSNP	www.ncbi.nlm.nih.gov/projects/SNP	I, II, III
Ensembl	www.ensembl.org	I, II, III
HapMap	http://hapmap.ncbi.nlm.nih.gov/	I, II, III
Patrocles	http://patrocles.org/	I
SNPper	http://snpper.chip.org/	I, II, III
UCSC Genome Browser	http://genome.ucsc.edu	I, II, III

4.1 STUDY SAMPLES

Four different case-control study samples with different properties and from four different countries were used in the original publications of this study. Their characteristics are summarized in Table 8, and they are described in more detail in the following sections.

The Finnish Health 2000 anxiety disorder study sample (I, II and III)

The core sample of all three studies was an anxiety disorder sample derived from the population-based epidemiological Health 2000 Study, carried out in 2000-2001 by investigators of the National Public Health Institute (currently the Institute for Health and Welfare). Its aim was to assess major public health problems, functioning, and their determinants in adult Finns (≥ 30 years of age; Heistaro, 2008). The 12-month prevalence of DSM-IV mental disorders was estimated in a representative sample (N = 6986) of the Finnish general adult population by structured psychiatric interview (Munich Composite International Diagnostic Interview; M-CIDI; Pirkola et al., 2005b). No ethnic groups were excluded during the recruitment, but only approximately 2% of the Finnish population was of foreign descent at the time of the study, and the interview was conducted in Finnish, excluding all non-fluent language speakers. The assessed mental disorders were: major depressive disorder (MDD), dysthymia, GAD, PD with or without agoraphobia, agoraphobia, social phobia and alcohol abuse and dependence. Among the total number of reliably performed mental health interviews (N = 6005), the prevalence of DSM-IV anxiety disorders was 4.1%. As lifetime prevalences were not assessed, OCD and PTSD were not diagnosed, and M-CIDI dropouts had somewhat higher scores in the Beck Depression Inventory and General Health Questionnaire-12 (indicating increased depressive symptoms and psychic distress, respectively) this figure is an underestimate of the true prevalence of anxiety disorders in the Finnish population.

We used the Health 2000 cohort to form an anxiety disorder sample for genetic analyses according to the following. We first selected all individuals meeting the criteria for a DSM-IV anxiety disorder diagnosis during the previous year (N = 295). In addition, we further broadened the definition of anxiety disorder subjects to include individuals meeting the criteria for DSM-IV sub-threshold diagnoses (N = 40). We subsequently selected two control individuals per case, matched based on sex, age (± 1 year), and university

Table 8. ***Main characteristics of the four human study samples used in the present study***

	Health 2000	Barcelona	BAMSE	VATSPSUD
Used in publication(s)	I,II,III	II	II	III
Place of origin	Finland, nation-wide	Barcelona, Spain	Stockholm, Sweden	Virginia, USA
Ascertainment	Population-based epidemiological cohort	Clinical recruitment of psychiatry outpatients and population controls	Population-based birth cohort	Population-based twin study
Diagnostic instruments	Composite International Diagnostic Interview (M-CIDI)	Structured Clinical Interview for DSM-IV Disorders: Clinical Version (SCID-CV)	EuroQol 5D (EQ-5D)	Structured Clinical Interview for DSM-III-R (SCID)
Anxiety phenotypes	12-month DSM-IV anxiety disorder diagnoses	Active DSM-IV panic disorder	Parent-reported moderate/extreme anxiety or depression at age 8	Lifetime DSM-III-R anxiety disorder diagnoses
Sample size in epidemiological study	6005	N/A	2033	9270
Sample size in genetic studies	321 anxiety disorder patients. 653 (or 1317 for some markers) matched controls.	188 panic disorder patients. 315 unscreened population controls.	138 children with moderate/ extreme anxiety or depression. 1882 children with no anxiety or depression.	589 cases and 539 controls scoring at the extremes of a genetic risk factor reflecting shared susceptibility across a range of anxiety disorders, MDD, and neuroticism
Sex distribution	63% females, 37% males	75% females, 25% males	48% females, 52% males	39% females, 61% males
Mean age ± SD	49.8 ± 12.7	35.5 ± 9.3	8.4 ± 0.5	36.5 ± 8.5

BAMSE = Barn Allergi Miljö Stockholm Epidemiologi; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders; MDD = major depressive disorder; SD = standard deviation; VATSPSUD = Virginia Adult Twin Study of Psychiatric and Substance Use Disorders

hospital catchment area (5 in the entire country; each with approximately 1 million inhabitants). Controls lacked anxiety or major mental disorders and had explicit negative diagnoses for all symptoms of anxiety. The majority replied negatively to all questions of the General Health Questionnaire-12, further indicating absence of psychic distress. The final sample size after accounting for DNA availability was 974 (321 cases with specific diagnoses as detailed in Table 9, and 653 controls). Some SNPs in study II were genotyped in an extended control sample totaling 1317 controls, with the age matching criterion relaxed to ± 2 years.

Table 9. ***Characteristics of the Health 2000 anxiety disorder study sample (N =974)***

	Cases							
Diagnostic group	**Core**	**Sub-threshold**	**Total**	**Controls**	**Total**	**Men**	**Women**	**Mean age ± SD**
Any anxiety disorder[a]	282	39	321	653	974	357 (36.7%)	617 (63.3%)	49.8 ± 12.7
Panic disorder with or without agoraphobia	108	0	108	218	326	106 (32.5%)	220 (67.5%)	46.7 ± 11.3
Generalized anxiety disorder	73	30	103	206	309	122 (39.5%)	187 (60.5%)	50.6 ± 12.6
Social phobia	58	7	65	133	198	99 (50.0%)	99 (50.0%)	45.4 ± 10.2
Agoraphobia without a history of panic disorder	31	15	46	94	140	60 (42.9%)	80 (57.1%)	52.8 ± 13.0
Phobia, not otherwise specified	58	0	58	121	179	54 (30.2%)	125 (69.8%)	54.6 ± 13.3

[a] Includes subjects with any of the diagnoses listed below. Note that 41 individuals met criteria for more than one anxiety disorder.

Disease comorbidities are important to acknowledge in any study sample. 35.9% of the identified anxiety disorder cases met the criteria for a comorbid depressive disorder (MDD and/or dysthymia), and 22.4% had a comorbid alcohol use disorder (alcohol abuse and/or dependence). Among the anxiety disorders, the most frequent comorbidity was between panic disorder and social phobia (N = 18; 6.4%). Altogether 41 subjects (14.5%) met the criteria for more than one anxiety disorder.

We performed genetic power calculations with an online tool (Purcell et al., 2003) to demonstrate the power of the sample to detect associations. They indicated > 80% power to detect a genotypic relative risk of 1.48-2.62 with disease allele frequencies of 1-60%.

In study II, we evaluated the comorbidity of asthma and specific DSM-IV anxiety disorders in the whole Health 2000 sample (N = 6005). For this purpose, we used consensus diagnoses of asthma that were made based on physicians' clinical examination, spirometry, and register data on use of health care services and reimbursed asthma medication.

In study III, we used information on the subjects' early childhood (≤ age 16) social environment in GxE models testing for interactions between

genetic polymorphisms and the number of childhood adversities in predicting onset of anxiety disorders. Childhood adversities were assessed with an 11-item self-report questionnaire that subjects completed in their own homes (Table 10; Pirkola et al., 2005a). They were instructed to choose "no", "yes", or "cannot say" in reply to the questions and only "yes" answers were coded positive. The highest observed sum score was 9, and the correlation between responses to individual questions was moderate (Crombach's alpha = 0.67; Kananen et al., 2010). In order to have approximately equal sample sizes in each group, subjects were categorized into three groups based on the number of experienced childhood adversities: 0 (N = 360), 1 (N = 216), and ≥ 2 (N = 281). This partition was the best one available, because the distribution of sum scores was highly left-skewed as most subjects reported no experienced childhood adversities (Figure 8).

Table 10. ***Self-report questionnaire for assessment of childhood adversities in the Health 2000 study sample***

Choose "no", "yes", or "cannot say" in response to the following questions. "When you think about your growth years, i.e., before you were aged 16, …"
1. Did your family have long-term financial difficulties?
2. Was your father or mother often unemployed although they wanted to work?
3. Did your father or mother suffer from some serious disease or disability?
4. Did your father have alcohol problems?
5. Did your mother have alcohol problems?
6. Did your father have any mental health problem, e.g., schizophrenia, other psychosis, or depression?
7. Did your mother have any mental health problem, e.g., schizophrenia, other psychosis, or depression?
8. Were there any serious conflicts within your family?
9. Did your parents divorce?
10. Were you yourself seriously or chronically ill?
11. Were you bullied at school?

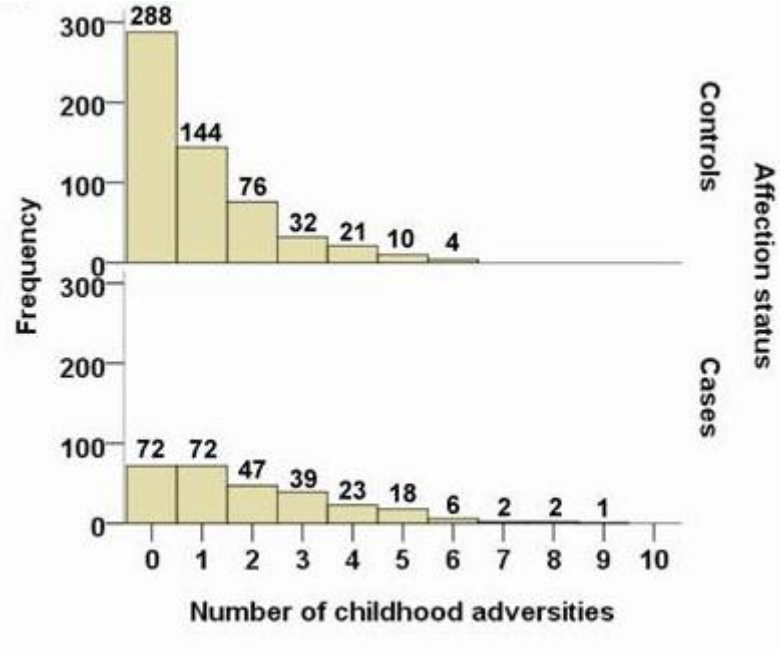


Figure 8 Distribution of childhood adversity sum scores in the Health 2000 sample. As the distribution was highly left-skewed, a categorized grouping of subjects (0, 1 and ≥ 2 experienced childhood adversities) was chosen for the gene x environment interaction analyses in study III.

Barcelona panic disorder sample (II)

In study II, we sought for a replication sample in which to follow up on association findings in the genes *NPSR1* and *NPS* that were observed in primary analyses done in the Health 2000 anxiety disorder sample. We established collaboration with the group of Xavier Estivill in Barcelona, Spain. Estivill and colleagues had collected a PD sample, consisting of 188 adult (> 18 years of age) Spanish Caucasians from the Psychiatry outpatient unit in Hospital del Mar, Barcelona (Table 8, page 61). As controls, they used 315 ethnicity-matched blood donors recruited from the Blood and Tissue Bank of the Catalan Health Service. Notable differences between the Barcelona sample and the Health 2000 sample are: exclusion of subjects with current comorbid DSM-IV axis disorders apart from other anxiety disorders, exlusion of subjects with lifetime history of mood disorder, psychiatrically unscreened controls, and the nature of recruitment (clinical vs. population-based).

The Swedish BAMSE cohort (II)

In study II, we also turned our attention to a Swedish population-based birth cohort (BAMSE), in which some of the same SNPs from our candidate genes of interest (*NPSR1* and *NPS*) had been genotyped (Table 8, page 61). The BAMSE birth cohort originally included 4089 children from the central and north-western parts of Stockholm (Melen et al., 2005; Wickman et al., 2002). For our study, we used phenotypic information collected in conjunction with a clinical examination performed when children were 8 years old. After exclusion of subjects with incomplete questionnaire information, or no DNA available, our study sample consisted of 2020 children (49% of the original birth cohort size).

Our interest in the BAMSE sample was to evaluate the epidemiological comorbidity between asthma and anxiety, and to further test for genetic associations between the *NPSR1* and *NPS* genes and anxiety. The asthma phenotype was defined as at least four episodes of wheezing during the last 12 months, or at least one episode of wheezing during the same period if the child was receiving inhaled steroids (Ostblom et al., 2008). Unfortunately, no information from specific anxiety symptom questionnaires was available to us. We therefore used a question from the parent-completed EuroQol (EQ-5D) questionnaire (The EuroQol Group, 1990) to assess anxiety/depression in the BAMSE children. Parents were asked: "Indicate which statement best describes your child's health condition today", and the reply options were: 1) is not anxious or depressed; 2) is moderately anxious or depressed; or 3) is extremely anxious or depressed. Only 0.1% of the children were extremely anxious or depressed and we therefore combined them with moderately anxious or depressed. We thus used a dichotomous assessment of parent-reported anxiety/depression (N positive = 138; N negative = 1882) as a phenotype in the genetic analyses of the BAMSE sample.

The most notable differences between the BAMSE sample and the other samples used in this study are the age of the subjects, and the unspecificity of the anxiety phenotype. We nevertheless thought that analysis of the EQ-5D anxiety/depression question had some additional value for two reasons: 1) Parent-reported emotional problems (anxiety, shyness, and withdrawal) in 8-year olds are specifically associated with a 2.6-fold risk for an anxiety disorder in early adulthood (Sourander et al., 2007), and 2) although the EQ-5D question in itself is unspecific in nature, it showed considerable overlap with the specific diagnoses of agoraphobia, GAD, and social phobia in the Health 2000 sample (in which the EQ-5D questionnaire had also been included).

The Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (III)

In study III, we again first conducted a primary genetic association analysis in the Health 2000 sample. Using meta-analytic methods, we subsequently combined our positive association findings in the glutamate decarboxylase 1 (*GAD1*) gene with data from the study in which prior evidence for association to the gene was originally reported (Hettema et al., 2006). In the prior study, *GAD1* SNPs were examined in a sample derived from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD; Table 8, page 61). VATSPSUD is a population-based study consisting of 9270 twin subjects, all Caucasian and born in Virginia, USA. In that cohort, a multivariate genetic analysis was performed to identify a latent phenotype reflecting shared genetic susceptibility across a broad range of anxiety and mood phenotypes: GAD, PD, agoraphobia, social phobia, neuroticism, and MDD. This genetic risk factor was then used to create a case-control sample by selecting one member of each twin pair as a case or control based upon scoring above the 80th or below the 20th percentile, respectively, of the genetic risk factor. The resulting sample consisted of 1128 subjects (589 cases and 539 controls). The cases had the following frequencies of mental disorders: MDD 80.1%, GAD 53.8%, PD 20.5%, agoraphobia 14.1%, and social phobia 17.5%, whereas the controls lacked any of the listed.

Notable differences between the VATSPSUD and the Health 2000 samples is the nature of the phenotype (derived from genetic risk factor modeling), the use of lifetime diagnoses compared to 12-month ones, the high MDD prevalence (more than twice the Health 2000 one), and a large difference in sex distribution among cases (59% males, compared to 37% in Health 2000). Nevertheless, when pooling association data from the Health 2000 sample with the VATSPSUD sample, we had the opportunity to study a combined sample of 1985 individuals (871 cases and 1114 controls) in analyses reflecting effects of genetic susceptibility across a broad range of internalizing disorders.

4.2 CANDIDATE GENE SELECTION

Througout this study, a total of 30 potential susceptibility genes for anxiety disorders were examined for disease-predisposing genetic variation represented by a total of 333 SNP markers (Table 11). The rationale for studying the genes was different in each of the original publications (I-III), and as described in the following sections.

Table 11. ***Candidate genes for anxiety disorders examined in the present study***

Gene symbol	Gene name	Total N SNPs examined	Publi-cation(s)
ACE	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	2	III
ADORA2A	adenosine A2a receptor	6	III
ALAD	aminolevulinate, delta-, dehydratase	18	I
BDNF	brain-derived neurotrophic factor	3	III
CCKBR	cholecystokinin B receptor	3	III
CDH2	cadherin-2, type 1, N-cadherin (neuronal)	23	I
COMT	catechol-O-methyltransferase	5	III
CPSF4	cleavage and polyadenylation specificity factor 4, 30kDa	4	I
CREM	cAMP responsive element modulator	2	III
CRH	corticotropin releasing hormone	4	III
DYNLL2	dynein, light chain, LC8-type 2	7	I
EPB41L4A	erythrocyte membrane protein band 4.1 like 4A	44	I, III
EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	13	I
GAD1	glutamate decarboxylase 1 (brain, 67kDa)	8	III
GLO1	glyoxalase I	15	I
GSR	glutathione reductase	22	I
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A	2	III
NPS	neuropeptide S	16	II
NPSR1	neuropeptide S receptor 1	43	II
NPY	neuropeptide Y	10	III
PDE4D	phosphodiesterase 4D, cAMP-specific	6	III
PLXNA2	plexin A2	11	III
PSAP	prosaposin (variant Gaucher disease and metachromatic leukodystrophy)	19	I
PTGDS	prostaglandin D2 synthase 21kDa (brain)	10	I
RGS2	regulator of G-protein signaling 2, 24kDa	2	III
S100A10	S100 calcium binding protein A10	5	I
SCN1B	sodium channel, voltage-gated, type I, beta	5	I
SLC15A2	solute carrier family 15 (H+/peptide transporter), member 2	12	I
SLC6A3	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	11	III
SLC6A4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	2	III

Murine anxiety candidate genes (I)

In study I, we focused on a set of candidate genes that had been identified using inbred mouse strains that differ in their innate levels of anxiety-like behaviour as a model (Hovatta et al., 2005). Iiris Hovatta and colleagues had combined behavioural testing of six inbred mouse strains with gene expression profiling of seven brain regions involved in the regulation of anxiety to identify genes with an expression level that might regulate anxiety-like behavior (Figure 9). Their strategy was to look for genes with an expression profile that correlated (either positively or negatively; Pearson correlation ≥ 0.75 or ≤ -0.75) with a behavioural vector representing anxiety levels across the six chosen mouse stains. An additional criterion used to prioritize genes with the potentially largest phenotypic effects, was that the fold difference in gene expression between the least and the most anxious strains (C57BL/6J and FVB/NJ vs. A/J and DBA/2J) had to be at least 50%.

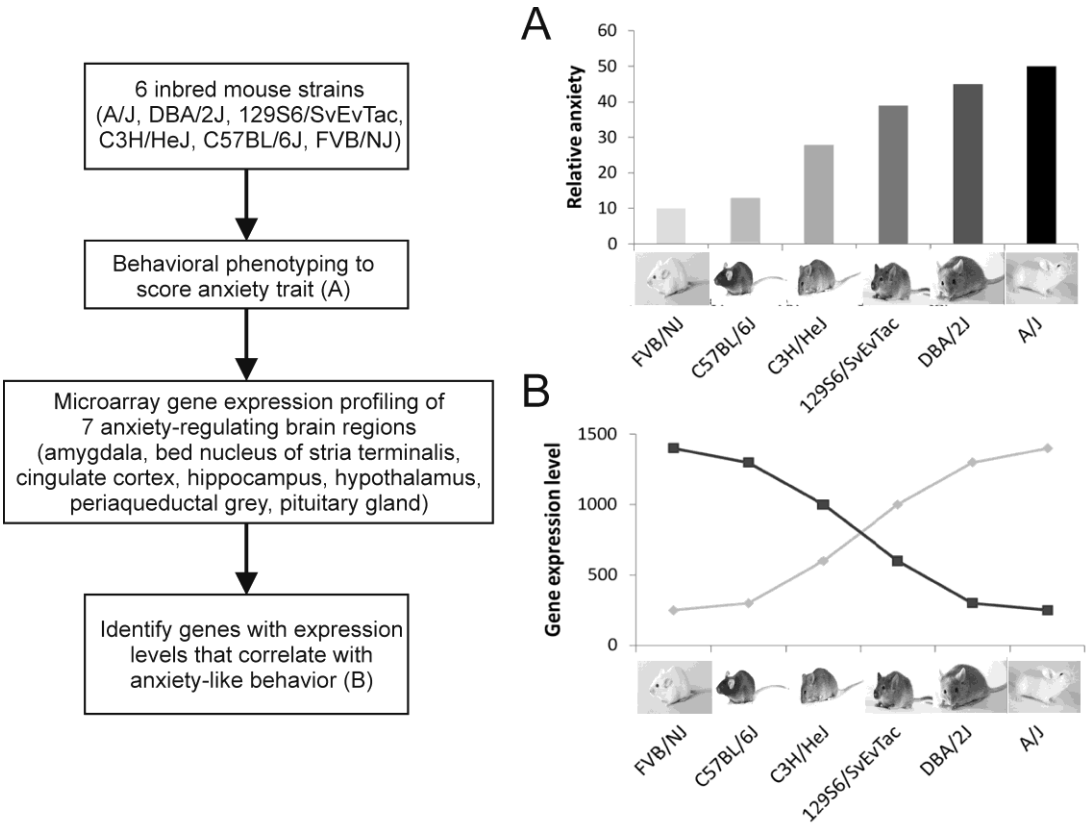


Figure 9 A strategy combining behavioural testing with gene expression profiling for identification of anxiety candidate genes in the mouse. First, inbred mouse strains with different innate characteristics are phenotyped by behavioural testing and ranked according to their order of relative anxiety (A). Second, gene expression profiling of brain regions relevant for anxiety is carried out to identify genes with expression levels that correlate with the anxiety phenotype (B). Potential candidate genes are either expressed at a higher level in more anxious strains than in strains with lower anxiety levels, or vice versa. Figure based on (Hovatta et al., 2005).

With the strategy described above, 17 genes were identified. The most convincing proof-of-principle that the approach was able to identify mouse genes relevant for anxiety came from subsequent functional studies on two of the 17 genes, glyoxalase 1 (*Glo1*) and glutathione reductase (*Gsr*). These genes encode important antioxidant enzymes involved in cellular defense against oxidative stress. By overexpressing or silencing them in the mouse cingulate cortex with lentivirus-mediated gene transfer, the anxiety-like behaviour of mice could be altered (Hovatta et al., 2005). This functionally demonstrated that the expression level of *Glo1* and *Gsr* regulates anxiety in mice.

For my own study in humans (I), we selected the 13 known human homologues of the 17 anxiety candidate genes identified in the mouse (Table 11). We considered them excellent previously unexamined candidate genes for human anxiety disorders based on the link between their expression level and anxiety in the mouse model, and the supporting functional evidence described above.

Asthma-related candidate genes (II)

In study II, we examined the genes encoding a previously identified asthma-susceptibility factor (neuropeptide S receptor 1; *NPSR1*) and its ligand (neuropeptide S; *NPS*) as potential candidate genes for anxiety disorders. Although the G-protein coupled receptor encoding *NPSR1* (formerly *GPR154*) was originally identified by positional cloning as a susceptibility gene for asthma and atopy (Laitinen et al., 2004), later identification of and studies on its ligand NPS made it clear that the receptor system is an important regulator of neurobiological phenotypes as well. At the time when our study was initiated, it was known that *Npsr1* is highly expressed in rat brain regions regulating arousal, anxiety, learning, and memory (Xu et al., 2007). Moreover, it was shown that NPS had a unique behavioural profile of simultaneously inducing wakefulness/arousal and anxiolysis when administered to the rodent brain (Xu et al., 2004).

Another line of evidence that prompted us to select the NPS-NPSR1 system for investigation came from epidemiological studies. Comorbidity between asthma and anxiety disorders had been observed in numerous clinical and community-based samples (e.g., Roy-Byrne et al., 2008; Goodwin et al., 2004; Goodwin et al., 2003; Goodwin, 2003). Other studies showed that subjects having both an anxiety disorder and asthma had poorer asthma control, more functional impairment, and decreased quality of life when compared to asthma patients without a psychiatric diagnosis (McCauley et al., 2007; Richardson et al., 2006; Lavoie et al., 2005). However, the underlying biological mechanisms of comorbidity are poorly known and shared genetic vulnerability represented one attractive hypothesis.

Based on the independent evidence implicating the NPS-NPSR1 system in both anxiety and asthma, we reasoned that the system could be a genetic link between the disorders and one potential mechanism behind their comorbidity. In study II, we therefore assessed the role of *NPSR1* and *NPS* as susceptibility genes for human anxiety disorders.

Candidate genes previously implicated in human anxiety (III)

In study III, we chose a set of 15 putative human anxiety susceptibility genes for investigation, aiming to replicate previous association findings in them in the Health 2000 sample. We performed a MEDLINE literature search for published human candidate gene studies reporting associations with anxiety disorders or anxiety-related personality traits during the previous 15 years. The 15 genes that were selected for investigation represented some of the most relevant findings within our field of research. One of the reasons for discrepant findings in psychiatric genetics is that most of the published studies, in particular in the earlier days of association studies, used small sample sizes. They were likely underpowered to detect the small effect sizes that are currently believed to be conferred by individual genetic risk variants, and consequently many early studies published with loosely defined significance criteria may represent spurious false positive findings. Therefore, and in general, the keys to evaluating the relevance of genetic association findings are replication in independent samples, and functional studies demonstrating causality. In particular, well-characterized population-based samples are rare within the anxiety disorders. We therefore thought that the Health 2000 study sample represented a valuable resource in which to attempt replication of some of the previous key findings of the field.

4.3 MARKER SELECTION

Throughout this study, SNP markers were used to represent the genetic variation of their surrounding genomic regions. The following general principles and tools were used to choose SNPs for genotyping:

1) For each gene of interest, we retrieved SNP information using the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP/) and the SNPper bioinformatics tool (Riva and Kohane, 2002). For additional examination of genes of interest, we also used the Ensembl (http://www.ensembl.org) and UCSC Genome Browser (http://genome.ucsc.edu) databases.

2) We first selected all non-synonymous SNPs for genotyping, reasoning that they would be prime candidates for having functional effects.

3) We then used the Tagger algorithm in Haploview (Barrett et al., 2005; de Bakker et al., 2005) to select complementary tagSNPs that would most efficiently capture the remaining genetic variation of the loci. We used genotype information for the HapMap European-derived Caucasian/European ancestry (CEU) population as a basis for the selection (The International HapMap Consortium, 2003). The criteria for selecting tagSNPs was that they had to have minor allele frequencies ≥ 0.05 and capture the genetic variation of as many other SNPs with minor allele frequencies ≥ 0.05 as possible by $r^2 \geq 0.8$. Due to the large size of some genes, we had to limit the number of tagSNPs selected from them to 1 SNP/exon.

4) In study I, we additionally used the Patrocles database (Hiard et al., 2010) to select SNPs representing polymorphic microRNA binding sites, i.e. SNPs with alleles that create novel or disrupt existing microRNA target sites. We considered the identified SNPs good potential candidates for influencing gene expression.

5) As study III was a replication attempt of previously published findings, we prioritized SNPs that had been genotyped in prior studies in the selection.

4.4 GENOTYPING

As a part of the Health 2000 Study, peripheral venous blood samples were collected from participants that consented. DNA was extracted from them at the DNA extraction core facility of the National Public Health Institute, with the majority of samples processed with the Puregene manual DNA extraction kit (Gentra Systems, Minneapolis, MN, USA). DNA quality control measures included sex-specific polymerase chain reaction to verify that samples were not cross-contaminated. The order of case and control samples was randomized across all plates, and the plates contained both internal duplicate and cross-plate genotyping controls. No template controls were also included to verify specific amplification of PCR products.

Genotyping of all but one SNP in the Health 2000 sample was carried out using Sequenom MassARRAY high-throughput genotyping technology with either iPLEX, or iPLEX Gold chemistry in accordance with the manufacturer's instructions (Sequenom, San Diego, CA, USA). This genotyping technology is based on single-base pair primer extension, in combination with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of the extension products (Figure 10). Final genotype calls are made from the mass spectrometry spectrum, based on the allele-specific masses of the extension products. A total of 12.5 – 15 ng of genomic DNA on 384-well plates was used as template for the genotyping.

We used software provided by Sequenom for both assay design (Assay Design), and for making preliminary genotype calls (Typer Analyzer). The Assay Design software aids combination of the SNPs into multiplex pools by designing compatible PCR- and extension primers. The average pool size in our study was 22 SNPs. The preliminary genotype calls made by Typer Analyzer for all SNPs were further manually verified by two independent investigators who verified that genotype clusters were distinguishable and unclear genotypes excluded. Routine genotyping quality control procedures further included checking no template control samples for absence of allele peaks and verifying consistencies in genotype calls of duplicates samples. In addition, Mendelian inheritance of marker alleles was verified by separate genotyping in a sample of 60 anonymous parent-offspring trios that was analyzed with the Pedcheck software (O'Connell and Weeks, 1998). As a general principle, marker assays with low success rates (< 85%) or deviation from Hardy-Weinberg equilibrium ($P < 0.05$) in the control sample were disregarded. Furthermore, all genotype data for individuals with genotype calls for less < 75% of the analyzed markers was excluded from the analysis.

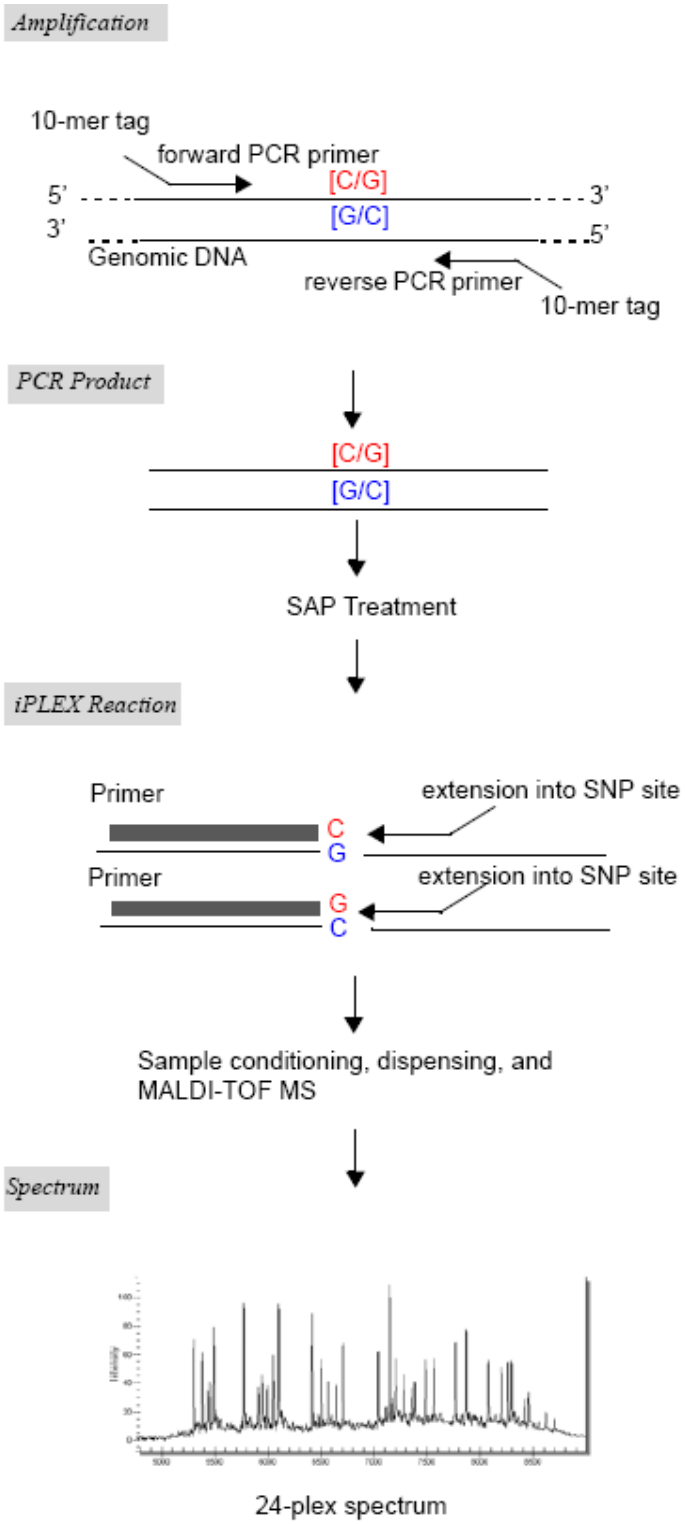


Figure 10 Principle of the Sequenom iPLEX genotyping technology. The SNP marker regions are first amplified in PCR multiplexes, followed by shrimp alkaline phosphatase (SAP) treatment for dephosphorylation of unincorporated dNTPs to prevent them from reacting in subsequent steps. In the actual iPLEX single base pair extension reaction, an extension primer anneals to each SNP site and addition of only one nucleotide to the primer using the SNP site as a template takes place due to the use of terminator ddNTPs (dideoxynucleotide triphosphates). Extension products are desalted and dispensed onto bioarrays for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis. Final genotype calls are made from the mass spectrometry spectrum using the Typer Analyzer software, based on the allele-specific masses of the extension products. Figure modified from (Oeth et al., 2005).

One additional SNP of functional relevance (rs16147) from the neuropeptide Y gene was genotyped with the 5' nuclease cleavage assay (TaqMan assay) according to the manufacturer's instructions (Applied Biosystems, Carlsbad, CA, USA). For this purpose 10 ng of DNA template was used on 384-well plates. PCR runs for allelic discrimination of the fluorescently labeled allele signals (either FAM or VIC) were performed on a CFX384 thermal cycling instrument (Bio-rad, Hercules, CA, USA). The Bio-rad CFX Manager software was used to interpret the fluorescent genotype signals, and genotyping quality control was performed as detailed above.

For the other samples of this study (BAMSE, Barcelona and VATSPSUD), DNA was processed according to the routine procedures of the respective institutions. In the BAMSE sample, genotyping was performed with Sequenom technology at the Karolinska Institute, Stockholm, Sweden. In the Barcelona sample, SNPs were genotyped at the Centro Nacional de Genotipado, Genoma España, Spain with the SNPlex multiplex genotyping system (Applied Biosystems). In the VATSPSUD sample, SNPs were genotyped with the 5' nuclease cleavage assay at the Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, USA.

4.5 STATISTICAL ANALYSES

The statistical procedures used in this study were mainly methods used to evaluate genetic associations in case-control samples, both on the level of individual SNPs and haplotypes. Moreover, the produced SNP and haplotype data was used for purposes of gene x environment interaction modeling and meta-analysis. The use of these genetic methods is described in more detail in the following sections. In addition, conventional statistical tests such as the chi square test, Fisher's exact test and its Freeman-Halton extension, t-test and factorial analysis of variance (ANOVA) were used.

Pointwise association analyses

Genetic association analyses of individual SNPs were carried out similarly in all studies. We used a 2 x 2 contingency table likelihood-ratio test for case-control material to test the independence of SNP allele counts in cases and controls (J. D. Terwillliger and T. Hiekkalinna, unpublished). The same test was extended to a 2 x 3 table to test independence of genotype counts. We always performed 10000 permutations of the dataset to compute empirical P-values. In most instances, we described results with empiral $P \leq 0.01$ as showing some evidence for association. The results obtained with the likelihood-ratio test were in concordance with those of a conventional chi square test.

Haplotype association analyses

Haploview (Barrett et al., 2005) and Locusview (T. Petryshen, A. Kirby and M. Ainscow, unpublished) softwares were used to examine and illustrate the LD structures of the examined genes visually. There is no universally optimal gold standard method for definition of haplotype blocks for haplotype association analyses. Thus, varying definitions were used throughout this study.

In study I, we performed global haplotype association tests on 2- and 3-marker sliding windows.

In study II, we restricted the analysis to regions of relatively stronger LD by first defining haplotype blocks with the algorithm of Gabriel et al. (Gabriel et al., 2002) in Haploview. This algorithm is based on 95% confidence intervals of D'-values, and it creates a block whenever 95% of informative SNP comparisons are categorized as being in strong LD. We then tested the specific haplotypes within the identified blocks for association.

In study III, we analyzed haplotype blocks consisting of all genotyped SNPs for each gene, and thus spanning the full length of the loci of interest. The major reasons for this approach were that many of the prior studies that we based our SNP selection on had performed such analyses, and only a limited number of SNPs (2-3) were studied for many of the genes.

In all studies, we used the Unphased software (Dudbridge, 2008) to perform the haplotype association analyses. Unphased estimates haplotype frequencies from unphased genotype data with an expectation-maximization algorithm. In global analyses, Unphased tests the null hypothesis that the odds ratios (ORs) of all haplotypes are equal with a likelihood-ratio test. In analyses of specific haplotypes, Unphased uses a score test to test whether there is a difference in OR between the test haplotype and all others pooled together. We always performed 10000 permutations of the dataset to obtain empirical P-values.

Phylogenetic analyses

The relation between individual SNP and haplotype associations can be better understood by interpreting findings in a phylogenetic context. For these purposes, we used the maximum parsimony algorithm in MEGA software to determine the most likely haplotype phylogeny (Tamura et al., 2007). As the amount of SNP information for each gene of interest was limited, we reasoned that the relatedness of haplotypes would be best explained by the phylogenetic tree that requires the least amount of evolutionary change to explain the observed data. The statistical support for each node on the phylogenetic trees was evaluated by bootstrapping.

Gene x environment interaction modelling

We evaluated GxE interaction effects in the Health 2000 anxiety disorder sample in study III. Childhood adversities represent some of the strongest known environmental risk factors for anxiety disorders, and this effect has been observed also in the Health 2000 sample (Pirkola et al., 2005a). We thus had strong prior evidence for main effects of childhood adversities, which motivated these analyses.

We used a binary logistic regression model in PASW Statistics Software (SPSS, Chicago, Illinois) to evaluate interactions. The model was based on one of the early seminal GxE examinations in mental disorders (Caspi et al., 2003). We aimed to explain anxiety disorder diagnosis with SNP genotypes, childhood adversities, and their interaction as covariates:

Logit[Prob(Any anxiety disorder)] = b_0 + b_1(SNP genotype) + b_2(Childhood adversities) + b_3(SNP genotype*Childhood adversities), where:

b_0 is a constant; b_1 is genotype classes of each SNP coded 0, 1, and 2 to reflect minor allele dosage; b_2 is the number of childhood adversities (categorized to groups of 0, 1, and 2 or more adversities to have as equal subgroup sizes as possible); and b_3 is the product of b_1 and b_2.

We did not include sex in the models, as it was a criterion for matching cases and controls. We also carried out haplotype-based GxE analyses for genes implicated by the individual SNP findings by substituting SNP genotype with haplotype copy number in the model.

We decided not to evaluate specific anxiety disorder subdiagnoses separately, as the major challenge of these analyses was the limited subgroup sample size that resulted from dividing the sample by both number of experienced childhood adversities and genotype. To further avoid spurious findings caused by limited sample size, and to obtain further statistical support for their modulating effects, we also conducted secondary analyses for SNPs showing significant interaction effects ($P \leq 0.01$). We tested their genotype distributions within the three categories of experienced childhood adversities (0, 1, ≥ 2) for significant deviations from the expected with either a chi square test or the Freeman-Halton extension of Fisher's exact test for 2 x 3 contingency tables, when appropriate.

Inverse-variance meta-analysis

In study III, we observed association to the same variants as investigators in a previous study of the *GAD1* gene (Hettema et al., 2006). This inspired us to carry out a meta-analysis to more accurately assess the overall contribution of *GAD1* to susceptibility to internalizing disorders (e.g., anxiety and mood disorders). We combined our genotype data from the Health 2000 sample

with data from the VATSPSUD sample, in which the prior study of *GAD1* was undertaken. Due to some inherent differences between the samples (Table 8, page 61), a broadly defined phenotype was chosen for unbiased pooling of both samples. We combined the whole Health 2000 sample with the VATSPSUD sample in order for the meta-analysis to reflect effects of genetic susceptibility shared by a broad range of internalizing disorders. The validity of this approach was also supported by models suggesting that comorbidity between internalizing disorders is explained, to a large extent, by shared genetic factors (Hettema et al., 2006). The final sample size for the joint analysis was 871 cases and 1114 controls.

We used the inverse-variance method (Borenstein et al., 2009) to compute weighted means for the effect sizes of risk alleles and and haplotypes observed in the two studies. The weighting coefficients for each sample were equal to the inverse variance of the observed effect estimates (1/variance). Weighted effect size means, and the combined variance, were calculated according to the formulas in Figure 11. The null hypothesis that the combined effect size is zero was evaluated with a 1 degree of freedom chi square test.

$$M = \frac{\sum_{i=1}^{k} W_i Y_i}{\sum_{i=1}^{k} W_i} \qquad V_M = \frac{1}{\sum_{i=1}^{k} W_i} \qquad SE_M = \sqrt{V_M}$$

Figure 11 Formulas used for inverse-variance meta-analysis. Weighted combined mean effect sizes (*M*) are calculated by dividing the sum of the products of sample effect size (*W*) multiplied by sample weight (*Y* = 1 / sample variance) with the sum of the sample weights. The variance of the weighted mean effect size (V_M) is the reciprocal of the sum of weights, and the standard error (SE_M) is the square root of the variance (Borenstein et al., 2009).

4.6 ELECTROPHORETIC MOBILITY SHIFT ASSAY

In study II, we tested whether the most significantly associated SNPs in the *NPSR1* gene influence transcription factor binding to their surrounding DNA sequence in an allele-specific manner. These experiments were carried out in the research group of Juha Kere at the Karolinska Institute, Stockholm, Sweden. We used electrophoretic mobility shift assay (EMSA) for this purpose (Cold Spring Harbor Laboratory Press, 2005). This method is used to study the DNA-binding properties of proteins, and we used it to determine possible sequence-dependent DNA-binding. Briefly, ^{32}P-radiolabeled DNA probes specific for each allele were first allowed to bind to a nuclear extract from the SH-SY5Y neuroblastoma cell line. The nuclear extract represents a total mixture of transcription factor proteins. The DNA-protein complexes were subsequently separated on a nondenaturing 6% polyacrylamide gel, and visualized by autoradiography. Whenever proteins have bound to the radiolabeled probe, its migration is altered on the gel. Thus, possible allele-specific protein binding can be discerned by comparing gel runs for probes corresponding to the different alleles of interest.

Having identified both qualitative and quantitative differences in DNA-protein binding for two of our SNPs of interest, we further attempted to identify the specific transcription factors involved. We bioinformatically predicted likely candidates with SNPInspector and MatBase software (Genomatix Software, Munich, Germany) and chose the most promising candidates, based on suggested involvement in neuronal function or mental disorders, for functional verification. For this purpose, we used a so-called "supershift" version of the EMSA, in which DNA-protein complexes are further allowed binding to an antibody for the protein of interest. If the radiolabeled probe has formed a complex with the hypothesized protein, its migration will be further retarded on the gel.

4.7 IMMOBILIZATION STRESS AND QUANTITATIVE REAL-TIME PCR

Also in study II, we examined whether gene expression responses are altered in immobilization stress-exposed mice deficient of neuropeptide S receptor 1 (*Npsr1*$^{-/-}$). These experiments were carried out in collaboration with the research groups of Juha Kere and Harri Alenius at the Karolinska Institute/University of Helsinki and Finnish Institute of Occupational Health, respectively. We used 12-16 wk old male mice on a heterogeneous C57BL/6 x 129/SvEvBrd background (Lexicon Genetics, The Woodlands, Texas). The mice were housed in standard cages with access to food and water *ad libitum*.

We used the immobilization stress paradigm as an inducer of physiological and psychological stress responses. Immobilization stress is one of the most frequently used methods for this purpose, and it is based on restricting the free movement of the test animal for a desired amount of time (Buynitsky and Mostofsky, 2009). Although the methods to induce immobilization are physical, the paradigm is considered to be a primary psychological stressor and thus a good model for psychiatric stress, with any physical discomfort the animal experiences being secondary. Physiological and molecular effects of immobilization stress include increased ACTH-response and corticosterone secretion, decreased heart rate, abnormalities in blood pressure, increased tumor necrosis factor-α (TNF-α) levels, increased c-fos expression, and increased oxidative damage to proteins and lipids (Buynitsky and Mostofsky, 2009), which together indicate increased stress. Behavioral changes induced by immobilization stress are increased anxiety-related behavior, reduced activity and exploration, increased pain response, increased acoustic startle response, decreased memory retention and reconsolidation. As any animal model, the immobilization stress paradigm is thought to model some aspects of human stress response, but results are difficult to directly extrapolate to human conditions.

We used an acute (1 h) immobilization stress paradigm, with sacrifice by CO_2 inhalation either immediately, or after a 1 h recovery period. Immobilization stress was carried out in 50 ml conical tubes put into darkened plastic boxes.

We used quantitative real-time PCR (qRT-PCR) to evaluate immobilization stress-induced changes in gene expression in *Npsr1*$^{-/-}$ and wild type mice. Brain regions (hypothalamus, cerebellum, hippocampus, striatum, cortex, and midbrain) were dissected immediately after sacrifice, frozen on dry ice, and stored at -80°C. Total RNA was extracted from them with TRI reagent (Molecular Research Center, Inc., Cincinnati, OH), and cDNA was synthesized from 500 ng of RNA with SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Quantitative RT-

PCR was performed with ABI Prism 7900 or ABI Prism 7500 Fast instruments using either TaqMan probes or self-designed primers (Table 12) for SYBR Green assay (Applied Biosystems). PCR amplifications were performed with 0.07 nM primer concentrations in the final reaction and a protocol consisting of initial denaturation at 95°C for 10 min, followed by 39 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Expression levels were normalized to the levels of the endogenous control genes *Gapdh* or *Rn18s*. The genes of interest were, in addition to *Npsr1* and *Nps*, either:

1) Putative stress-related downstream target genes of NPS-NPSR1 signaling (Vendelin et al., 2006); namely *Bhlhb2* (basic helix-loop-helix domain containing, class B2), *Egr1* (early growth response 1), *Fos* (FBJ osteosarcoma oncogene), *Gal* (galanin), *Inhba* (inhibin beta-A), *Junb* (jun-B oncogene), *Klf10* (Kruppel-like factor 10), *Nab1* (Ngfi-A binding protein 1) and *Tac1* (tachykinin 1), or
2) Genes well known to be involved in stress response; *Il1b* (interleukin 1 beta), *Ntf3* (neurotrophin 3) and *Tnf* (tumor necrosis factor)

Table 12. ***Sequences of self-designed mouse primers for quantitative real-time PCR***

Gene symbol	Forward primer sequence (5'->3')	Reverse primer sequence (5'->3')
Bhlhb2	TTGGGTCACTTGGAAAAAGC	TTTCTTCCCGACAAATCACC
Egr1	ACCCGTTCGGCTCCTTTC	GCAGCATCATCTCCTCCAGTTT
Fos	GGAATGGTGAAGACCGTGTCA	TCAGGAGATAGCTGCTCTACTTTGC
Gal	TGGAGGAAAGGAGACCAGGAAG	GCCTCTTTAAGGTGCAAGAAACTG
Inhba	AGGCGGCGCTTCTCAAC	CCTCTATCTCCACATACCCGTTCT
Junb	GGCTTTGCGGACGGTTTT	GGCGTCACGTGGTTCATCT
Klf10	GCCTGTCACACCAGTGTCTG	GGCTGTAAGGTGGCGTTAAA
Nab1	TCTATGGGCGATTTGACTCC	CAGGGCAAAAAGCTCATCTC

In addition to the gene expression experiment, we collected blood from the mice for serum corticosterone measurement. This was carried out with the OCTEIA Corticosterone HS Enzyme immunoassay (Immunodiagnostic Systems, Boldon, UK) according to the manufacturer's instructions.

5 RESULTS

5.1 SIX MURINE ANXIETY GENES MAY INFLUENCE SUSCEPTIBILITY TO HUMAN ANXIETY DISORDERS (I AND III)

The human homologues of thirteen candidate genes for modulating murine anxiety-like behavior, identified based on their up- or downregulation in brain regions of more anxious mouse strains (Hovatta et al., 2005), were tested for association to human anxiety disorders. These analyses were carried out in the Health 2000 anxiety disorder study sample, which was described for the first time in original publication I.

Two-stage association analysis identifies six prime candidates

Pointwise analyses

The association analysis was carried out in two stages, with an initial first screening round with less markers/gene (Figure 12). A second stage of fine-mapping was then done for the top genes from stage I. The criteria for including a gene in the stage II analysis were: 1) at least two SNPs with empirical $P \leq 0.05$ in any of the analyzed diagnostic groups; or 2) a haplotype window with empirical $P \leq 0.05$ in any of the analyzed diagnostic groups. Eight of the thirteen genes were selected for analysis in stage II based on the criteria above (Figure 12).

Finally, data from a total of 144 SNPs from 13 genes was analyzed. We tested them for association to both core (only subjects with DSM-IV diagnoses), and extended (including DSM-IV subthreshold diagnoses) anxiety disorder diagnoses of any anxiety disorder, PD, GAD and social phobia. At empirical $P \leq 0.01$ in the final association analysis of the combined data from stages I and II, five genes showed evidence for association in the pointwise SNP analyses: *ALAD* (δ-aminolevulinate dehydratase), *CDH2* (cadherin 2), *EPB41L4A* (erythrocyte membrane protein band 4.1 like 4a), *PSAP* (prosaposin), and *PTGDS* (prostaglandin D2 synthase). These individual SNP findings are summarized in Table 13. Notably, some of the most significantly associated SNPs either conferred amino acid or triplet codon change (in *EPB41L4A* and *PTGDS*, respectively), or were located in potential regulatory regions (3'-untranslated region [UTR] in *ALAD* and promoter in *PSAP*).

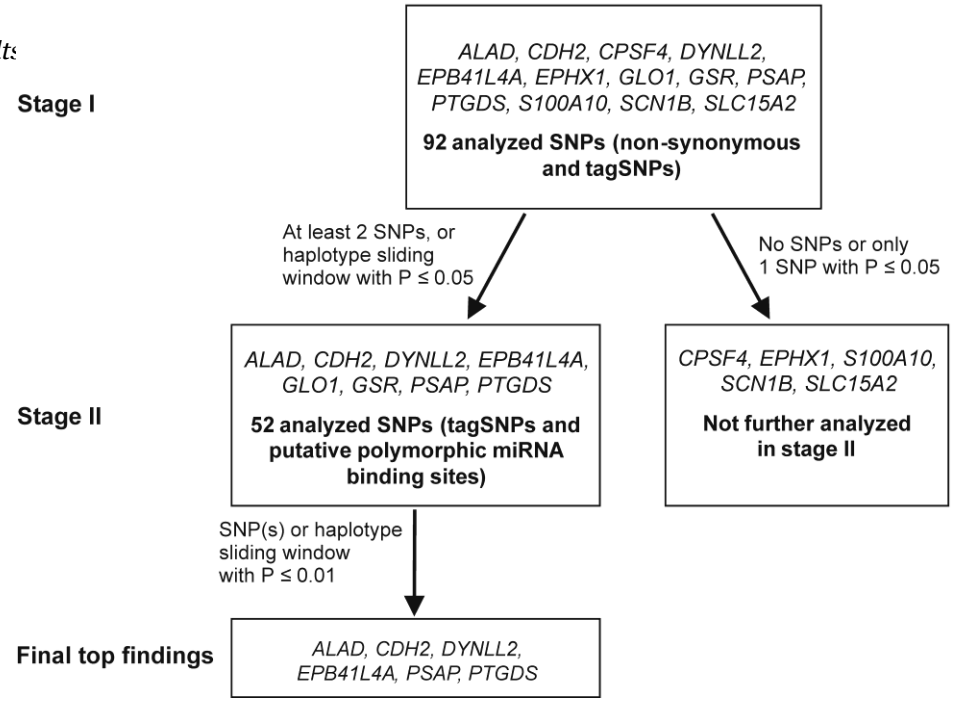


Figure 12 Schematic of results from a two-stage association analysis of 13 murine anxiety candidate genes. Eight of 13 genes were examined further after stage I. Finally, six genes showed evidence for association by P ≤ 0.01. *ALAD* = δ-aminolevulinate dehydratase; *CDH2* = cadherin 2; *CPSF4* = cleavage and polyadenylation specificity factor 4; *DYNLL2* = dynein light chain LC8-type 2; *EPB41L4A* = erythrocyte membrane protein band 4.1 like 4a; *EPHX1* = epoxide hydroxylase 1; *GLO1* = glyoxalase 1; *GSR* = glutathione reductase; miRNA = microRNA; *PSAP* = prosaposin; *PTGDS* = prostaglandin D2 synthase; *S100A10* = S100 calcium binding protein A10; *SCN1B* = voltage-gated sodium channel type I beta; *SLC15A2* = solute carrier family 15 (H+/peptide transporter), member 2.

Table 13. ***Murine candidate gene SNPs with P ≤ 0.01 in tests for allelic association***

Gene	SNP	SNP location/type	Alleles [A1/A2]		Allele frequencies A1	Allele frequencies A2	Allelic LRT P-value[c]	Allelic OR (CI 95%)	Phenotype
ALAD	rs818702	3'UTR	A/G	Cases	0.884	0.116	0.008	2.40 (1.25 - 4.60)	SOCPH[a]
				Controls	0.761	0.239			
CDH2	rs7240351	Intronic	A/G	Cases	0.333	0.667	0.006	1.90 (1.21 - 3.00	SOCPH[b]
				Controls	0.488	0.513			
EPB41L4A	rs7719346	Non-synonymous (Tyr/His)	A/G	Cases	0.140	0.860	0.008	2.03 (1.17 - 3.54)	GAD[a]
				Controls	0.248	0.752			
	rs1464766	Intronic	A/G	Cases	0.255	0.745	0.010	1.57 (1.09 - 2.27)	PD
				Controls	0.350	0.651			
	rs12657885	Intronic	C/T	Cases	0.918	0.083	0.010	1.98 (1.13 - 3.49)	GAD[b]
				Controls	0.849	0.151			
PSAP	rs4746097	Predicted promoter	C/T	Cases	0.729	0.271	0.004	1.68 (1.17 - 2.40)	PD
				Controls	0.616	0.384			
	rs11597008	Predicted promoter	C/T	Cases	0.615	0.385	0.008	1.59 (1.13 - 2.22)	PD
				Controls	0.502	0.498			
PTGDS	rs4880179	Synonymous (Thr/Thr)	A/G	Cases	0.053	0.947	0.010	3.74 (1.36 - 10.27)	GAD[b]
				Controls	0.015	0.985			

ALAD = δ-aminolevulinate dehydratase; *CDH2* = cadherin 2; CI = confidence interval; *EPB41L4A* = erythrocyte membrane protein band 4.1 like 4A; GAD = generalized anxiety disorder; LRT = likelihood-ratio test; OR = odds ratio; PD = panic disorder; *PSAP* = prosaposin; *PTGDS* = prostaglandin D2 synthase; SOCPH = social phobia

[a] DSM-IV core diagnosis

[b] DSM-IV extended diagnosis

[c] Empirical p-values from 10 000 permutations are shown.

Haplotype analyses

Haplotype analyses of 2- and 3-SNP sliding windows yielded further support for associations in *ALAD*, *CDH2* and *PSAP*. One additional gene not implicated by P ≤ 0.01 in the pointwise analyses, *DYNLL2* (dynein light chain LC8-type 2), reached this level of significance when analyzing haplotypes. The results from the sliding window analyses of the six genes with P ≤ 0.01 in pointwise and/or haplotype analyses are depicted in Figure 13, which also shows their genomic structure. Test statistics for haplotype blocks with empirical global P ≤ 0.01 are further detailed in Table 14.

Sixteen additional SNPs in the large (257 kb) *EPB41L4A* gene were genotyped in study III to increase coverage. The haplotype figures and analyses shown here were updated to include all studied SNPs from the gene.

Table 14. ***Murine candidate gene 2- or 3-marker haplotype blocks with P ≤ 0.01 in a likelihood-ratio test for global haplotype association***

Gene	SNPs	Empirical global P-value[c]	Haplo-types	Haplotype frequencies		Haplotype-specific P-value	OR (CI 95%)	Phenotype
				Cases	Controls			
ALAD	rs11789221 - rs8177822 - rs818708	0.006	C-A-C	0.014	0.002	0.002	8.12 (1.72 - 38.36)	Any anxiety disorder[a]
			C-C-C	0.578	0.607	0.246		
			C-C-T	0.408	0.391	0.505		
	rs818702 - rs11789221 - rs8177822	0.0009	A-C-A	0.036	0.000	0.005	N/A	SOCPH[a]
			A-C-C	0.848	0.766	0.079		
			G-C-C	0.116	0.234	0.010		
CDH2	rs1041985-rs7240351	0.001	C-G	0.317	0.160	0.0006	2.44 (1.45 - 4.09)	SOCPH[b]
			C-A	0.333	0.487	0.006		
			T-G	0.350	0.353	0.956		
DYNLL2	rs10132 - rs9900038 - rs9902118	0.009	A-G-C	0.131	0.061	0.004	2.31 (1.30 - 4.12)	GAD[b]
			A-G-T	0.621	0.717	0.017		
			G-A-C	0.248	0.222	0.480		
PSAP	rs7092990 - rs4746097 - rs11597008	0.008	C-C-C	0.612	0.552	0.014	1.28 (1.05 - 1.56)	Any anxiety disorder[b]
			C-C-T	0.100	0.116	0.298		
			C-T-T	0.197	0.259	0.003		
			T-T-T	0.091	0.073	0.185		
	rs7092990 - rs4746097	0.008	C-C	0.729	0.616	0.004	1.68 (1.17 - 2.40)	PD
			C-T	0.196	0.310	0.002		
			T-T	0.075	0.074	0.975		

ALAD = δ-aminolevulinate dehydratase; *CDH2* = cadherin 2; CI = confidence interval; *DYNLL2* = dynein light chain LC8-type 2; SOCPH = social phobia; GAD = generalized anxiety disorder; OR = odds ratio; PD = panic disorder; *PSAP* = prosaposin

[a] DSM-IV core diagnosis

[b] DSM-IV extended diagnosis

[c] Empirical p-values from 10 000 permutations are shown.

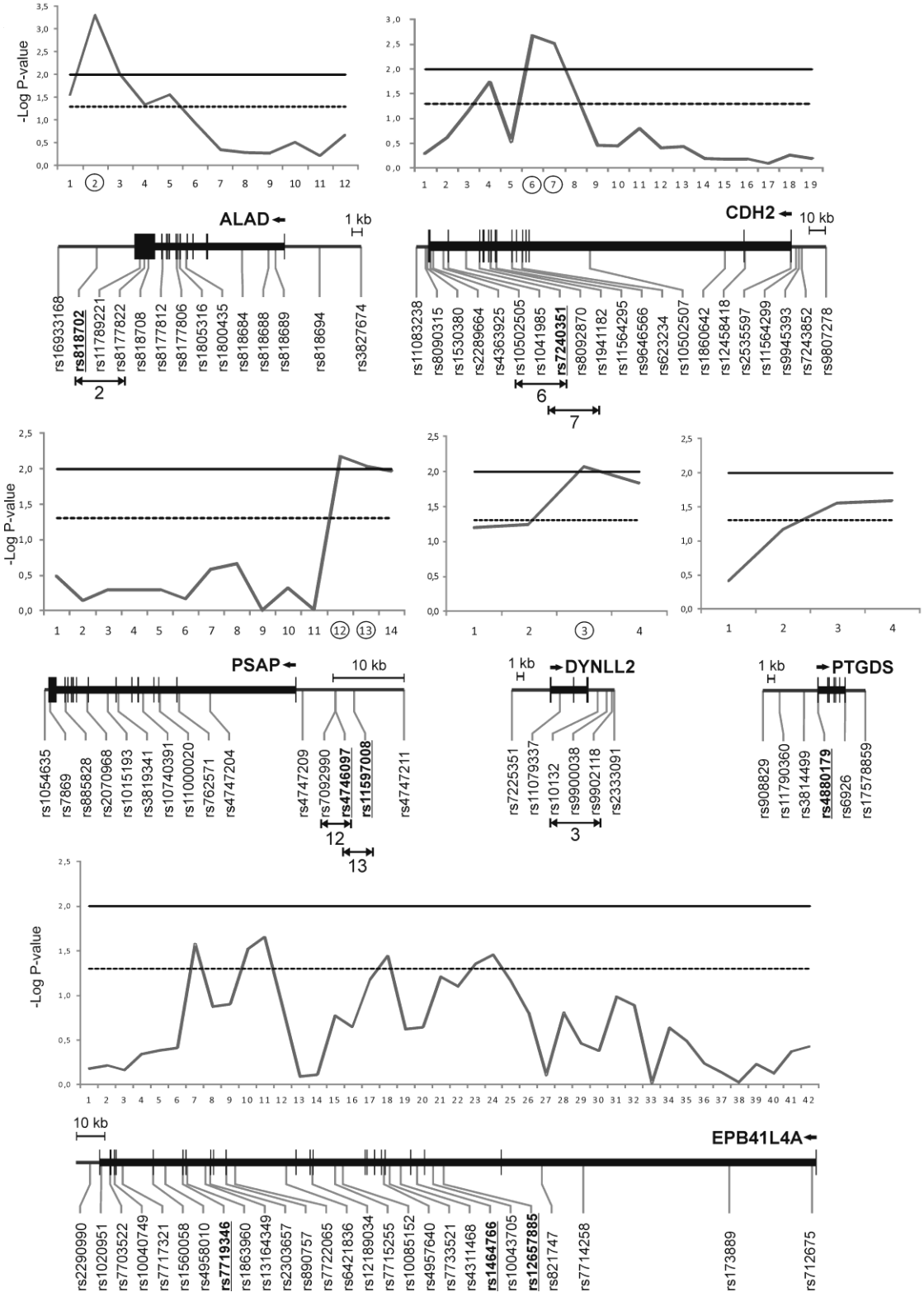


Figure 13 Results from haplotype analyses of sliding SNP windows, and genomic structure, for murine anxiety candidate genes with P ≤ 0.01 in pointwise and/or haplotype analyses. Plots show –log of the P-value for each analyzed haplotype block, identified by a running number from left to right.The solid horizontal line corresponds to P = 0.01, and the dotted line to P = 0.05. Results are shown for the analysis type (either 2- or 3-marker sliding window) and phenotypes that each gene was most significantly implicated in (social phobia for *ALAD* and *CDH2*, generalized anxiety disorder for *DYNLL2*, *EPB41L4A* and *PTGDS*, and panic disorder for *PSAP*). Individual SNPs with P ≤ 0.01 are shown bold and underlined. Haplotype windows with P ≤ 0.01 are circled, and their marker composition is identified with the corresponding number under the gene structure.

Trend analysis further supports EPB41L4A findings

Since we had conducted a total of 1008 statistical tests when analyzing the individual SNPs (7 phenotypes x 144 SNPs), our results did not survive a Bonferroni-based multiple testing correction. Therefore, to provide further support for relevance, we performed a supplementary analysis to examine if any of the investigated genes was overrepresented among the top findings across all tests. We ranked all obtained P-values from most to least significant (1 – 1008) and divided them into four classes: top decile, second decile, second quintile and bottom 60%. For each gene, we then compared the observed distribution of P-values across the classes to that expected under the null hypothesis of randomly distributed P-values. We also performed the same analysis minimized across all tests for each marker, i.e. including only the smallest observed P-value for each SNP when taking all 7 analyzed phenotypes into account. The reason for the minimized analysis was that we wanted to acknowledge that the 7 analyzed phenotypes are in fact not independent as for instance the core and extended diagnostic groups include largely the same subjects. The statistical significances of deviations from the expected P-value distribution were evaluated with a 3 df likelihood-ratio test.

Both the analysis of all ranked P-values and the minimized analysis indicated that *EPB41L4A* SNPs were overrepresented among the top findings (P_{all} = 0.0003; $P_{minimized}$ = 0.008; Figure 14). When comparing only the most relevant class of findings, i.e. the top quintile of P-values, to the remainder with a 1-tailed likelihood-ratio test, the significance of *EPB41L4A* further increased (P_{all} = 9.46 x 10^{-6}; $P_{minimized}$ = 0.002). The trend analysis thus highly supported that SNPs in *EPB41L4A* yield more significant P-values than expected by chance, further suggesting relevance for the gene in anxiety disorder predisposition.

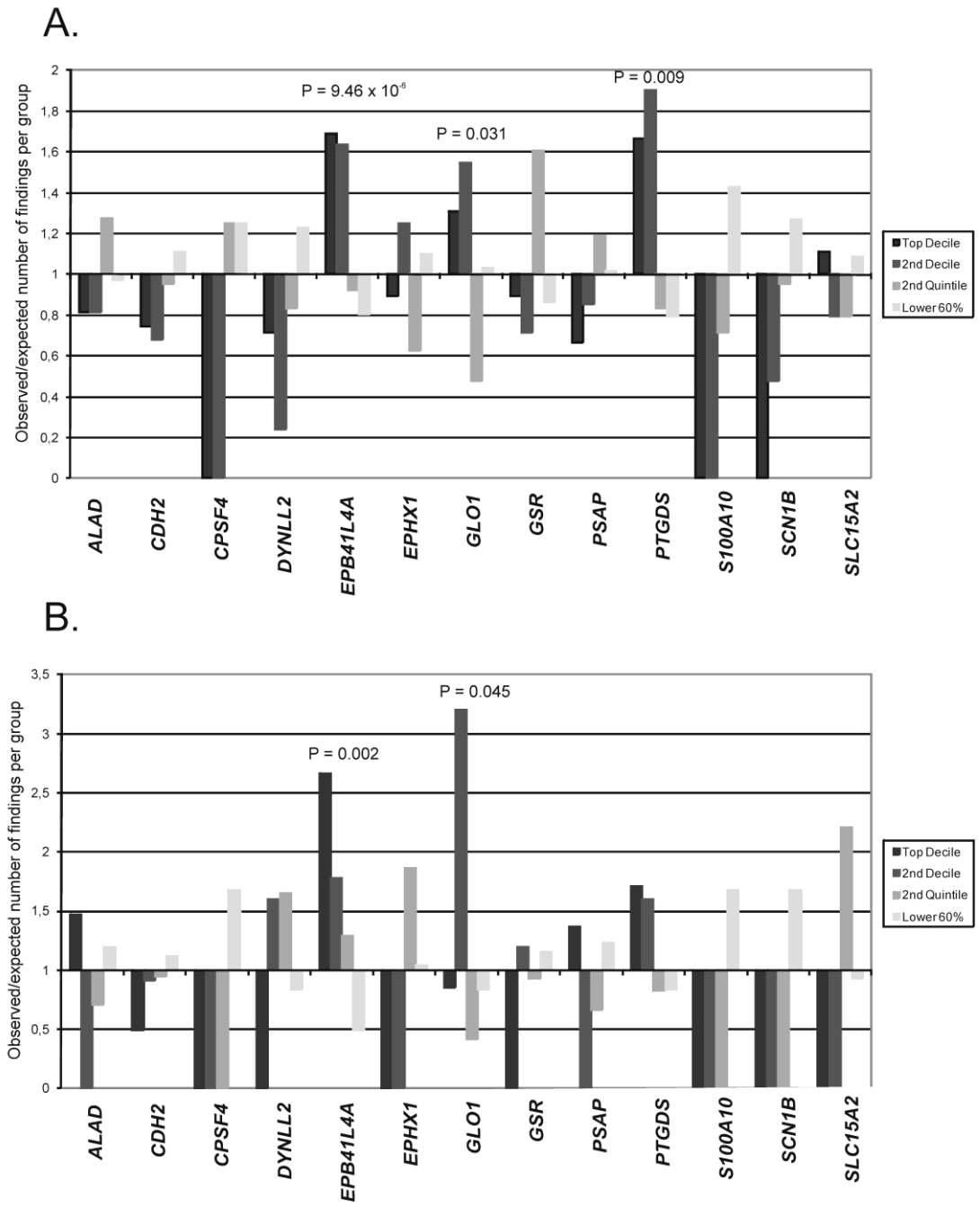


Figure 14 Trend analysis comparing the distribution of ranked P-values across classes representing top and bottom findings. The significance of overrepresentation of a gene among the top 20% of findings was evaluated with a likelihood-ratio test, and P-values for genes with P ≤ 0.05 are shown. The same analysis was carried out using all obtained P-values for each SNP (A), and minimized by including only the smallest observed P-value for each SNP to elimate redundancy (B). Both analyses most significantly implicated *EPB41L4A* as being overrepresented among the top findings, compared to a random distribution of P-values.

Summary of results

Of 13 candidate genes identified based on their differential expression level between non-anxious and anxious mouse strains, we found that six showed some evidence for involvement in susceptibility to specific human anxiety disorders (Table 15). The most significant evidence for association was for a haplotype block in the 3'-UTR of *ALAD* with social phobia (P = 0.0009). A role for *EPB41L4A* in anxiety predisposition was further supported by overrepresentation of SNPs from this gene among the most significant associations.

Table 15. ***Summary of murine and human results for the six identified novel anxiety disorder candidate genes***

	Mice[a]		Humans	
Gene	**Expression in more anxious mouse strains**	**Average fold change[b]**	**Associated diagnostic group(s)**	**Most significant association P-value**
ALAD	↑ in hippocampus, periaqueductal grey	2.17	Social phobia, any anxiety disorder	0.0009
CDH2	↑ in pituitary	1.72	Social phobia	0.001
DYNLL2	↑ in periaqueductal grey	1.85	GAD	0.009
EPB41L4A	↑ in pituitary	3.98	PD, GAD, social phobia	0.006
PSAP	↑ in periaqueductal grey	1.73	PD, any anxiety disorder	0.004
PTGDS	↓ in bed nucleus of stria terminalis, periaqueductal grey	-2.67	GAD	0.01

ALAD = δ-aminolevulinate dehydratase; *CDH2* = cadherin 2; *EPB41L4A* = erythrocyte membrane protein band 4.1 like 4A; GAD = generalized anxiety disorder; PD = panic disorder; *PSAP* = prosaposin; *PTGDS* = prostaglandin D2 synthase

[a] Data from Hovatta et al., 2005

[b] Average fold change for comparison between the two most anxious (DBA/2J and A/J) vs. the two least anxious (C57BL/ 6J and FVB/NJ) mouse strains. In cases of multiple brain regions with differential expression, an average of them is shown.

5.2 NEUROPEPTIDE S AND ITS RECEPTOR PREDISPOSE TO PANIC DISORDER (II)

We aimed to evaluate the potential role of the asthma-predisposing NPS-NPSR1 signaling system in susceptibility to anxiety disorders. Genetic variation in the NPSR1 and NPS encoding genes was tested for association with anxiety disorders in adults, and parent-reported anxiety/depression in children. The association analyses were done in three different study samples (Table 8, page 61): the population-based Health 2000 anxiety disorder sample from Finland, a clinical PD replication sample from Spain, and a Swedish birth cohort (BAMSE). In the two epidemiological samples, we also aimed to confirm previously reported comorbidity between asthma and anxiety. We further wanted to provide evidence for functional relevance of the most significantly associated SNPs, and carried out EMSA experiments to examine whether they might influence transcription factor binding to their surrounding genomic region. We also tested whether gene expression responses are altered in *Npsr1*-deficient mice exposed to acute stress.

Comorbidity of asthma and anxiety

We confirmed previously reported comorbidity between anxiety and asthma in both the adults (≥ 30 years) of the Health 2000 study, and in the 8-year old children of the BAMSE birth cohort. All anxiety disorders pooled did not significantly associate with asthma in the Health 2000 sample, but we found that subjects with agoraphobia had asthma significantly more often than expected by chance (14.1% of subjects with agoraphobia had asthma, compared to 4.0% in subjects without agoraphobia; $\chi^2 = 20.1$, df = 1, P =3.0 x 10^{-4}). In a logistic regression model of determinants of asthma, the only significant predictors were female sex (P = 0.001; OR = 5.17, CI95% 2.23 – 12.00) and agoraphobia (P = 1.20 x 10^{-5}; OR = 1.64, CI95% 1.24 – 2.17).

In the BAMSE sample, 11.3% of children with asthma were reported by their parent as being anxious or depressed. The corresponding frequency among children without asthma was 6.5%. Anxiety was a significant predictor of asthma in a logistic regression model (unadjusted P = 0.026, P = 0.049 when adjusted for sex, heredity for allergic diseases, maternal smoking during pregnancy and/or at childbirth and maternal age at study enrollment).

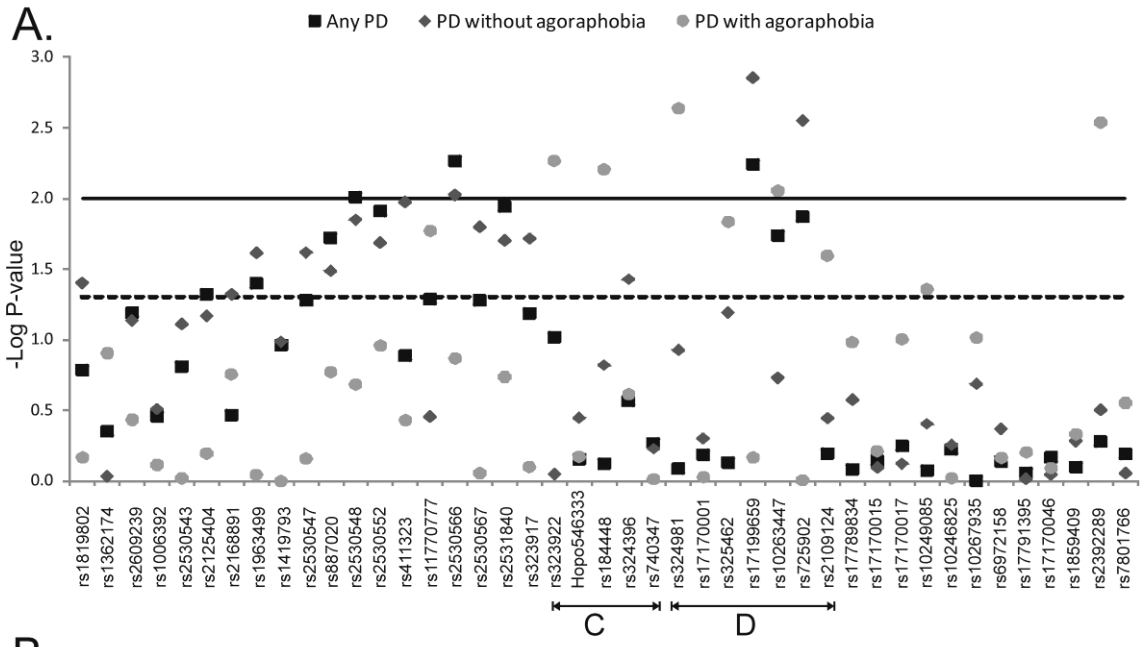
Genetic associations with panic disorder and anxiety/depression

Primary association analysis in the Health 2000 sample

In an initial examination in the Health 2000 sample, we found that 17 of the examined 43 *NPSR1* SNPs, and 3 of the 16 examined *NPS* SNPs showed evidence for association at $P \leq 0.05$ with either PD (including both PD with and without agoraphobia), or its subdiagnosis category PD without agoraphobia (summarized in Figures 15 and 16). Analyses of PD with agoraphobia were not given great emphasis, as the size of this diagnostic subgroup was limited (N = 30). However, they suggest that associations in *NPSR1* cannot be directly attributed to any subtype of PD, whereas *NPS* is more specifically associated with the subdiagnosis PD with agoraphobia.

We further identified eight haplotype blocks in *NPSR1* and *NPS*, and examined specific haplotypes within them for association to PD. We found risk haplotypes for either PD or PD without agoraphobia within three of the blocks ($P \leq 0.05$; Figures 15C-D and 16C). The potentially functionally most relevant finding in *NPSR1* was for a haplotype carrying a common and well-studied gain-of-function amino acid substitution (rs324981; Asn107Ile). The Ile-allele, which we found associated with PD (empirical P = 0.03), is known to increase the sensitivity of the *NPSR1* receptor for *NPS* by 10-fold (Reinscheid et al., 2005).

In *NPS*, we found one specific risk haplotype that accounted for all the individual SNP findings and spanned the entire small locus of the gene (empirical P = 0.015). Notably, it was tagged by the Leu-allele of a non-conservative amino acid substitution (rs990310; Ser14Leu) in the signal peptide motif of the NPS precursor protein.



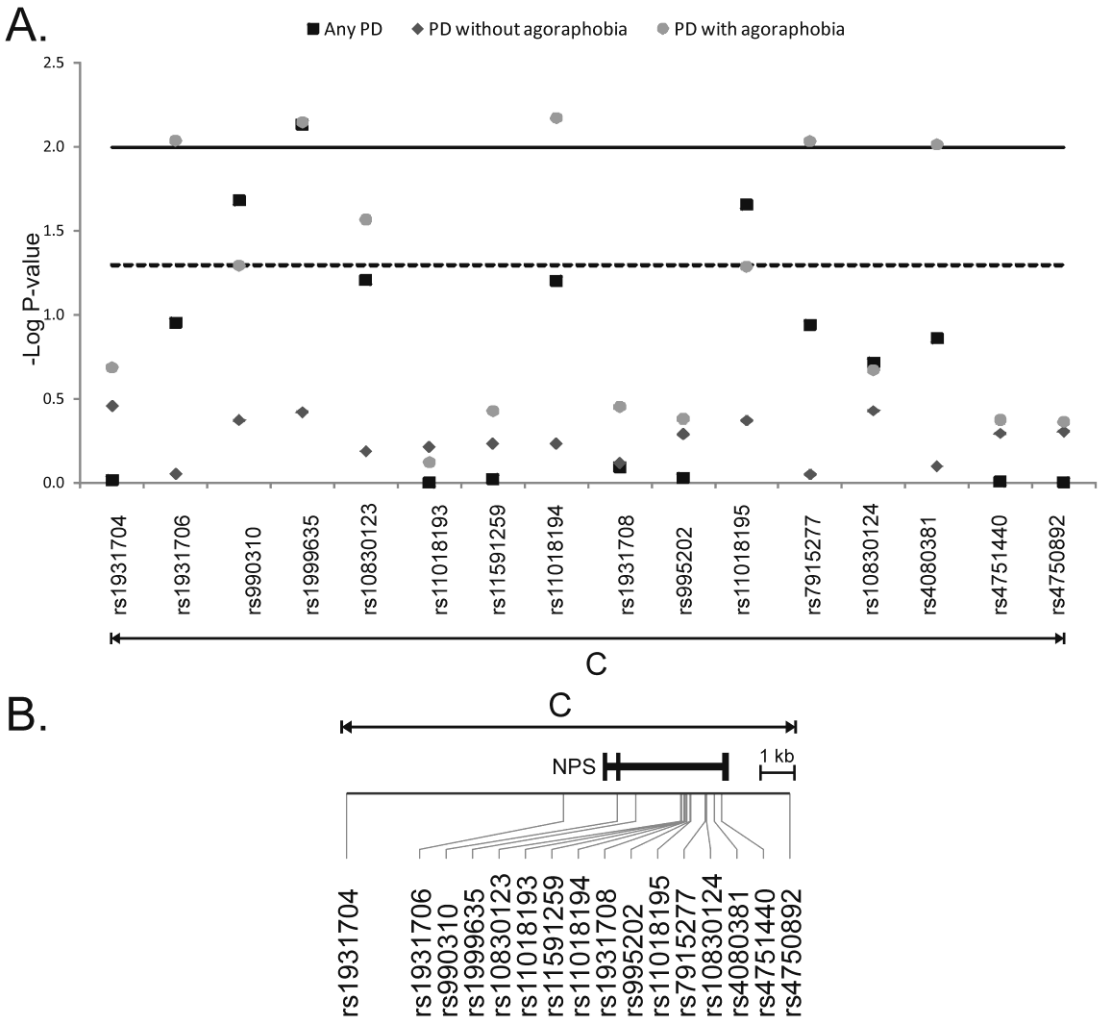
C.

	rs323922	Hopo 546333	rs324396	rs740347	Haplotype frequency Cases	Controls	Theoretical P-value	Empirical P-value
H1	G	G	T	G	0.341	0.386	0.279	
H2	C	G	C	G	0.284	0.332	0.221	
H3	G	G	C	G	0.240	0.131	0.0005	0.002
H4	C	A	C	C	0.087	0.077	0.681	
H5	C	G	C	C	0.048	0.075	0.204	

D.

	rs324981	rs17170001	rs325462	rs17199659	rs10263447	rs725902	rs2109124	Haplotype frequency Cases	Controls	Theoretical P-value	Empirical P-value
H1	A	G	A	T	G	A	G	0.326	0.379	0.289	
H2	T	G	T	T	C	A	A	0.208	0.254	0.300	
H3	T	G	T	C	G	G	A	0.208	0.104	0.003	0.030
H4	A	G	A	T	G	A	A	0.104	0.128	0.466	
H5	T	A	T	T	G	G	A	0.118	0.082	0.228	
H6	T	A	T	T	G	A	A	0.007	0.022	0.264	
H7	A	G	A	T	G	G	A	0.000	0.018	0.105	

Figure 15 Graphical summary of genetic association findings in the *NPSR1* gene in the Finnish Health 2000 study sample. (A) Plot showing the –log association P-value of each individual SNP tested. Results are shown for panic disorder (PD), and its subdiagnoses PD with and without agoraphobia. The solid horizontal line corresponds to P = 0.01, and the dotted line to P = 0.05. (B) Genomic structure of *NPSR1*, and positions of the analyzed SNPs. (C and D) Detailed structure of blocks containing haplotypes showing evidence for association with either any PD (C) or PD without agoraphobia (D). Risk alleles of individual SNPs also implicated by P ≤ 0.05 in either any PD or PD without agoraphobia are shaded grey. Spans of haplotype blocks shown in (C) and (D) are indicated in panels (A) and (B) with their corresponding letter.



C.

	rs1931704	rs1931706	rs990310	rs1999635	rs10830123	rs11018193	rs11591259	rs11018194	rs1931708	rs995202	rs11018195	rs7915277	rs10830124	rs4080381	rs4751440	rs4750892	Haplotype frequency: Cases	Controls	Theoretical P-value	Empirical P-value
H1	G	T	C	G	G	G	T	T	G	A	A	T	C	C	G	A	0.288	0.313	0.532	
H2	G	T	C	G	G	G	T	T	A	A	A	T	C	C	G	A	0.328	0.293	0.378	
H3	G	T	C	G	G	A	T	C	G	A	A	T	A	C	G	A	0.081	0.118	0.161	
H4	A	C	C	G	G	A	T	T	G	C	A	C	C	A	C	C	0.086	0.086	0.989	
H5	A	C	C	G	C	A	G	T	G	A	A	C	C	A	G	A	0.081	0.076	0.848	
H6	G	T	C	G	G	A	T	C	G	A	A	T	C	C	G	A	0.045	0.076	0.152	
H7	G	C	T	A	C	A	T	T	G	A	G	C	C	A	G	A	0.091	0.037	0.006	0.015

Figure 16 Graphical summary of genetic association findings in the *NPS* gene in the Finnish Health 2000 study sample. (A) Plot showing the –log association P-value of each individual SNP tested. Results are shown for panic disorder (PD), and its subdiagnoses PD with and without agoraphobia. The solid horizontal line corresponds to P = 0.01, and the dotted line to P = 0.05. (B) Genomic structure of *NPS*, and positions of the analyzed SNPs. (C) Detailed structure of a locus-spanning haplotype block showing evidence for association with any PD. Risk alleles of individual SNPs also implicated by P ≤ 0.05 in either any PD or PD with agoraphobia are shaded grey. The span of the haplotype shown in (C) is shown in panels (A) and (B).

Replication attempt in the Barcelona panic disorder sample

We subsequently aimed to replicate the observed associations with PD in a Spanish sample of PD outpatients (Table 8, page 61). No significant evidence for association to *NPSR1* was observed, but two *NPS* SNPs, representing the same S14L-haplotype as in the Finnish sample, associated with PD without agoraphobia (P ≤ 0.05, summarized in Figure 17). However, the risk alleles were opposite compared to the Finnish sample. No haplotype associations with empirical P ≤ 0.05 were found in the Spanish sample.

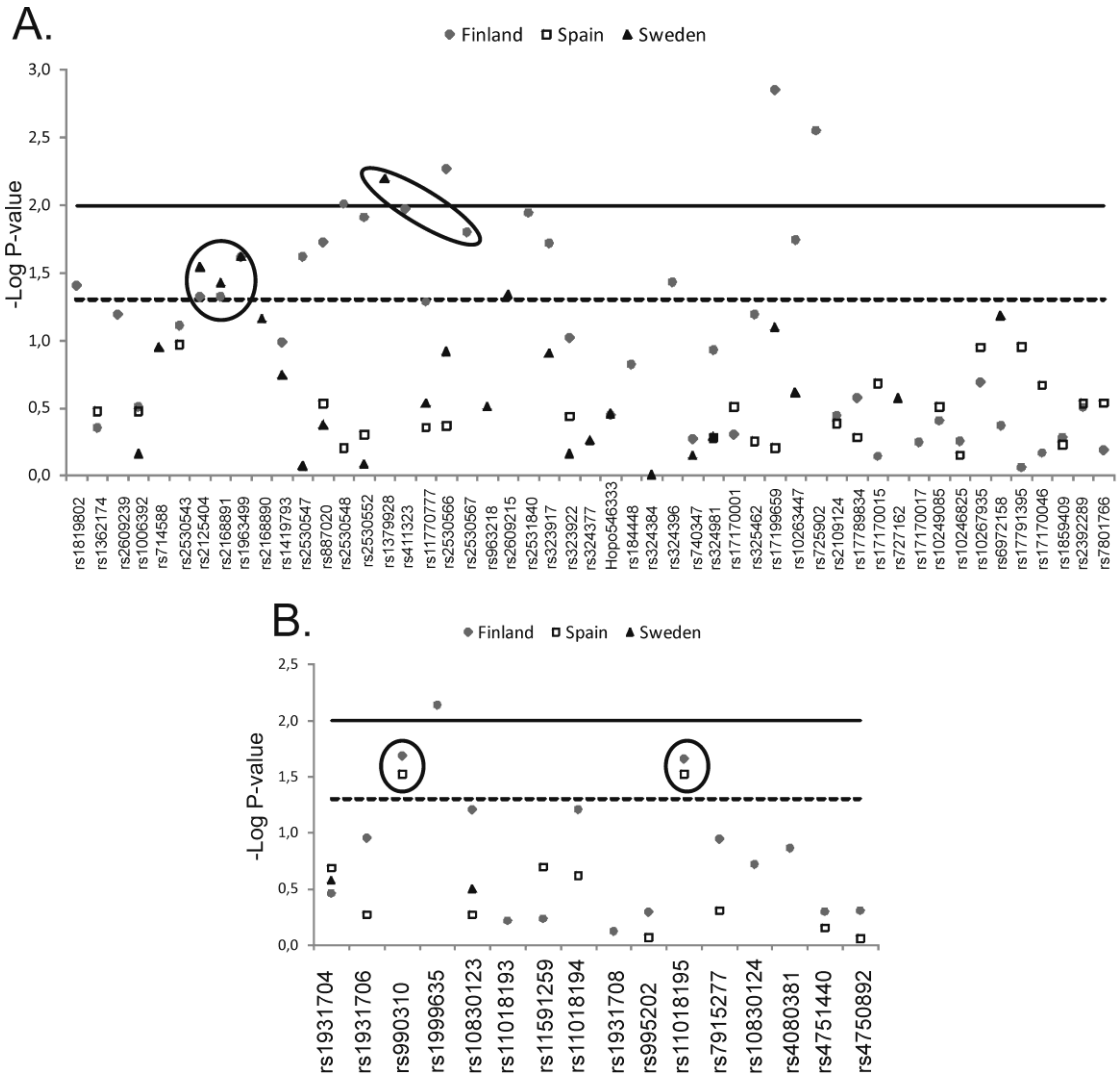


Figure 17 Graphical summary of *NPSR1* (A) and *NPS* (B) genetic association findings in three independent samples. Results (-log P-values for each individual SNP) are shown for the Finnish Health 2000 sample, the Spanish panic disorder (PD) sample, and the Swedish BAMSE birth cohort. The shown P-values are minimized from analyses of any PD and PD without agoraphobia in the Finnish sample, and any PD and its subdiagnoses PD with or without agoraphobia in the Spanish sample. Marker regions where evidence for association (P ≤ 0.05) overlaps between at least two of the samples are circled. Note that although the same two *NPS* SNPs are implicated in Finland and Spain, the risk alleles are different.

Further associations between NPSR1 SNPs and parent-reported anxiety/depression

Twenty-six SNPs from *NPSR1* and two from *NPS* had been genotyped in children of the Swedish BAMSE birth cohort. Based on our findings with PD, we evaluated their association with a parent-repored measure of anxiety/depression from the EQ-5D questionnaire. Five *NPSR1* SNPs associated with this phenotype ($P \leq 0.05$, Figure 17). Three of them, and a fourth proxy for one of the Swedish SNPs, were also implicated in PD in the Health 2000. No haplotype associations with empirical $P \leq 0.05$ were found.

Potential functional relevance of NPSR1 SNPs

We aimed to show that the *NPSR1* SNPs with evidence for association might have functional consequences. We selected five SNPs, all with $P \leq 0.01$ in either the Health 2000 sample or in the BAMSE sample, and tested them for allele-specific binding to nuclear proteins in EMSAs. Alleles of two of the SNPs, rs2530548 and rs2530566, showed both qualitative and quantitative differences in protein binding. As nuclear extracts are rich in transcription factors (TFs), they might be the proteins involved in the allele-specific binding. We therefore attempted to identify the specific TFs responsible for the DNA-protein interactions. According to bioinformatic predictions, rs2530548 might affect binding of SOX-5 (sex determining region Y-box 5), whereas rs2530566 might influence binding of a number of TFs like growth factor independent 1 transcription repressor (GFI1), OVO-like 1 (OVOL1), homeobox transcription factors A9 and B9 (HOXA9, HOXB9) and runt-related transcription factor 2 (RUNX2). We prioritized SOX-5 and RUNX2 for functional testing, as they had the clearest prior evidence for suggested involvement in neuronal function and mental disorders (Kwan et al., 2008; Benes et al., 2007). However, antibodies for these two TFs did not shift the gel migration of the DNA-protein complexes in supershift EMSAs, and they are thus unlikely the proteins involved in the interactions.

Npsr1-deficiency alters molecular stress responses in mice

We evaluated immobilization stress-induced gene expression changes in *Npsr1*$^{-/-}$ mice and wild types. Altogether, we tested whether 14 genes were differentially regulated in response to stress due to *Npsr1*-deficiency. Expression changes of these genes were evaluated in at least one of four brain regions (cortex, hippocampus, hypothalamus, and striatum). The treatment groups consisted of mice killed immediately after 1 h immobilization stress, mice subjected to 1 h restraint stress and killed after a 1 h recovery period, and unstressed controls killed immediately after home cage removal. Results from factorial ANOVA analyses evaluating main effects of stress treatment, *Npsr1* genotype, and their interaction are shown in Table 16, page 96.

We found experiment-wide significant (P ≤ 0.0019 [0.05/26 hypotheses tested]) main effects of stress treatment for serum corticosterone levels and the expression of six genes in at least one brain region (*Egr1*, *Fos*, *Il1b*, *JunB*, *Klf10* and *Tnf*; Figure 18). This was an expected result, as these genes were mainly immediate early genes already previously linked to stress response. However, together with the significant increase in serum corticosterone, these findings indicated that our stress treatment was valid and working.

Our main interest was rather in identifying genes that showed main effects of *Npsr1* genotype or genotype x stress treatment interactions. Such at least nominally significant effects were observed for *Nps*, *Fos*, *JunB* in the cortex, for *Il1b* in the cortex and hypothalamus, and for *Ntf3* in the cortex and striatum (Figures 18 and 19). The experiment-wide significant, and thus most relevant, results were for *Il1b* and *Ntf3*. *Il1b* was upregulated in the cortex of *Npsr1*$^{-/-}$ mice after stress exposure as compared to wild types (1.9 fold immediately after immobilization and 2.7 fold after a 1 h recovery; P = 0.0005). *Npsr1*-deficient mice further lacked the stress-following induction of *Ntf3* that was seen in the striatum of wild types (7.5 fold immediately after immobilization and 2.5 fold after a 1 h recovery; P = 0.0006).

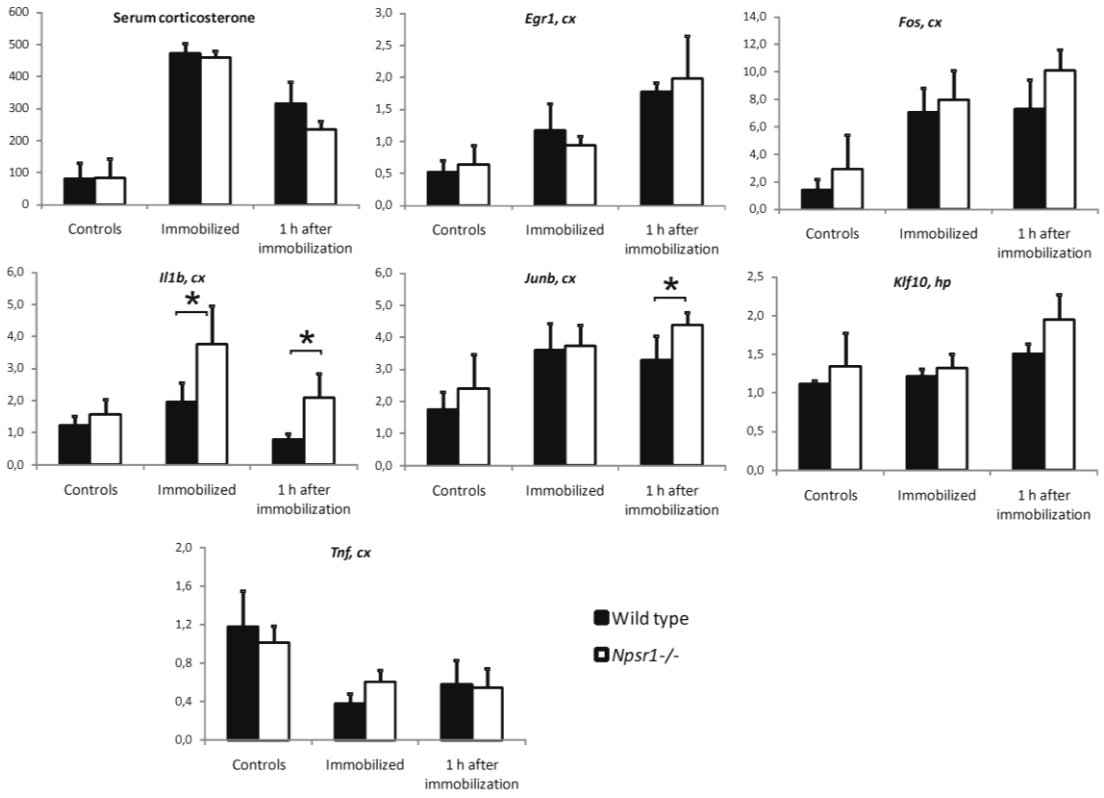


Figure 18 Serum corticosterone, and brain gene expression levels of genes showing experiment-wide significant effects (P ≤ 0.0019) of immobilization stress treatment in *Npsr1*$^{-/-}$ and wild type mice. Units are ng/ml for corticosterone, and expression relative to either *Gapdh* or *Rn18s* for the genes. Please refer to Table 16 for test statistics, and full gene names. Note that *Fos*, *Il1b* and *Junb* also show nominally significant main effects of *Npsr1* genotype. Significant post-hoc comparisons (P ≤ 0.05) between genotypes in Student's T-test are indicated (*). Cx = cortex; hp = hippocampus.

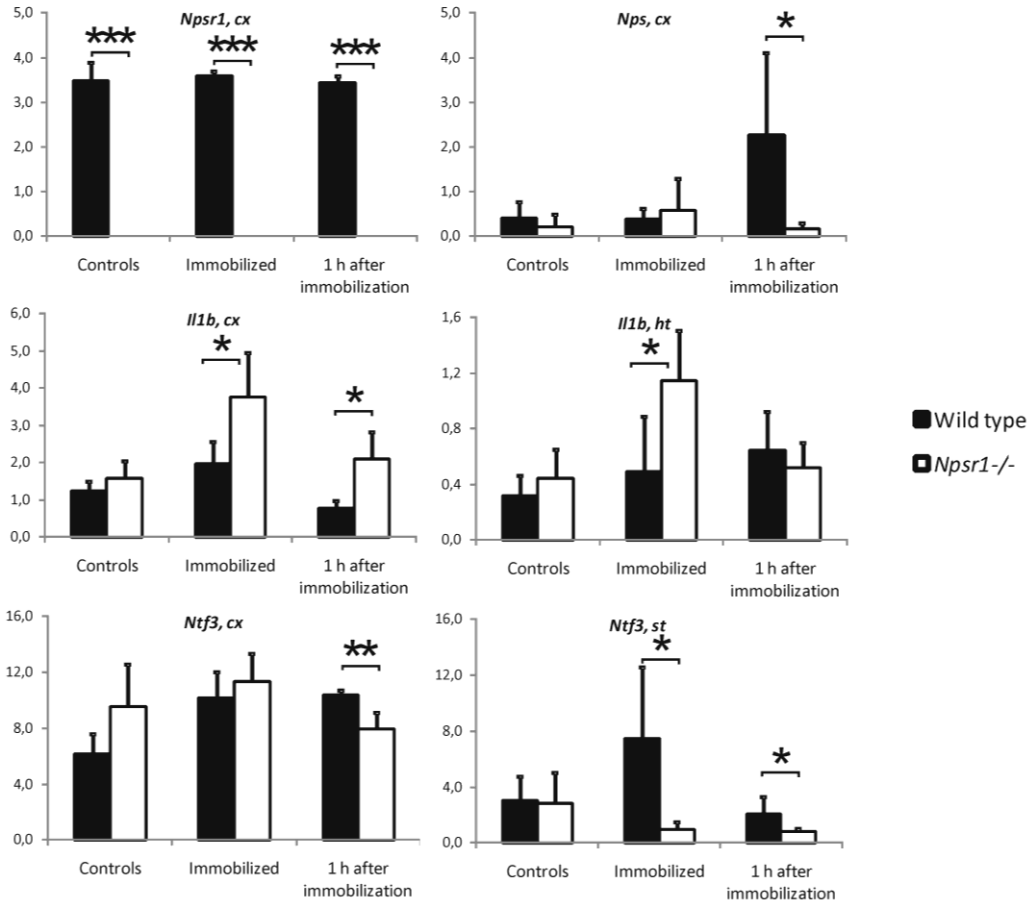


Figure 19 Brain gene expression levels of genes showing at least nominally significant (P ≤ 0.05) effects of *Npsr1* genotype, or stress treatment x *Npsr1* genotype interactions. Units are expression relative to either *Gapdh* or *Rn18s*. Please refer to Table 16 for test statistics, and full gene names. Note that *Fos* and *Junb*, plotted in Figure 18, also show nominally significant main effects of *Npsr1* genotype. Significant post-hoc comparisons between genotypes in Student's T-test are indicated (* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001). Cx = cortex; ht = hypothalamus; st = striatum.

Summary of results

We replicated prior epidemiological findings of comorbidity between anxiety and asthma in both adults and children. SNPs and haplotypes within both *NPSR1* and *NPS* were associated with PD in adults. *NPSR1* SNPs were also associated with parent-reported anxiety/depression in children. Evidence from three human study samples thus supports a role for the NPS-NPSR1 system in modulating anxiety predisposition.

We further suggest that the associated *NPSR1* polymorphisms may influence transcription factor binding to an intronic region. This might influence the expression level of *NPSR1* if it affects a regulatory element. However, the specific transcription factors involved remain to be identified.

Finally, *Npsr1*-deficient mice differ from wild types most significantly regarding the induction of two known stress-related genes, *Il1b* and *Ntf3*.

Table 16. ***Serum corticosterone levels [ng/ml], and relative brain expression levels of genes related to stress and anxiety in Npsr1$^{-/-}$ and wild type mice after immobilization stress.*** *Results are shown for controls killed after home cage removal, mice killed after 1 h stress exposure (Immobilized), and mice killed after 1 h stress and a 1 h recovery (1 h after immobilization). Mean values are based on three – six animals per treatment group. P-values ≤ 0.05 are highlighted in bold.*

			Wild type						*Npsr1* $^{-/-}$						Factorial ANOVA test statistics					
			Controls		Immobilized		1 h after immobilization		Controls		Immobilized		1 h after immobilization		Genotype main effects		Treatment main effects		Genotype x treatment effects	
Gene/hormone	**Symbol**	**Tissue**	**M**	**±SD**	**M**	**±SD**	**M**	**±SD**	**M**	**±SD**	**M**	**±SD**	**M**	**±SD**	$F_{df=1}$	**P**	$F_{df=2}$	**P**	$F_{df=2}$	**P**
Corticosterone	N/A	Serum	80.2	50.4	471.9	30.7	316.0	68.6	83.6	60.9	460.3	19.9	234.3	26.0	2.31	0.148	141.0	**6.92×10^{-11}**	1.62	0.228
Basic helix-loop-helix domain containing, class B2	*Bhlhb2*	Ht	1.45	0.05	1.36	0.14	1.31	0.25	2.14	0.53	1.36	0.18	2.06	1.29	3.85	0.069	1.40	0.278	0.99	0.396
Early growth response 1	*Egr1*	Cx	0.52	0.18	1.17	0.42	1.77	0.14	0.64	0.30	0.94	0.14	1.99	0.66	0.04	0.849	29.45	**2.11×10^{-6}**	0.64	0.540
		Hp	1.19	0.34	1.47	0.31	1.32	0.31	1.05	0.20	1.10	0.14	2.00	0.78	0.01	0.936	3.79	**0.042**	3.31	0.060
		Ht	1.45	0.68	5.83	1.13	9.20	3.90	0.85	0.07	4.72	0.79	15.56	8.43	0.97	0.337	17.45	**6.12×10^{-5}**	2.36	0.123
FBJ osteosarcoma oncogene	*Fos*	Cx	1.40	0.81	7.06	1.78	7.33	2.12	2.94	2.46	7.96	2.15	10.11	1.51	5.13	**0.036**	27.47	**3.40×10^{-6}**	0.52	0.605
		Hp	1.16	0.30	2.54	0.62	3.03	1.33	1.15	0.34	3.11	1.32	4.02	1.13	1.10	0.307	22.47	**1.28×10^{-5}**	0.47	0.633
		Ht	0.80	0.32	8.03	2.33	6.34	2.94	1.00	0.28	8.92	2.33	4.52	1.12	0.08	0.788	24.95	**5.31×10^{-5}**	0.85	0.451
Galanin	*Gal*	Ht	2.97	4.88	0.42	0.17	0.45	0.27	1.04	1.18	0.52	0.16	0.31	0.02	0.16	0.692	2.69	0.095	0.34	0.716
Interleukin 1 beta	*Il1b*	Cx	1.23	0.28	1.97	0.59	0.78	0.20	1.58	0.47	3.76	1.20	2.10	0.75	17.62	**0.0005**	12.43	**0.0004**	2.42	0.118
		Ht	0.32	0.14	0.50	0.39	0.65	0.28	0.44	0.21	1.15	0.36	0.52	0.18	3.74	0.069	5.14	**0.017**	4.15	**0.033**
Inhibin beta-A	*Inhba*	Cx	3.21	0.54	2.37	0.38	2.14	0.50	3.16	0.93	2.36	0.56	2.33	0.54	0.04	0.852	5.83	**0.011**	0.09	0.913
		Hp	1.76	0.79	1.76	0.40	1.68	0.40	1.88	0.30	1.50	0.14	1.83	0.54	0.02	0.884	0.26	0.773	0.63	0.542
		Ht	1.48	0.50	1.14	0.18	1.87	1.11	1.38	0.23	1.22	0.20	1.74	0.47	0.02	0.877	2.56	0.105	0.07	0.933
Jun-B oncogene	*Junb*	Cx	1.74	0.55	3.60	0.83	3.28	0.75	2.42	1.05	3.73	0.67	4.39	0.38	4.51	**0.048**	13.87	**0.0002**	0.87	0.434
		Hp	0.90	0.20	1.17	0.20	2.23	1.28	1.08	0.22	1.72	0.66	2.79	0.89	3.78	0.068	14.01	**0.0002**	0.10	0.903
		Ht	0.97	0.59	3.11	0.77	1.83	1.11	0.92	0.17	3.42	0.94	4.03	5.09	0.37	0.553	8.27	**0.003**	0.08	0.922
Kruppel-like factor 10	*Klf10*	Hp	1.12	0.04	1.22	0.09	1.50	0.13	1.35	0.43	1.33	0.18	1.95	0.33	4.22	0.056	8.14	**0.003**	0.44	0.651
		Ht	1.28	0.64	0.76	0.20	0.99	0.18	1.06	0.22	0.99	0.19	2.08	2.39	0.62	0.443	0.84	0.452	0.50	0.617
Ngfi-A binding protein 1	*Nab1*	Hp	1.50	0.89	1.09	0.37	1.13	0.23	1.23	0.46	1.18	0.47	1.33	0.33	0.00	0.980	0.46	0.639	0.50	0.617
		Ht	0.75	0.07	0.59	0.13	0.77	0.06	0.90	0.19	0.83	0.16	0.84	0.19	3.68	0.058	1.11	0.354	0.54	0.592
Neuropeptide S	*Nps*	Cx	0.41	0.37	0.38	0.25	2.28	1.83	0.22	0.29	0.58	0.72	0.17	0.14	1.03	0.331	0.23	0.799	8.17	**0.006**
Neuropeptide S receptor 1	*Npsr1*	Cx	3.47	0.42	3.59	0.11	3.44	0.16	0.00	0.00	0.00	0.00	0.00	0.00	N/A	N/A	0.32	0.730	N/A	N/A
Neurotrophin 3	*Ntf3*	Cx	6.15	1.45	10.15	1.90	10.36	0.35	9.56	3.04	11.39	1.96	7.95	1.22	0.98	0.335	5.04	**0.018**	5.09	**0.018**
		St	3.06	1.73	7.51	5.11	2.10	1.24	2.87	2.20	1.00	0.51	0.84	0.19	14.96	**0.0006**	2.65	0.088	4.25	**0.024**
Tachykinin	*Tac1*	Cx	4.80	0.49	4.38	0.81	4.65	0.52	4.84	0.81	5.03	2.26	5.05	0.84	0.63	0.439	0.04	0.962	0.15	0.865
Tumor necrosis factor	*Tnf*	Cx	1.18	0.37	0.38	0.10	0.58	0.26	1.01	0.17	0.61	0.12	0.55	0.20	0.01	0.914	17.13	**6.82×10^{-5}**	1.58	0.233

ANOVA = analysis of variance; Cx = cortex; Hp = hippocampus; Ht = hypothalamus; M = mean; N/A = not applicable; SD = standard deviation; St = striatum.

Mean values shown are based on three-six animals per experimental group. P-values ≤ 0.05 were highlighted in bold.

5.3 GLUTAMATE DECARBOXYLASE 1 AND NEUROPEPTIDE Y MODULATE ANXIETY SUSCEPTIBILITY (III)

We analyzed 92 SNPs from 15 putative susceptibility genes for anxiety disorders or anxiety-related traits, aiming to replicate some of the most relevant findings within the field in the Health 2000 sample. Childhood adversities are known strong risk factors for anxiety disorders, and GxE interactions may be important in explaining why some individuals are more stress resilient than others. Therefore, we further examined whether any of the studied genetic variants modulates the effect of the number of experienced childhood adverse life events on anxiety disorder risk.

Primary analysis implicates GAD1 in susceptibility to phobias

We tested SNPs for association to any anxiety disorder, PD, GAD, and phobias (combining social phobia, agoraphobia, and phobia not otherwise specified into one phenotype in this study). We also chose to focus our analyses only on the subjects with DSM-IV core diagnoses, excluding subjects with subthreshold diagnoses. The only gene showing evidence for association at empirical P ≤ 0.01 was glutamate decarboxylase 1 (*GAD1)*, in which three SNPs associated with phobias (Table 17). An additional 9 genes were implicated with P ≤ 0.05, and these findings are summarized in Table 18. Although these 9 genes only showed modest evidence for genetic association, the results are shown here as the purpose of the investigation was to replicate prior findings and they may therefore be of some relevance.

On the haplotype level, the only gene showing evidence for association at empirical P ≤ 0.01 was similarily *GAD1* with phobias. In fact, the three individual SNP findings in the gene were not independent, as they all represented the same specific risk haplotype (Figure 20, page 99).

Table 17. ***Genetic associations (P ≤ 0.01) in the GAD1 gene with phobias***

SNP	SNP type	Alleles [A1/A2]		Allele frequencies A1	Allele frequencies A2	Allelic LRT P-value[a]	Allelic OR (CI 95%)
rs769407	Intronic	C/G	Cases	0.385	0.615	0.0005	1.79 (1.30-2.48)
			Controls	0.259	0.741		
rs3791851	Intronic	G/A	Cases	0.390	0.610	0.0009	1.64 (1.21-2.23)
			Controls	0.280	0.720		
rs769395	3'-UTR	C/T	Cases	0.388	0.612	0.002	1.64 (1.21-2.22)
			Controls	0.278	0.722		

CI = confidence interval; LRT = likelihood-ratio test; OR = odds ratio

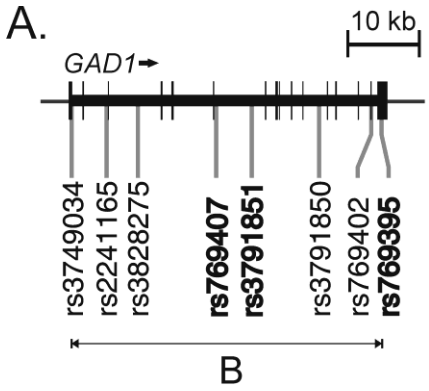
[a] Empirical p-values from 10 000 permutations are shown.

Table 18. ***Genetic associations with P ≤ 0.05 in 9 putative susceptibility genes for anxiety disorders***

Gene	SNP	SNP type	Alleles [A1/A2]		Allele frequencies A1	Allele frequencies A2	Allelic LRT P-value[a]	Phenotype	Previously associated with (references)
ADORA2A	rs1003774	5'-UTR	G/T	Cases	0.583	0.417	0.028	GAD	PD, caffeine-induced anxiety, harm avoidance (Hamilton et al., 2004; Childs et al., 2008; Deckert et al., 1998)
				Controls	0.473	0.527			
	rs5751876	Synonymous (Tyr/Tyr)	C/T	Cases	0.479	0.521	0.014	GAD	
				Controls	0.603	0.397			
	rs1041749	3'-UTR	C/T	Cases	0.478	0.522	0.012	GAD	
				Controls	0.609	0.391			
BDNF	rs6265	Non-synonymous (Met/Val)	A/G	Cases	0.120	0.880	0.030	Phobias	OCD, neuroticism, harm avoidance (Montag et al., 2010; Frustaci et al., 2008)
				Controls	0.177	0.823			
COMT	rs737865	Intronic	A/G	Cases	0.774	0.226	0.038	Any anx.dis.	PD, genetic susceptibility shared by anxiety spectrum phenotypes, harm avoidance, phobia, low extroversion (Hettema et al., 2008)
				Controls	0.816	0.184			
				Cases	0.774	0.226	0.026	Phobias	
				Controls	0.837	0.163			
	rs1544325	Intronic	A/G	Cases	0.416	0.584	0.044	PD	
				Controls	0.498	0.502			
CRH	rs11997416	3'-UTR	C/T	Cases	0.945	0.055	0.037	GAD	Behavioral inhibition (Smoller et al., 2005; Smoller et al., 2003)
				Controls	0.887	0.113			
NPY	rs16135	Intronic	C/T	Cases	0.939	0.061	0.023	PD	Anxiety disorders, harm avoidance (Zhou et al, 2008)
				Controls	0.885	0.115			
PDE4D	rs35305	Intronic	G/T	Cases	0.530	0.470	0.039	Phobias	Neuroticism (Calboli et al., 2010; Shifman et al., 2008)
				Controls	0.604	0.396			
PLXNA2	rs12094123	Intronic	A/T	Cases	0.495	0.505	0.030	PD	Anxiety disorders, neuroticism, psychological distress, anxiety severity (Wray et al., 2007; Coric et al., 2010)
				Controls	0.581	0.419			
SLC6A3	rs27072	3'-UTR	A/G	Cases	0.224	0.776	0.044	PD	PTSD, GAD, SOCPH (Rowe et al., 1998; Segman et al., 2002)
				Controls	0.160	0.840			
	rs403636	Intronic	G/T	Cases	0.840	0.160	0.027	GAD	
				Controls	0.913	0.087			
SLC6A4	rs6354	5'-UTR	A/C	Cases	0.776	0.224	0.049	PD	PD, OCD, anxiety and/or MD, neuroticism, trait anxiety (Wray et al., 2009; Costas et al., 2010; Strug et al., 2010)
				Controls	0.838	0.162			

ADORA2A = adenosine A2a receptor; *BDNF* = brain-derived neurotrophic factor; *COMT* = catechol-O-methyltransferase; *CRH* = corticotropin releasing hormone; GAD = generalized anxiety-disorder; LRT = likelihood-ratio test; MD = major depression; *NPY* = neuropeptide Y; OCD = obsessive-compulsive disorder; PD = panic disorder; *PDE4D* = phosphodiesterase 4D, cAMP-specific; *PLXNA2* = plexin A2; PTSD = post-traumatic stress disorder; *SLC6A3* = solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; *SLC6A4* = solute carrier family 6 (neurotransmitter transporter, serotonin), member 4; SOCPH = social phobia

[a] Empirical p-values from 10 000 permutations are shown.



B.

								Haplotype frequencies			
	rs3749034	rs2241165	rs3828275	rs769407	rs3791851	rs3791850	rs769395	Cases	Controls	Theoretical P-value	Empirical P-value
H1	C	A	A	G	A	C	T	0.353	0.450	0.022	
H2	T	G	G	G	A	T	T	0.210	0.230	0.431	
H3	C	A	G	C	G	C	C	0.357	0.221	0.0001	0.0009
H4	C	A	G	G	A	C	T	0.026	0.038	0.396	
H5	T	G	G	C	G	C	C	0.021	0.021	0.999	
H6	C	A	A	C	G	C	C	0.013	0.005	0.733	

Figure 20 Genomic structure of *GAD1*, and haplotype association finding with phobias in the Finnish Health 2000 study sample. (A) Genomic structure of *GAD1* and positions of the analyzed SNPs. SNPs associating with phobias with P ≤ 0.01 are highlighted in bold. (B) Detailed structure of a locus-spanning haplotype block. One specific risk haplotype (H3) associates with phobias and accounts for the individual SNP findings. Risk alleles of individual SNPs also implicated by P ≤ 0.01 are shaded grey. The span of the haplotype block is shown in (A).

Meta-analysis suggests involvement of GAD1 in susceptibility to a broad range of internalizing disorders

The *GAD1* risk haplotype we discovered was the same one that was associated with genetic susceptibility shared by anxiety disorders, MD and neuroticism in a prior study (Hettema et al., 2006) done in the VATSPSUD sample (Table 8, page 61). We therefore used meta-analytic methods to incorporate the VATSPSUD findings with ours, with the aim to obtain a more accurate cross-study assessment of the contribution of *GAD1* to susceptibility to internalizing disorders. As there were several inherent differences between the Health 2000 sample and the VATSPSUD one (please refer to section 4.1 for details), our best option for unbiased pooling of the samples was to combine the entire samples with each other. Four of the same *GAD1* SNPs had been genotyped in both samples. In the resulting combined analysis (N = 1985) of a broad phenotype reflecting shared genetic susceptibility across a range of internalizing disorders and neuroticism, significance of the aforementioned specific risk haplotype increased compared to the analysis of either sample alone (Table 19). Altogether, this haplotype increased the risk for internalizing disorders by an OR = 1.3 (CI 95% 1.11 – 1.52).

Table 19. ***Meta-analysis of GAD1 risk alleles and haplotypes with a broad phenotype reflecting shared genetic susceptibility across internalizing disorders and neuroticism***

		Health 2000 sample (N = 857)[a]			VATSPSUD sample (N = 1128)[b]			Combined sample (N = 1985)	
SNP(s)	Risk allele/ haplotype	Risk allele freq.	OR (CI 95%)	P-value	Risk allele freq.	OR (CI 95%)	P-value	OR (CI 95%)	P-value
rs2241165	A	0.746	1.04 (0.82-1.32)	0.765	0.748	1.37 (1.12-1.68)	0.001	1.22 (1.05-1.42)	0.011
rs769407	C	0.288	1.19 (0.94-1.50)	0.160	0.236	1.27 (1.05-1.53)	0.008	1.23 (1.07-1.43)	0.005
rs3791851	G	0.308	1.13 (0.91-1.40)	0.264	0.238	1.24 (1.02-1.49)	0.013	1.19 (1.03-1.37)	0.019
rs3791850	C	0.761	0.98 (0.77-1.24)	0.878	0.749	1.41 (1.15-1.72)	0.0006	1.21 (1.04-1.41)	0.014
rs2241165-rs769407-rs3791851-rs3791850	A-C-G-C	0.221	1.27 (1.00-1.61)	0.044	0.200	1.32 (1.08-1.62)	0.007	1.30 (1.11-1.52)	0.0009

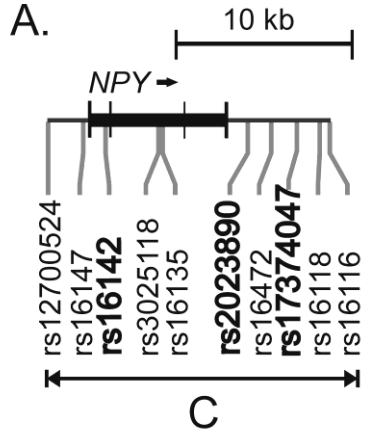
CI = confidence interval; GAD = generalized anxiety disorder; *GAD1* = glutamate decarboxylase 1; MDD = major depressive disorder; OR = odds ratio; PD = panic disorder; VATSPSUD = Virginia Adult Twin Study of Psychiatric and Substance Use Disorders

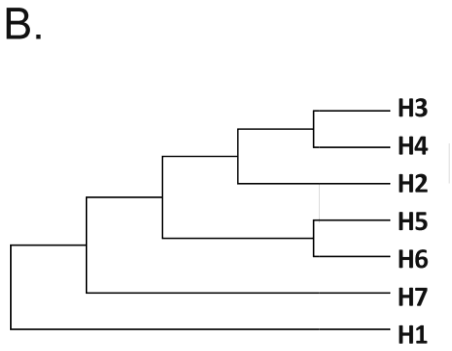
[a] 282 cases with PD, GAD, social phobia, agoraphobia and phobia not otherwise specified, and 575 controls

[b] 589 cases and 539 controls scoring at the extremes of a genetic risk factor reflecting shared susceptibility to MDD, GAD, PD, agoraphobia, social phobia, and neuroticism

Anxiety-predisposing effects of NPY are conditional on early life stress exposure

Of all examined genes in the Health 2000 sample, only *NPY* showed solid gene x environment interaction effects. Three SNPs in this gene, representing two phylogenetically related risk haplotypes hereafter referred to as H3 and H4 (Figure 21), modulated the effect of childhood adverse life events on susceptibility to anxiety disorders ($P \leq 0.01$; Table 20 and Figure 22). The most significant GxE effect was observed when analysing the related H3 and H4 jointly to increase subgroup sample size, thus comparing subjects with any other haplotype configuration to H3/- or H4/- heterozygotes, and a pooled group of H3/H3, H4/H4 homozygotes and H3/H4 compound heterozygotes ($b = 0.47$, SE = 0.16, $P = 0.003$; Figure 22F). Among subjects who had experienced at least two childhood adversities, the H3 or H4 heterozygotes had a 3.76-fold risk for an anxiety disorder compared to subjects with other haplotype configurations, whereas the corresponding risk for individuals homozygous for H3 or H4, or compound heterozygotes, was 6.00-fold.





C.

	rs12700524	rs16147	rs16142	rs3025118	rs16135	rs2023890	rs16472	rs17374047	rs16118	rs16116	Frequency in controls
H3	A	T	C	C	C	T	C	G	G	C	0.113
H4	A	T	C	C	C	T	C	A	G	C	0.113
H2	G	T	T	C	C	C	C	A	G	C	0.125
H5	A	T	T	A	C	C	T	A	C	C	0.068
H6	A	T	T	C	T	C	T	A	C	C	0.055
H7	A	C	T	C	T	T	C	A	C	C	0.050
H1	A	C	T	C	C	T	C	A	C	T	0.468

Figure 21 Genomic structure of *NPY*, and haplotype phylogeny and structure of the locus in the Finnish Health 2000 study sample. (A) Genomic structure of *NPY* and positions of the analyzed SNPs. SNPs showing gene x environment (GxE) interaction effects with childhood adversities in influencing anxiety susceptibility ($P \leq 0.01$) are highlighted in bold. (B) Maximum parsimony-based phylogeny analysis for the seven haplotypes spanning the *NPY* locus (designated H1 – H7, in order of population frequency). Particular support was obtained for relatedness of H3 and H4, and H5 and H6. (C) Detailed structure of the locus-spanning haplotype block. Risk alleles of individual SNPs showing GxE interactions are shaded grey. Only one haplotype (H3) carries all three risk alleles, whereas its closest relative H4 carries two of them. The span of the haplotype block is shown in (A).

Table 20. ***Logistic regression analysis of gene x environment interactions between NPY and childhood adversities in influencing susceptibility to anxiety disorders.*** *Results shown for SNPs and haplotypes with interaction effects with $P \leq 0.01$.*

	Childhood adversities			SNP genotype/ haplotype copy			Childhood adversities x SNP genotype		
SNP/haplotype	***b***	**SE**	**P-value**	***b***	**SE**	**P-value**	***b***	**SE**	**P-value**
rs16142	0.498	0.111	7.22×10^{-6}	-0.333	0.206	0.105	0.427	0.151	0.005
rs2023890	0.903	0.119	3.42×10^{-14}	0.321	0.204	0.115	-0.427	0.148	0.004
rs17374047	0.580	0.101	8.87×10^{-9}	-0.246	0.263	0.351	0.519	0.201	0.010
H3	0.584	0.102	1.10×10^{-8}	-0.344	0.279	0.218	0.586	0.210	0.005
H4	0.657	0.102	1.24×10^{-10}	-0.267	0.289	0.355	0.239	0.211	0.258
H3+H4	0.504	0.114	9.22×10^{-6}	-0.341	0.212	0.108	0.468	0.156	0.003

b = regression coefficient; SE = standard error of *b*

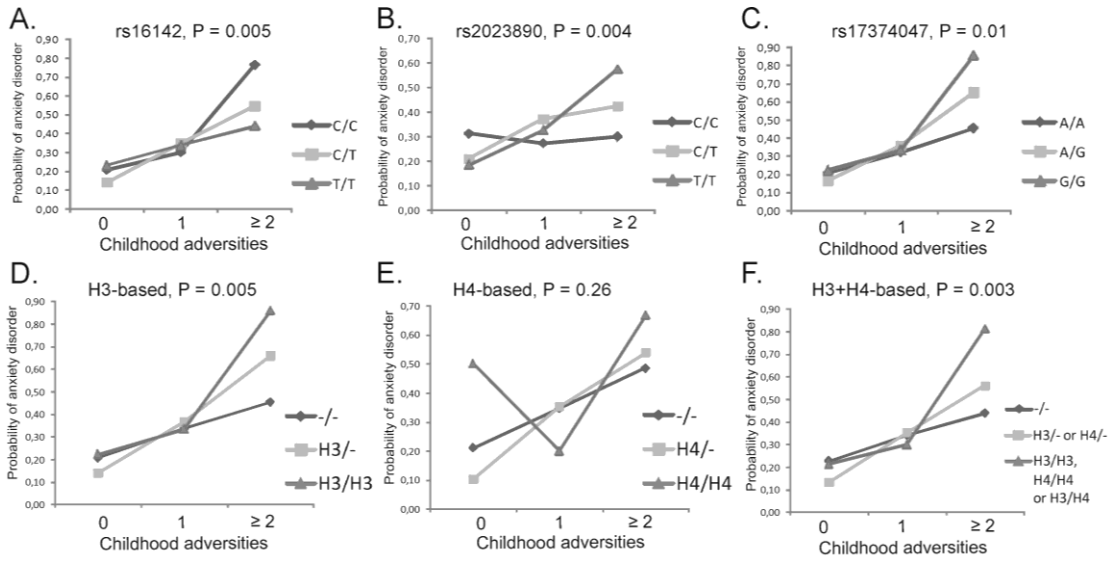


Figure 22 Probability of anxiety disorder diagnosis as a function of the number experienced childhood adversities, and *NPY* genotype. (A-C) Three SNPs in *NPY* showed significant effects (P ≤ 0.01) in logistic regression analysis of gene x environment (GxE) interactions. (D-F) Haplotype copy number-based analyses of two phylogenetically related haplotypes, H3 and H4 (as designated in Figure 21), supported the individual SNP findings. Haplotype H3 was tagged by rs17374047 (C) and thus yielded a similar result. Analysis of H4 alone was non-significant, while pooled analysis of H3 and H4 to achieve the largest subgroup sample size possible yielded the most significant observed GxE interaction. Sample sizes for the subcategories of experienced childhood adversities were: 0 (N = 360), 1 (N = 216), and ≥ 2 (N = 281).

Summary of results

Although the most significant findings in the *GAD1* gene were obtained with a phobia phenotype, it also appeared to contribute to susceptibility to internalizing disorders in a broad sense. These effects are mainly due to to one specific common risk haplotype. We also observed that genetic variation in *NPY*, attributable to two related risk haplotypes, interacts with childhood adverse life events to influence susceptibility to anxiety disorders. No main effects of *NPY* SNP genotype or haplotype were observed, indicating that its anxiety-predisposing effects are conditional on childhood stress exposure in our sample.

6 DISCUSSION

6.1 GENERAL IMPLICATIONS

The work presented in this study has lead to the identification of altogether ten potential susceptibility genes for anxiety disorders at the $P \leq 0.01$ significance level (Table 21). For seven of the ten implicated genes, our findings can be considered the first report of involvement in human anxiety. The identified genes encode proteins representing four different main categories: four enzymes catalyzing biochemical reactions important for brain functioning (ALAD, GAD1, PSAP and PTGDS), two neuropeptides and one neuropeptide receptor (NPY, NPS and NPSR1), two structural proteins involved in neuronal morphology and signaling (CDH2 and EPB41L4A) and a regulator of dynein complex assembly involved in axonal retrograde transport (DYNLL2). Although the 30 genes examined throughout this study naturally only represent a biased subset of all human genes, these findings illustrate the genetic and functional heterogeneity that likely underlies anxiety disorders.

The multiple testing problem and defining a true finding

Upfront, it should be acknowledged that some of the findings likely are statistical type I error false positives caused by limited sample size of the analyzed anxiety disorder subdiagnosis groups, in combination with the multiple statistical tests performed. We chose a permutation-based approach to correct for small sample deviations from chi-square assumptions in the association analyses. This should reduce the chance for spurious genetic associations. In addition, we explored the use of multiple testing corrections based on the total number of performed statistical tests (Bonferroni-correction; Bonferroni, 1932), or less conservatively, based on the actual efficient number of statistical tests as our SNPs were typically in LD and therefore not independent (Nyholt, 2004). However, it became clear that the penalty conferred by testing a large number of SNPs in multiple phenotypes is such that no multiple testing correction method would support the findings. Corrections based on the concept of false discovery rate (FDR; the rate by which significant features at a given threshold are truly false) are also advocated by many as proving a good and easily interpreted balance between the number of true and false positive findings (Storey and Tibshirani, 2003). We did not perform FDR-analyses, but they could have been helpful for critical interpretation of our findings. Unfortunately, there is no gold standard methodology for multiple testing correction in datasets like ours, and the development of such standard guidelines is one of the current major challenges of genetic epidemiology. Even the question on what an

appropriate threshold for a genome-wide significant finding is remains somewhat unresolved, but the order of magnitude for Western populations seems to be in the range of 5-7 x 10^{-8} (Dudbridge and Gusnanto, 2008; Risch and Merikangas, 1996). Some argue that almost equally stringent criteria should be applied also in candidate gene studies by correcting for all the statistical tests that were not performed in reality – after all, had we not discovered anything, we would have moved on to another genomic position to look further (Joseph D. Terwilliger, personal communication).

Table 21. ***Summary of genes showing evidence for association (P ≤ 0.01) with anxiety disorders.***

Gene symbol	Gene name	Function	Most significant P-value	Phenotype
GAD1	glutamate decarboxylase 1 (brain, 67kDa)	Enzyme synthesizing GABA	0.0005[a]	Phobias (social phobia, agoraphobia, phobia NOS)
ALAD	aminolevulinate, delta-, dehydratase	Enzyme of heme metabolism	0.0009[b]	Social phobia, any anxiety disorder
CDH2	cadherin-2, type 1, N-cadherin (neuronal)	Neural cell-cell adhesion	0.001[b]	Social phobia
NPSR1	neuropeptide S receptor 1	Receptor for NPS	0.001[a]	PD
NPY	neuropeptide Y	Regulation of emotional homeostasis, stress coping, cognitive processes, food- and ethanol intake and sexual behavior	0.003[b]	Any anxiety disorder (GxE interaction with childhood adversities)
PSAP	prosaposin (variant Gaucher disease and metachromatic leukodystrophy)	Neurotrophic factor, repair of neural injury and enzyme for hydrolysis of sphingolipids	0.004[a]	PD, any anxiety disorder
EPB41L4A	erythrocyte membrane protein band 4.1 like 4A	Interactions between plasma membrane and cytoskeleton	0.008[a]	Social phobia, GAD, PD
NPS	neuropeptide S	Regulation of arousal and anxiety, food and ethanol intake and immunological phenotypes	0.007[a]	PD
DYNLL2	dynein, light chain, LC8-type 2	Axonal retrograde transport, dynein complex assembly	0.009[b]	GAD
PTGDS	prostaglandin D2 synthase 21kDa (brain)	Enzyme for synthesis of prostaglandin D2, a neuromodulator and neurotrophic factor	0.01[a]	GAD

GxE = gene x environment; GABA = gamma-aminobutyric acid; GAD = generalized anxiety disorder; NOS = not otherwise specified; PD = panic disorder

[a] Pointwise

[b] Haplotype-based

It is clear based on the above that the threshold we chose as a criterion for evidence for association ($P \leq 0.01$) is an arbitrary one, used primarily to identify the most relevant SNPs or genes from a larger dataset. Therefore, extreme caution is needed in interpreteting them as true findings. The best way to obtain further confidence in the findings would be to replicate them in independent samples and preferably in the strictest sense (same allele, same phenotype). Moreover, the many hypotheses and questions that the genetic variation showing evidence for association raise should be experimentally evaluated. For instance, among the associated variation are synonymous SNPs, non-synonymous SNP, SNPs in promoter regions, SNPs in 3'-UTRs, intronic SNPs that potentially influence transcription factor binding and haplotypes spanning the entire loci of the investigated genes. Functional experiments could be designed to evalute the effects of these alleles on gene expression, and protein structure and function, *in vitro* and *in vivo*. Genetic imaging studies to assess whether the identified genetic variants influence responses in brain regions relevant for anxiety would be an important addition. Finally, transgenic animal models with either overexpression or silencing of the identified genes, preferably in relevant brain regions, would be important for understanding their potential impact on behavior.

To summarize, a combination of replication and functional approaches is needed before our gene findings can be considered true. Encouragingly, for some of our top candidate genes (e.g., *GAD1, NPY*, *NPS* and *NPSR1*) there is already published functional evidence from various sources that supports their role in modulating the same phenotypes that we found associated with the genes. These specific findings are discussed in sections to come.

Common versus rare variants in anxiety disorder susceptibility

We identified 15 risk variants showing evidence for association with $P \leq 0.01$ in pointwise SNP tests. Of these, 13 can be considered common (frequency > 0.05) and 2 rare (frequency < 0.05). The average OR was 1.79 for the common risk variants, and 3.15 for the rare variants. This is a sensible result, as we had very limited statistical power to detect variants with smaller effects with the sample sizes available to us. Moreover, being common was a selection criterion for many of the investigated SNPs (i.e., all tagSNPs). The ORs and risk allele frequencies observed by us are also in line with those reported in other candidate gene studies of anxiety disorders (e.g., Hohoff et al., 2010; Hettema et al., 2008; Wray et al., 2007). Our limited data from a few candidate genes does not allow for any conclusions regarding whether the CD-CV, or the CD-RV hypothesis is more appropriate for explaining genetic susceptibility to anxiety disorders. The most likely answer is that anxiety, as other complex diseases, is influenced by a spectrum of allelic variation that includes both common and rare variants (Smoller, 2011; Bodmer and Bonilla, 2008). One factor that could influence this allelic spectrum, and in which subjects with anxiety disorders differ from patients

with some other psychiatric diseases, is that they may not have as dramatically reduced fecundity as in e.g. schizophrenia or autism (Bundy et al., 2011; Reichenberg et al., 2006). This would imply less negative selection keeping frequencies of anxiety risk variants low in the population.

Shared or disorder-specific genetic susceptibility and the phenotype problem

Whether the clinical DSM-IV grouping of anxiety disorders accurately reflects their underlying biology and genetics is a matter of ongoing debate. The best answer is "yes and no", as on one hand DSM-IV defined anxiety disorders exhibit familial aggregation and are heritable entities (Smoller et al., 2008a). On the other hand, the genetic boundaries between disorders are blurry and anxiety-proness phenotypes transcend the categorical DSM-IV boundaries. Multivariate structural equation models based on a large twin sample suggest that there are both genetic susceptibility factors that are shared between many anxiety disorders, and disorder-specific factors (Hettema et al., 2005). In this thesis work, we had the opportunity to examine both a pooled sample of many anxiety disorders, as well as some of the most common anxiety disorders separately. Most of the associations discovered were to specific anxiety disorder diagnoses rather than to the group of all anxiety disorders pooled, supporting the existence of disorder-specific risk factors. This is somewhat surprising, as we clearly had the largest power to detect associations in the pooled sample. A likely explanation is that the "any anxiety disorder" group we used in the analyses was too phenotypically heterogeneous to be optimal for detection of susceptibility genes.

It remains unresolved what the optimal way would be to pool different types of anxiety disorders together to obtain the larger sample sizes needed to identify genetic risk variants with small effects. When studying a complex phenotype, a compromise between too modest subgroup sample size and excess phenotypic heterogeneity is necessary. In this thesis, we tried to extend subgroup sample sizes by both pooling anxiety disorders based on symptom level similarity, and by using extended anxiety disorder definitions (e.g., including subjects with DSM-IV subthreshold diagnoses). Grouping disorders with phobic symptoms (social phobia, agoraphobia, phobia NOS) yielded the most significant observed association of the study, namely for *GAD1*. Symptom level classification might thus be a good option for future biological and genetic studies of anxiety disorders, as the diagnostic classifications were in fact primarily made for clinical grouping purposes.

On the other hand, we had hypothesized that DSM-IV subthreshold cases, showing some signs of anxiety symptoms but not sufficiently for a full diagnosis, share the same underlying genetic susceptibility to anxiety as core cases. In our data, it was clearly not universally true that inclusion of the subthreshold subjects made associations stronger. This could reflect the

blurry line between what is considered “normal” and what “pathological” anxiety, and the difficulty of where to place a subject with a subtreshold diagnosis in that phenotypic spectrum.

To summarize, both susceptibility gene identification attempts using clinical diagnosis entities of anxiety, as well as attempts using phenotypes that transcend DSM-IV boundaries, likely provide clues about the mechanisms underlying anxiety-like behavior.

Implications due to inherent characteristics of the Health 2000 sample

The discovery sample featured in all studies of this thesis, the Health 2000, has several characteristics that should be considered when interpreting the results. For instance, the study did not actively exclude individuals of foreign descent. This raises the question whether population stratification may be a source of spurious associations. We do not believe that this is the case, as the Finnish population in general is genetically homogeneous and only 2% of it was of foreign descent at the time of the study, and proficiency in Finnish was a prerequisite for a successful mental health interview.

It is notable that the methodology of the Health 2000 was not designed to assess lifetime diagnoses of anxiety disorders, and we have therefore likely not identified subjects that are in remission. Likewise, we have missed subjects with OCD and PTSD diagnoses as these phenotypes were not assessed. Also, the fact that dropouts from the mental health interview had personality inventory scores indicating poorer psychosocial functioning suggests that some subjects with more severe forms of anxiety disorders may inadvertently have been excluded from the study. The consequence of all the exlusions above is likely reduced signal from true findings and therefore lower power to detect them.

Finally, the availability of a measure of experienced childhood stress is one considerable asset of the Health 2000 sample. Its limitation is its self-reported nature. It is possible that anxiety disorder subjects recall their childhood in a different light than healthy individuals, seeking an explanation for their symptoms. The large age range of the study subjects (30-87 years) also makes it possible that there is a cohort effect present in the sample. A further limitation is that some severe stressors, such as childhood abuse and parental death were not assessed. However, such traumatic events may represent strong individual triggers of anxiety disorders on their own. The events we did assess are relatively more mildly linked causally to the onset of anxiety disorders, and therefore perhaps even better suited for the analysis of additive effects in GxE interactions.

In the following sections, I will focus on some of the more specific conclusions and implications that result from the data presented in this thesis. I will also highlight biological mechanisms that could explain how the identified susceptibility genes might influence anxiety.

6.2 A CROSS-SPECIES APPROACH FOR IDENTIFICATION OF ANXIETY-PREDISPOSING GENES

Traditional selection of candidate genes for influencing a trait, which are then experimentally evaluated, is typically based on earlier linkage information and/or prior presumptions about the physiology and molecular biology that underlies the trait of interest. Although such selections are scientifically sound, and have lead to the identification of genetic variation relevant for disease susceptiblity, the limitation is that this may lead to an "information bottleneck" (Zhu and Zhao, 2007) that hampers understanding of the biology of the studied trait. This may be particularly relevant in psychiatric disorders, where the underlying molecular and genetic mechanisms are extremely complex in nature, and we acknowledge that our knowledge of them is incomplete. Based on prior assumptions, most candidate gene studies in anxiety disorders have examined genes involded in neurotransmitter metabolism and signaling, genes encoding proteins targeted by anxiolytics, and genes involved in stress response.

With current technology, there are now ways to circumvent information bottlenecks. Global hypothesis-free approaches for the examination of whole genomes, transcriptomes, or proteomes in disease susceptibility have become available. Cross-species approaches using such methodological tools in animal models, and subsequently applying the gained knowledge to the study of human disease phenotypes may be particularly beneficial. The benefits of cross-species approaches here is that animal models provide access to sample material from the tissue most relevant for the disease for e.g. functional genomics studies. Moreover, a human complex trait such as anxiety is influenced by marked genetic and environmental heterogeneity. Both of these can be minimized when studying the trait in animals: genetic heterogeneity by using inbred animals that are genetic clones, and environmental exposures by equalizing conditions across animals. The main limitation of cross-species approaches is that the animal findings may not translate directly to the human disease due to the species difference. However, an evolutionarily strongly conserved phenotype, such as anxiety-related behavior, for which pharmacologically validated animal paradigms also exist, may be particularly suitable for cross-species studies.

In study I of this thesis, we carried out an investigation in which candidate gene selection was based on a cross-species approach, unbiased by previous knowledge. Of 13 genes differentially expressed between anxious and non-anxious mice (Hovatta et al., 2005), six showed some evidence for association with anxiety disorders at $P \leq 0.01$. These findings necessarily require replication in other independent samples to be appropriately evaluated, as our study is the first one to directly link the identified genes

(*ALAD*, *CDH2*, *DYNLL2*, *EPB41L4A*, *PSAP* and *PTGDS*) to anxiety phenotypes. Functionally, all of them can be imagined to be very important genes for proper functioning of the brain (Table 21, page 104). However, the most important consequence of study I has been to further narrow down the list of interesting candidate genes derived from a gene expression study in mice to a subset that may be most relevant for human anxiety. This will in the future lead to prioritization of the six top candidate genes for functional studies that aim to unravel their mechanistic link to anxiety.

Others have also taken advantage of a variety of cross-species approaches for the identification of anxiety candidate genes. There have been one human linkage study and a few association studies that have examined human chromosomal loci syntenic to murine QTLs for anxiety (Fullerton et al., 2007; Smoller et al., 2001; Smoller et al., 2001). The linkage study provided suggestive evidence for linkage (LOD ≥ 1.9) with PD/agoraphobia or anxiety proness on 10q, 12q13 and 1q (Smoller et al., 2001). A family-based association analysis of behavioral inhibition examined four candidate genes (*GAD2* [glutamate decarboxylase 2, the adenosine receptor encoding genes *ADORA1* and *ADORA2A*, and *PENK* [preproenkephalin]), selected based on murine QTL-data or functional evidence from mouse models with features of the phenotype (Smoller et al., 2001). The only gene that showed suggestive evidence for association (P = 0.05) was *GAD2*, a GABA synthesizing enzyme. However, the most promising candidate gene to have emerged from cross-species approaches is *RGS2*, that was originally identified from a murine QTL for emotionality (Yalcin et al., 2004). The gene has now been associated with human PD, PTSD and behavioral inhibiton (Otowa et al., 2011; Amstadter et al., 2009; Smoller et al., 2008b). A genetic imaging study has demonstrated that a SNP in *RGS2* has a strong effect on amygdala and insular cortex activation in response to emotional faces (Smoller et al., 2008b), and studies addressing the functional link between anxiety and *RGS2* are ongoing (e.g., Salim et al., 2011).

Among other animal-model based approaches for identification of anxiety susceptibility factors are transcription profiling, proteomics and metabolomics experiments in mouse strains selectively bred for high- or low anxiety-related behavior. These have already resulted in the identification of several potential biomarkers for anxiety, with *Glo1* expression and protein levels emerging as one prime candidate (Czibere et al., 2011; Filiou et al., 2011; Hovatta et al., 2005; Krömer et al., 2005). So far, attempts to establish relevance for findings from these global profiles in human anxiety disorders have been limited. This should be one focus of future research, and in the present study we add to this field by reporting suggestive associations ($0.01 < P < 0.05$) between SNPs in *GLO1* and "any anxiety disorder".

The availability of genome-wide genotype data and different types of global functional profiles are now spurring large-scale integrative approaches that combine animal and human datasets with the aim to identify the most

relevant genes and functional pathways for a trait. In anxiety disorders, three notable recent studies were carried out.

The first study used a pharmacogenomic mouse model, in which global gene expression changes in response to treatment with an anxiogenic (yohimbine) or an anxiolytic (diazepam) drug where evaluated in anxiety-regulating brain regions and in blood (Le-Niculescu et al., 2011). The new data was integrated with published human (association/linkage findings, expression evidence) and animal data (expression evidence, QTLs, data from transgenics) in a translational strategy for cross-matching and prioritizing findings. The identified top set of anxiety-relevant pathways included cAMP-, glucocorticoid receptor-, and CRH-signaling. Interestingly, *PTGDS* and *DYNLL2*, linked to anxiety by our human association findings and gene expression evidence, emerged among the top candidate genes.

In another study, a systems biological approach was used to identify molecular pathways that are stably enriched in anxiety- and depression-related phenotypes (Gormanns et al., 2011). For this purpose, the authors combined data from a large number of publicly available human and mouse phenomes and transcriptomes, and reported that the most significantly dysregulated pathways in anxiety were related to carbohydrate metabolism, tight junction signaling and phosphatidylinositol signaling.

Finally, a third recent study systematically combined genome-wide rodent and human data to select a set of candidate genes for association testing in a large human sample (Hettema et al., 2011). First, a GWA analysis was used to identify QTLs for fear-related behavior in heterogeneous stock mice. Findings were priority-ranked based on murine linkage and knockout studies, a meta-analysis of human linkage scans and a human GWA of anxiety. The top-ranked regions were finally examined in subjects with anxiety disorders, high neuroticism and MDD. Evidence for association was found in *PPARGC1A* (peroxisome proliferator-activated receptor gamma coactivator 1alpha), which plays a role in energy expenditure and neuroprotection. *Ppargc1a*$^{-/-}$ mice show GABAergic dysfunction, making the gene a promising novel candidate for modulating anxiety-related traits.

To summarize, global hypothesis-free cross-species approaches can give important clues about regulatory pathways important for anxiety. The present study examined murine candidate genes, selected based on gene expression profiling in brain regions of inbred mouse strains, in a human sample. We implicated novel candidate genes for anxiety disorders beyond the usual suspect genes directly involved in neurotransmitter metabolism and signalling or stress response. Ours, and other studies, suggest that cross-species approaches represent a potentially effective strategy to identify molecular pathways that influence a trait.

6.3 THE NEUROPEPTIDE S SYSTEM IN PANIC DISORDER

Our interest in the genes of the neuropeptide S signaling system, *NPS* and *NPSR1,* arose due to the link between the G-protein coupled receptor NPSR1 and asthma predisposition (Melen et al., 2005; Laitinen et al., 2004), accompanied by epidemiological comorbidity between asthma and anxiety (Roy-Byrne et al., 2008; Goodwin, 2003). As there was also rodent evidence that NPS, the 20 amino acid neuropeptide ligand of NPSR1, has a unique behavioral profile of inducing anxiolysis while increasing wakefulness and arousal (Xu et al., 2004), we aimed to examine whether the system represents a biological link between anxiety and asthma.

We confirmed that asthma is comorbid with anxiety in adults of the Health 2000 sample, and children of the BAMSE study. Several explanations for such a comorbidity have been proposed, e.g., that increased anxiety is due to living with a chronic potentially life threatening condition like asthma, shared respiratory abnormalities, misinterpretation of asthma symptoms as panic attacks, shared genetic susceptibility, or anxiogenic effects of asthma medication (Goodwin et al., 2003). Agoraphobia was the only specific anxiety disorder that co-occurred with asthma in our study. A specific asthma-agoraphobia link was previously seen in adolescents, and suggested to be due to fear conditioning by previous asthma episodes in a public place, or parental overprotection of a child with asthma (Katon et al., 2007). Unfortunately, no longitudinal data was available to us, and we could not assess whether asthma or agoraphobia was the primary diagnosis in our subjects. A bidirectional link between the two disorders could have given more support to the theory that they share some biological risk factors.

The genetic part of our study supported the hypothesis that the NPS-NPSR1 system could influence susceptibility to not only asthma, but also anxiety. Both *NPSR1* and *NPS* associated with PD diagnosis, while *NPSR1* also associated with parent-reported anxiety/depression in children. It is particularly noteworthy that we unbiasedly set out to investigate several subtypes of anxiety disorders, and then observed the most significant findings with PD. First, *NPSR1* is located under one of the few PD linkage peaks (7p14-15) seen in more than one sample (Crowe et al., 2001; Knowles et al., 1998). Second, of the anxiety disorders, PD is the one most frequently comorbid with asthma (Katon et al., 2004; Goodwin et al., 2003). PD and asthma also share symptoms related to respiratory function, such as sensations of choking or suffocation and shortness of breath caused by hyperventilation (Zaubler and Katon, 1996). Intriguingly, it was shown that NPS-signaling regulates respiration via a CNS-mediated pathway, as central NPS increased respiratory rate while decreasing tidal volume in mice (Zhu et al., 2011). Such changes are hallmarks of the fight-or-flight response and

could cause the sensations of shortness of breath seen in asthma- and panic attacks. However, the *NPSR1* haplotypes that we found associated with PD were not the same ones that had been previously associated with asthma (Kormann et al., 2005; Melen et al., 2005; Laitinen et al., 2004), at least not in the part of the gene that had been examined in all studies. Therefore, we cannot directly conclude that asthma and anxiety share the same genetic susceptibility in a strict sense, although our results implicate a neuropeptide system that clearly could have biological relevance for both respiratory abnormalities and anxiety responses.

In parallel with our work, there was rapid progress in characterizing the physiological and neurobiological functions of the NPS-system (Guerrini et al., 2010; Pape et al., 2010; Reinscheid, 2008). Although NPS administration also has other behavioral consequences, such as reward-like effects (Cao et al., 2011), enhanced long-term memory (Okamura et al., 2011), and decreased food- and ethanol intake (Badia-Elder et al., 2008; Smith et al., 2006), a major role for it in fear processing is supported. Neuroanatomically, *Nps* and its only known receptor *Npsr1* are expressed in brain regions relevant for anxiety (Xu et al., 2007; Xu et al., 2004). Neural circuitry responsible for NPS action was identified, demonstrating that NPS-signaling modulates afferent and intrinsic glutamatergic and GABAergic transmission in the amygdala (Jungling et al., 2008; Meis et al., 2008). Amygdalar NPS injections block fear-potentiated startle responses and accelerate fear extinction (Fendt et al., 2010; Jungling et al., 2008). NPS also stimulates HPA-axis activity, increasing ACTH and corticosterone levels (Smith et al., 2006). Behavioral studies of *Npsr1*$^{-/-}$ mice have so far yielded inconsistent results, with mice on a 129S-background showing some signs of increased anxiety-like behavior (Duangdao et al., 2009), while mice on the C57BL/6 background show little or no anxiety-related alterations (Fendt et al., 2011). We have thus far not evaluated the anxiety-like behavior of our genetically heterogeneous *Npsr1*$^{-/-}$ mice, but we found that they differ from wild types regarding the induction of two stress-related genes, *Il1b* and *Ntf3*. Upregulation of the cytokine IL1B after stress may reflect an increased proinflammatory response due to lack of protective NPS-NPSR1 signaling. Lack of induction of the neurotrophic factor NTF3 suggests that *Npsr1*$^{-/-}$ mice have reduced capability to maintain neuroplasticity and brain integrity under stress. A further important function of NPS signaling may thus be to activate molecular pathways that counteract stress-induced brain damage.

A number of particularly exciting recent human genetic studies directly support the associations we observed between the NPS-NPSR1 system and PD. Much research was conducted on a functional SNP in *NPSR1* (rs324981; A/T, Asn107Ile), of which the T-allele (Ile) increases *NPSR1* expression and the sensitivity of the receptor for NPS by about 10-fold, leading to more active NPS-signaling (Bernier et al., 2006; Reinscheid et al., 2005). In a study concurrent with ours, the T-allele was associated with PD in two German case-control samples (Domschke et al., 2011). The same allele was

also associated with the dimensional trait anxiety sensitivity in patients. Healthy volunteers carrying the T-allele of rs324981 also evaluate their fear reactions to a conditioned stimulus predicting an electrical shock as stronger than A/A homozygotes (Raczka et al., 2010). We observed association between only one of three haplotypes carrying the T-allele and PD, whereas the variant did not show any evidence for association in pointwise analyses. A possible reason for this is that there may be other modulating variants in LD with rs324981 in the Finnish population that are also functionally important.

Behavioral tests and functional magnetic resonance imaging (fMRI) studies are now also shedding light on how rs324981 could influence anxiety phenotypes. PD patients carrying the T-allele have increased heart rate and anxiety symptom intensity during a stressful behavioral avoidance test (Domschke et al., 2011). T-allele carriers also show decreased activation of cortical regions involved in cognitive processing of threat-related stimuli (the dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate cortex), and increased responsiveness of the basolateral amygdala, during emotional processing of fearful faces (Dannlowski et al., 2011; Domschke et al., 2011). In addition, they have increased activation of the rostral dorsomedial prefrontal cortex (involved in conscious assessment of threat) in response to conditioned stimuli predicting pain (Raczka et al., 2010). Taken together, we are now beginning to understand the neurocircuitry and autonomous responses that are influenced by genetic variation in *NPSR1*.

All of the above supports that genetic variation in the NPS-NPSR1 system could influence its function and PD susceptibility. These effects are likely mediated by hyperresponsiveness of the amygdala, overinterpretation and distorted processing of stimuli that predict threat, and increased autonomic arousal and HPA-axis activity (Figure 23). Individuals that are genetically susceptible to increased or more sensitive NPS-signaling may have an innate tendency for increased arousal, and for cognitively misinterpreting responses to arousing or aversive stimuli (e.g., shortness of breath, palpitations) or situations/places in which such symptoms have occurred, as predicting threat (Dannlowski et al., 2011; Domschke et al., 2011; Raczka et al., 2010). This may lead to a vicious cycle of anxious arousal that manifests as a panic attack in a susceptible individual. The theory that increased NPS-signaling is associated with PD might at first appear contradictory with the anxiolytic yet arousal-provoking effects of NPS administration in rodents (Xu et al., 2004). However, PD is to large extent thought to be caused by a state of increased arousal (Blechert et al., 2007a). High NPS levels early in development could have detrimental effects for shaping neuroendocrine responses and later anxiety predisposition, while a dose or increase of NPS later in life could primarily have beneficial anxiolytic effects (Dannlowski et al., 2011). Our data suggests that some of the *NPSR1* SNPs most significantly associated with PD alter transcription factor binding and thereby possibly influence *NPSR1* expression. Such alterations represent one further possible

mechanism for innately more active NPS-signaling, in addition to the functional Asn107Ile polymorphism described above.

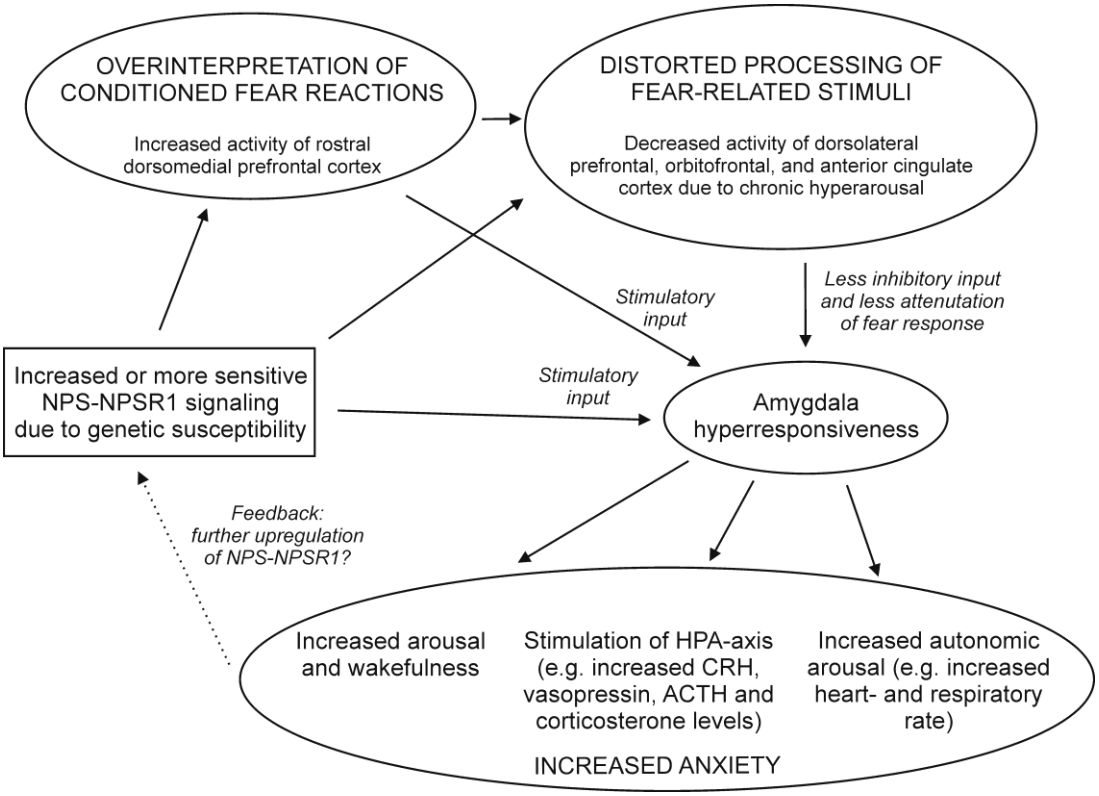


Figure 23 Hypothetical model of how genetic susceptibility to increased or more sensitive NPS-NPSR1 signaling could increase susceptibility to panic disorder. ACTH = adrenocorticotrophic hormone; CRH = corticotrophin-releasing hormone; HPA = hypothalamus-pituitary-adrenal; NPS = neuropeptide S; NPSR1 = neuropeptide S receptor 1

In summary, we implicated the NPS system in predisposition to PD. Concurrent and subsequent studies have supported our findings, and a wide variety of functional evidence further suggests that NPS-NPSR1 signaling plays a role in mediating anxiety predisposition. The possibility of using theurapeutic agents that target the system in treatment of anxiety disorders should be explored. Several synthetic antagonists of NPSR1 were already identified (Dal Ben et al., 2011). In rats, intranasal application of NPS has anxiolytic effects, and such a mode of application of the peptide could be feasible also in humans (Lukas and Neumann, 2012). However, at least two major problems need to be addressed first: 1) Given the widespread functions of the NPS-NPSR1 system, one would except a range of undesired side effects related to e.g. increased autonomic and behavioral arousal or apetite; 2) NPS could be a potent anxiolytic in subjects without a history of pathological anxiety, but would NPS administration actually rather trigger panic attacks by increasing arousal in sensitized PD patients?

6.4 REPLICATION OF GLUTAMATE DECARBOXYLASE 1 FINDINGS IN THE FINNISH POPULATION

In study III, we examined 15 putative susceptibility genes for anxiety, selected based on some of the most relevant findings from human samples, for association to anxiety disorders in the Finnish population. The only gene that showed evidence for association with $P \leq 0.01$ was *GAD1* in the analysis of phobias (social phobia, agoraphobia and phobia NOS). One specific locus-spanning *GAD*1 risk haplotype (out of six common haplotypes present in the population) increased risk for phobias by about 1.3-fold. Interestingly, the same haplotype was previously associated with genetic susceptibility shared by anxiety disorders, MD and neuroticism in an American sample from Virginia (Hettema et al., 2006); VATSPSUD. Of the specific anxiety disorder diagnoses in the VATSPSUD, the haplotype showed suggestive association with agoraphobia, supporting our observations in subjects with phobias. Although our finding cannot be considered as a replication by its strictest definition (same allele, same phenotype) as similar phenotype measurements were not available for the Health 2000 and VATSPSUD, we provide the first independent support for *GAD1* as a susceptibility gene for anxiety disorders. The fact that the same haplotype has now been associated with anxiety in two samples supports that it has true biological relevance.

By combining effects observed in the Finnish and American samples with a meta-analytic approach, we showed that the *GAD1* risk haplotype also associated with a broad phenotype reflecting shared genetic susceptibility across internalizing disorders and neuroticism. The subjects included in the broad phenotype had PD, GAD, social phobia, agoraphobia, MDD (in the Health 2000 only as comorbid with anxiety disorders), neuroticism (assessed only in the VATSPSUD) and phobia NOS (assessed only in Health 2000). Thus, although *GAD1* may particularly strongly modulate susceptibility to phobias, an observable effect remained when considering anxiety and mood phenotypes as a whole. Therefore, *GAD1* might be an example of a risk factor that is shared by multiple psychiatric disorders and anxious personality (see Figure 5, page 36). This would not be surprising, given the widespread and important role of the enzyme encoded by the gene in the CNS.

The GAD enzyme synthesizes GABA from glutamate, and is essential for the balance between the main excitatory neurotransmitter (glutamate) and the main inhibitory neurotransmitter (GABA) of the CNS. In general, there is a large body of evidence supporting a role for GABAergic neurotransmission in modulation of anxiety. Boosting GABA-signaling with agonists of the $GABA_A$-receptor (such as benzodiazepines) has anxiolytic effects, while attenuating it with inverse agonists (such as FG7142) has anxiogenic effects (Durant et al., 2010). Reduced GABA levels were seen in at least some brain regions of PD and social phobia patients (Pollack et al., 2008; Chang et al.,

2003). In social phobia, these levels were normalized after pharmacological treatment (Pollack et al., 2008). It was proposed that a disturbance in GABA metabolism is present in PD, and that it may be a consequence of GAD enzyme dysfunction (Goddard et al., 2004).

The two isoforms of the GAD enzyme, GAD67 and GAD65, are encoded by two separate genes (*GAD1* and *GAD2*, respectively). GAD67 is found throughout neurons while GAD65 is mainly found in axon terminals (Kaufman et al., 1991). GAD67 produces more than 90% of the basal GABA (Kash et al., 1999), but there are some brain-region specific differences in the relative expression of the isoforms (Feldblum et al., 1993). *Gad1*$^{-/-}$ mice have not been behaviorally studied, as they die after birth due to cleft palate (Asada et al., 1997), but *Gad2*$^{-/-}$ mice show increased anxiety-like behavior and reduced response to anxiolytics (diazepam and pentobarbital) that facilitate GABAergic neurotransmission (Kash et al., 1999). They also show impairments in threat estimation, fear memory consolidation and fear extinction (Sangha et al., 2009; Bergado-Acosta et al., 2008; Stork et al., 2003). Mice selectively bred for high anxiety-like behavior have increased amygdalar *Gad1* mRNA and protein levels, reflecting an attempt to boost GABA release in compensation for the high anxiety state (Tasan et al., 2011). Less maternal care during early development increases methylation of the *Gad1* promoter in the rat hippocampus, reducing its expression (Zhang et al., 2010). This indicates that environmental factors can have an impact on the development of the GABA system.

In humans, subjects with neuroticism or mood disorders show decreased plasma GAD activity (Kaiya et al., 1982). Reduced expression of *GAD1* is one of the more solid findings in post-mortem brain studies of schizophrenia patients (Torrey et al., 2005). Candidate gene studies have tested variants in *GAD1* for association to a range of psychiatric disorders, such as autism, bipolar disorder, depression and schizophrenia. The most solid findings are from studies examining schizophrenia-related phenotypes in three independent family-based samples (Straub et al., 2007; Addington et al., 2005). In those studies, allelic variation in *GAD1* also associated with cognitive measures and cortical grey matter volume loss in schizophrenia patients, and with activation of the prefrontal cortex during a working memory task in healthy individuals. Unfortunately, direct comparisons between our findings and these previously published ones are difficult to make as not all of the same SNPs were genotyped.

As we observed the strongest associations between phobias and *GAD1* in the Health 2000 sample, it is interesting to note an observation from a neurological disorder called stiff-person syndrome (SPS) that supports this link. SPS is characterized by autoantibodies against the GAD enzyme, which results in reduced levels of GABA in the brain and cerebrospinal fluid (Ameli et al., 2005; Henningsen and Meinck, 2003). Patients with SPS have phobias as a frequent non-motor symptom, to the extent that subjects are often misdiagnosed with a primary psychiatric disorder such as agoraphobia.

Human autoantibodies against GAD also induce anxiogenic-like behavior when passively transferred into rats (Geis et al., 2011).

To summarize, one attractive hypothesis is that genetic variation reducing GAD1 activity may have profound effects on neurotransmission by reduction of GABA levels (Figure 24). Future studies should experimentally address whether the *GAD1* risk haplotype now identified in two independent samples is associated with decreased GAD activity in plasma or post-mortem brain. It should be evaluated whether such a change is accompanied by altered GABA levels, as reduced GABAergic neurotransmission is one hallmark of anxiety disorders and other psychiatric diseases. Targeting of GABAergic metabolism, combined with better knowledge of $GABA_A$ receptor function, are important research fields within the development of novel anxiolytics (Durant et al., 2010; Uusi-Oukari and Korpi, 2010; Pillay and Stein, 2007).

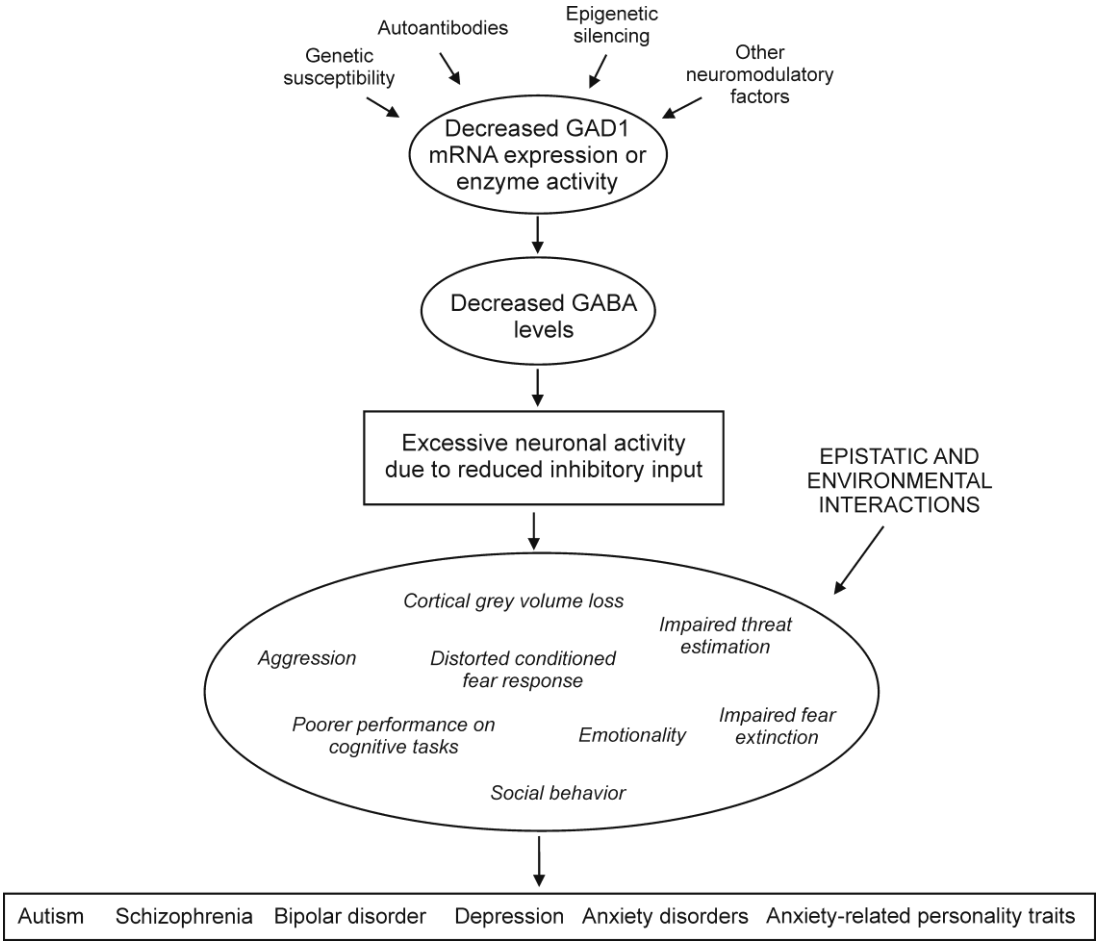


Figure 24 Hypothetical model for involvement of *GAD1* in neuropsychiatric disorders. Decreased GAD1 activity, caused by a number of potential factors, could reduce GABA levels. Alterations of GABAergic neurotransmission, in combination with other genetic and environmental modulatory factors, could have widespread effects in the brain. Such effects manifest as a variety of behavioral changes that are hallmarks of specific psychiatric disorders. GAD1 could therefore be involved in partially determining predisposition to a wide spectrum of more or less severe psychiatric disorders or influence personality. Figure based on references cited in the text and (Leppä et al., 2011; Stork et al., 2000). GABA = γ-aminobutyric acid; GAD1 = glutamate decarboxylase 1.

6.5 INTERACTION OF NEUROPEPTIDE Y WITH THE ENVIRONMENT IN MODULATING RISK FOR ANXIETY DISORDERS

We turned our attention to GxE interactions in attempt to gain better understanding of the joint effects of environmental and genetic factors on anxiety disorder susceptibility. More specifically, we explored whether any of the SNPs genotyped in the Health 2000 sample modulated anxiety disorder onset in interaction with childhood adversities. We had a particularly good prior justification for such analyses, as stressful events early in life are acknowledged to be some of the strongest known environmental risk factors for later anxiety disorders (Green et al., 2010). In particular, adversities of the so-called maladaptive family functioning cluster (parental mental illness, substance abuse disorder, criminality, violence, physical/sexual abuse and neglect) are strong predictors of anxiety disorders. Examinations of possible interactions between such well established and strong environmental risk factors and genetic variants may shed light on why some individuals are more stress resilient than others.

Of all the SNPs examined in the Health 2000 sample, the only ones robustly showing evidence for GxE interaction effects with childhood adversities in modulating anxiety disorder susceptibility were from the *NPY* gene. The effects could further be attributed to two phylogenetically related risk haplotypes spanning the whole gene locus. In general, the analyses were complicated by the limited subgroup sample size that resulted from partitioning the sample by both the number of experienced childhood adversities and SNP genotype. Some potential effects of SNPs with low MAFs may therefore have remained undiscovered, and much larger sample sizes would be needed for their reliable assessment.

Nevertheless, it is particularly interesting that *NPY* was identified as the most promising candidate gene for GxE effects out of all the ones examined. NPY is a highly evolutionarily conserved 36-amino acid neuropeptide and one of the most abundant peptides of the CNS. It is involved in regulation of a wide variety of processes, such as stress response and stress resilience, emotional homeostasis, cognitive processes, food- and ethanol intake, energy balance, sleep regulation, inflammatory processes, tissue growth and remodelling, and sexual behavior (Wu et al., 2011; Thorsell, 2008; Eaton et al., 2007). NPY-signaling is mainly anxiolytic and counteracts physiological, cellular, and behavioral effects of stress-promoting signals. Accordingly, higher NPY levels are associated with stress-resilience in both rodents and humans (Morgan et al., 2002; Thorsell et al., 2000). In mammals, five G-protein coupled receptors for NPY have been identified (Y1, Y2, Y4, Y5 and y6), and their signaling inhibits cAMP synthesis via inhibitory G-proteins (Wu et al., 2011; Berglund et al., 2003). The Y5 receptor was specifically suggested to be responsible for an anxiety disorder linkage peak on 4q31-34 (Domschke et al., 2008; Kaabi et al., 2006)

There is evidence from both animal and human studies suggesting that *NPY*, and genetic variation in it, modulates the effects of environmental stress on anxiety proneness. Transgenic rats overexpressing *Npy* performed similarly to controls in the elevated plus maze under baseline conditions, but lacked the anxiogenic response seen in wild types when the test was preceded by restraint stress (Thorsell et al., 2000). Of macaques exposed to social separation stress, carriers of a specific NPY promoter variant had lower CSF NPY levels, but higher arousal and alcohol consumption upon later stress (Lindell et al., 2010). In humans, haplotypes of *NPY* predict its mRNA levels in lymphoblasts and in post-mortem cerebellum, and plasma NPY levels (Zhou et al., 2008). Furthermore, lower NPY expression predicted higher emotion-induced activation of the amygdala, and reduced stress resilience. The same study reported that one promoter SNP (rs16147) accounted for a considerable proportion of the expression differences between different *NPY* haplotypes.

The functional rs16147 SNP was later examined for GxE interaction effects, and shown to interact with childhood adversities in influencing symptoms of anxiety and depression (Sommer et al., 2010). It also modulated risk for GAD in interaction with exposure to a natural disaster (hurricanes; Amstadter et al., 2010). Although both of the mentioned human GxE studies support involvement of NPY variation in determining stress resilience in interaction with the enviroment, they reported different risk genotypes (C/C in the former, vs. T/T in the latter study). The SNP also influences HPA-axis responsiveness to acute psychosocial stress, with T/T carriers exposed to high early childhood adversity showing the lowest ACTH and cortisol stress responses (Witt et al., 2011).

There are discrepancies regarding which of the alleles of rs16147, C or T, is associated with higher expression and protein levels of NPY, with most studies supporting that the C-allele is the high expressing one (Sommer et al., 2010; Shah et al., 2009; Zhou et al., 2008; Buckland et al., 2005; Itokawa et al., 2003). This has raised the question whether rs16147 is the actual causal variant, or simply in varying degree of LD with other functional variants. In our examination, we found no evidence of GxE effects for rs16147, but the risk haplotypes we observed carried its T-allele. The most likely scenario is that other genetic variation besides rs16147 influences NPY levels, and we suggest that only a subset of the T-allele carrying haplotypes confer increased risk for anxiety disorders in the Finnish population. Based on the majority of reports, they might be accompanied by reduced NPY expression and protein levels. Such a finding would be consistent with the decreased brain/CSF/plasma NPY levels observed in subjects with PTSD, depression, bipolar disorder and suicide victims (Wu et al., 2011). Genetic predisposition to reduced NPY levels offers one explanation for the reduced stress resilience (assessed by us as predisposition for any anxiety disorder under conditions of high early life stress) we observed in subjects with specific risk haplotypes

spanning the *NPY* locus. A hypothetical link between NPY levels and stress resilience is illustrated in Figure 25.

In summary, our findings provide further support that genetic variation in *NPY,* a neuropeptide system crucial for regulation of stress responses, modulates stress resilience in interaction with environmental factors. Knowledge of such effects is particularly important for understanding why some individuals get an anxiety disorder, while others do not, even under stressful life conditions. Targeting of the NPY system might offer a novel means of treatment for anxiety, mood- and stress-related disorders. Investigations of the effects of intranasal NPY administration in humans are underway, with no apparent reported side effects, but also no clear evidence yet that it enters the brain (Wu et al., 2011; Lacroix et al., 1996).

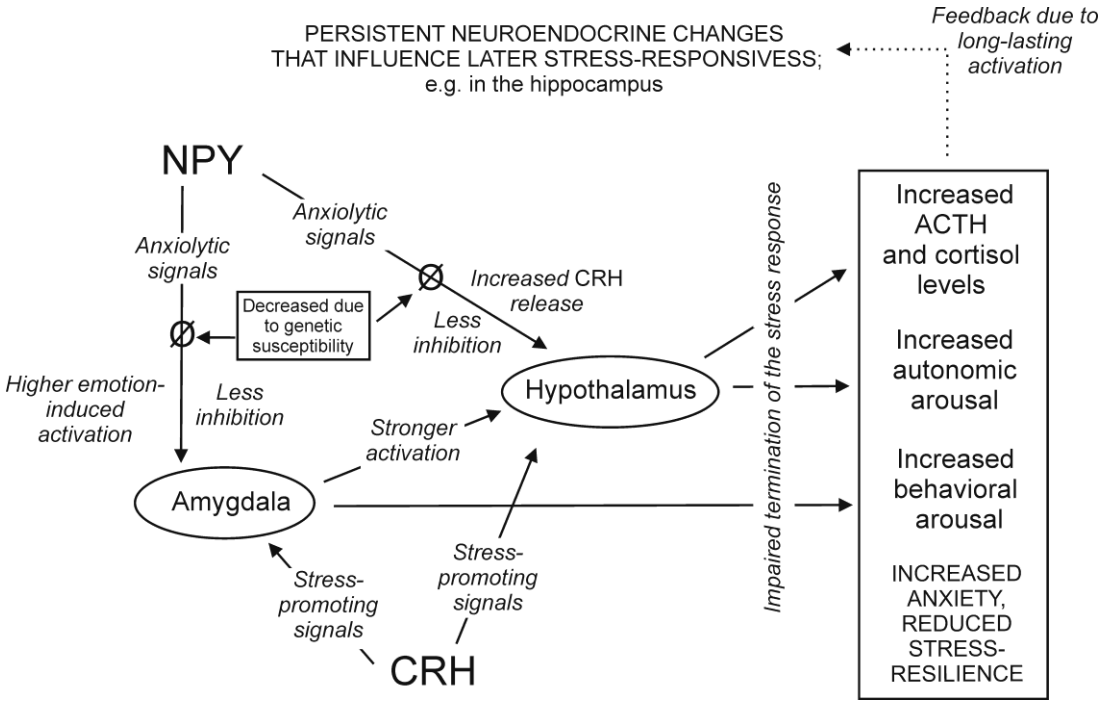


Figure 25 A hypothetical link between neuropeptide Y (NPY) and stress resilience. Normally, NPY is upregulated in response to stress to counteract the stress-promoting effects of increased CRH (corticotrophin-releasing hormone) release and restore emotional homeostasis. On a behavioral level, this terminates the stress response. Higher NPY levels are consequently associated with better coping under stress. Conversely, innately reduced NPY levels could be associated with impaired downregulation of neuroendocrine stress responses, and increased risk for anxiety disorders. Programming of neuroendocrine stress responses during early development influences later stress-responsiveness. ACTH = adrenocorticotropic hormone.

7 CONCLUDING REMARKS AND FUTURE PROSPECTS

The field of human genetics has moved forward with tremendous pace since it was proclaimed that the human genome sequence had been completed. We are living in an era of genomics where the main focus is shifting from first understanding the structure of genomes to understanding the biology of genomes, and finally to understanding the biology of disease (Green et al., 2011). There is hope that genomics will live up to its great expections in years to come by advancing the field of medicine and improving healthcare. Routine analysis of the whole genome of any individual will become commonplace soon – but are we ready to understand what the output is telling us? Thorough understanding of the biology of a trait will require adding additional layers of information to the genome sequence data, such as understanding of the regulatory mechanisms of genes (e.g., epigenetic mechanisms, post-transcriptional regulation by non-coding RNAs and alternative splicing of mRNAs), gene x gene and gene x environment interactions, and protein interactions. Technical developments within the large-scale "omic" -methods such as as transcriptomics, proteomics and metabolomics are now making such integrative approaches to understanding human disease-related biological pathways possible. Next-generation sequencing based methods will play an important role in the following years for not only sequencing of genomes, but also for analyzing quantitatively global profiles of mRNA and non-coding RNA expression.

Again, the field of genetic mapping of human disease genes is finding itself in a transition between current "trendy" methodological approaches (GWA => exome sequencing => whole-genome sequencing). Such shifts are inevitable, driven by curiosity and desire to improve human quality of life, and fueled by technical progress. However, with each new wave of studies performed using the concurrent "trendy" genetic mapping method, we have had to revise at least some of our basic hypothesis for explaning disease susceptibility. The present study was conducted during a time period that witnessed the research community rush into the era of GWA studies with great enthusiasm, as they became technically and economically feasible. We also witnessed many researchers, most of them psychiatric geneticists, disembark from their GWA project with considerably less enthusiasm than when it started. It became evident that the Finnish anxiety disorder sample that this thesis is based on would not be nearly large enough to genome-wide significantly detect the small effects now thought to be conferred by each of the hundreds or thousands of genetic variants that collectively influence susceptibility to a trait as complex as anxiety. Is there then a place for small candidate gene studies in this era of large hypothesis-free genome-wide studies and omics?

First, one main significance of this work has been to demonstrate that a focused human candidate gene study can at least be used to refine the information provided by one global hypothesis-free approach, namely gene expression profiling in mice. This combined approach lead to the identification of potential novel biological pathways that might not otherwise have received attention in concurrent anxiety research.

Second, by forming a hypothesis about a neuropeptide system (NPS-NPSR1) that could influence both susceptibility to asthma and anxiety, we discovered an intriguing biological link between PD and respiratory phenotypes. Later functional studies focusing on genes of the same system have been crucial for supporting our findings, and understanding the mechanisms involved.

Third, by a candidate gene replication finding, we support involvement of the *GAD1* gene in anxiety susceptibility. Solid candidate gene findings are scarce in anxiety disorders, and any findings that help to identify the most relevant susceptibility genes are valuable in that they provide important clues about disease etiology about what the focus of research into novel therapeutic agents could be.

Fourth, with a candidate gene finding in the *NPY*, we highlight the importance of understanding GxE interactions in susceptibility to psychiatric disorders. Demonstrating that such effects are indeed present, and disease-relevant, is an important step in explaining the "missing heritability" (Manolio et al., 2009) of complex diseases that remains unresolved by GWA studies, and that is particularly pronounced in mental disorders.

Thus, well-justified and well-designed candidate gene studies in comprehensively characterized samples, combined with functional experiments, should perhaps not yet be drowned in the omic sea. However, the genetic risk variants that were identified in this study conform to the same discussion as many of the results of the GWA studies performed to date: Will there be a clinical utility, when it comes to prediction of disease risk and treatment response, for risk variants with modest or small effect sizes? Will the novel knowledge about the biological mechanisms that they provide result in development of novel medications? Hopefully, future studies focusing on the top candidate genes identified in this study will help answer these questions.

Current research on the genetics of anxiety disorders should flourish, as all the ingredients are there: heritability, better knowledge of neurocircuitry and the molecular basis than in many other psychiatric phenotypes, well-validated animal models for discovery and validation of candidate genes, and an enormous importance for public health (Smoller, 2011). However, problems encountered include difficulties in drawing the line between normal and pathological anxiety, fuzzy boundaries between the anxiety disorders, combined with large genetic heterogeneity. Therefore, genetic studies in anxiety have been relatively scarce and a "fear of anxiety genetics" has prevailed (Smoller, 2011). The good news is that there is a light in sight.

The field is starting to bring anxiety research to the level of other major psychiatric disorders, making use of a variety of innovative approaches and methodologies.

In the future, exome and eventually genome-wide sequence data, will enable assessment of rare variants also in susceptibility to anxiety disorders. Anxiety disorders may be relatively more strongly influenced by environmental factors and early life experiences that modulate development of neural pathways than many other psychiatric disorders. Thus, stressful or traumatic life events likely have profound effects on later stress resilience and fear responses. I therefore envision that large-scale epigenetic studies and gene expression profiles, combined with attempts to understand GxE interactions will play a key role in explaining mechanisms behind anxiety susceptibility. Imaging genetics will continue to be important for understanding how sequence variation modulates brain function on a neural circuit level. Moreover, induced pluripotent stem cell technologies will enable human patient cell lines of the most relevant type for molecular studies. Sequence-based profiles of mRNA and miRNA transcription, and more efficient proteomics approaches should be pursued to obtain the most comprehensive view yet of regulatory networks and biological pathways relevant for anxiety. This will be a rich and crucial source of information for drug development.

Exciting times lay ahead for geneticists and anxiety researchers. It is my hope that the work presented in this thesis will have some part in the transition taking anxiety research to the next level, eventually leading to improved treatment of pathological anxiety.

8 ACKNOWLEDGEMENTS

This study was carried out between the years 2006 and 2011 at the University of Helsinki (Research Program of Molecular Neurology and Department of Medical Genetics) and the National Public Health Institute (later the National Institute for Health and Welfare; Departments of Mental Health and Substance Abuse Services, and Molecular Medicine). Past and current heads of these units are acknowledged for providing excellent research facilities and atmosphere.

This study was financially supported by the Helsinki Biomedical Graduate School, Nylands Nation vid Helsingfors Universitet, the Emil Aaltonen Foundation, the Jalmari and Rauha Ahokas Foundation, Svenska Kulturfonden, H. Lundbeck A/S, the Finnish Foundation for Psychiatric Research, and the University of Helsinki Funds. All are warmly thanked for their support.

I am where I am today due to a number of important people. I warmly remember Leena Palotie, who gave me my first real job in science. You will continue to be an inspiration and role model for generations of geneticists and scientists to come. Leena also introduced me to Iiris, and suddenly I found myself swept away by a wave of interesting anxiety-related projects. Iiris, you have been my mentor on the winding path towards becoming an independent researcher, and you have seen me grow along the way. I'm deeply thankful for your support in both good and bad times, for trusting me with your projects, and pushing me in the right direction when I was going wrong.

Professors Anu Wartiovaara and Esa Korpi, I highly value your scientific expertise and helpful suggestions in- and outside of thesis committee meetings. Anu is also thanked for accepting the role of kustos at my thesis defense, while Professor Martin Schalling is warmly acknowledged for accepting the role of opponent. Thanks are also due to Professor Erkki Isometsä and Dr. Marjo Kestilä for helpful suggestions and comments during the pre-examination of the thesis.

All others who contributed to this thesis work are warmly thanked for making it possible. I would like to thank Professors Jouko Lönnqvist and Sami Pirkola for their important role in collecting and characterizing the Health 2000 sample that was so central to this thesis. All study participants are also acknowledged. Professors Juha Kere and Harri Alenius, Drs. Sini Ezer and Mauro D'Amato, Mrs. Rita Helldan, and all other co-authors are thanked for a fruitful collaboration related to the NPS-NPSR1 signaling system. Dr. John M. Hettema and co-workers are thanked for collaborating on data related to *GAD1*. Drs. Samuli Ripatti and Joseph D. Terwilliger are acknowledged for discussions on statistical problems, and Dr. Kaisa Silander for technical advice. Professor Hannu Sariola and members of the Korpi

group – Anni-Maija, Teemu, Olga, Kati and Anne - are thanked for collaboration and guidance.

All of my colleagues in the Hovatta lab, I would not be here without you either. I will miss all of you, the Crazy Fridays and the (pseudo)scientific discussions. Tessa, my office mate for so many years with whom I've shared the good and the bad – every workplace should have a copy of you to cheer things up! Laura, I admire your technical and organizational skills that were essential for this work, and I just love your way of not taking things too seriously. Juuso, thanks for bringing some post doc experience into the lab, and for fun discussions on any topic. Kaisa, thanks for working together with me on numerous hopeless projects, and for making me feel less old by being my link to student life. Ilari, thanks for making me feel older by being the same. Mari, you have been a great addition to the group, always ready for work and non-work-related exchange of thoughts. Katherine, thanks for teaching me countless things about Excel and bioinformatics in your cheerful way. Jenni, thanks for helping me with my projects, and for bringing fresh ideas into the lab. Nina and Kirsi, thank you for the fantastic office company and the badly needed discussions on parenting. Our former lab members - Pia, Johanna, Petri, Helena, Ulla, Sirli, and Suvi – thanks to all of you for advice, support and, most importantly, friendship.

There are so many others I have encountered during my years at THL and the University of Helsinki. Henna, Tiia, Kaisu and Antti – I will never forget the start of my career in the Mickey Mouse office. Markus L, Karola, Nora, Siv, Minna, Jesper, Liisa, Annu, Virpi, Juho, Satu, Ansku, Tea, Anu K, Jarkko, PP, Olli, Jussi, Heidi N, Jonna, Ida, and Marine – thank you for being wonderful colleagues and company in the lab, on conference trips, and at lunches. To all people at the Research Program of Molecular Neurology – Padmini, Chris, Alessandro, Joni, Jani, Terhi, Brendan, Pentti, Ove, Tiina, Anu H, Ilse, Tuula, Henna, Markus I, Erika, Paula, Riikka J, Maarit, Uwe, Pilvi, Heidi, Kati, Riikka H, Liliya, Eino, Alex, Sanna, Sofia, Annamari, Jana, Valtteri, Emil, Heini, Shuichi and all I know I have forgotten – thank you for all these years.

Finally, I'm extremely grateful for my family and relatives. Without your support and help, even at times when you did not understand, I could not have made it through the feeding of cells on Christmas, or the long days ending in a failed experiment. Thank you for being there.

Jonas

Helsinki, February 2012

9 REFERENCES

1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature 2010. 467:1061-1073.

Abelson JL, Khan S, Liberzon I, Young EA. HPA axis activity in patients with panic disorder: Review and synthesis of four studies. Depress Anxiety 2007. 24:66-76.

Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb A, Greenstein D, Lenane M, Gochman P, Baker N, Balkissoon R, Vakkalanka RK, Weinberger DR, Rapoport JL, Straub RE. GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. Mol Psychiatry 2005. 10:581-588.

Alvarez RP, Biggs A, Chen G, Pine DS, Grillon C. Contextual fear conditioning in humans: Cortical-hippocampal and amygdala contributions. J Neurosci 2008. 28:6211-6219.

Ameli R, Snow J, Rakocevic G, Dalakas MC. A neuropsychological assessment of phobias in patients with stiff person syndrome. Neurology 2005. 64:1961-1963.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV-TR. 2000. Washington, DC: American Psychiatric Association.

Amin N, Schuur M, Gusareva ES, Isaacs A, Aulchenko YS, Kirichenko AV, Zorkoltseva IV, Axenovich TI, Oostra BA, Janssens AC, van Duijn CM. A genome-wide linkage study of individuals with high scores on NEO personality traits. Mol Psychiatry 2011.

Amstadter AB, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, Gelernter J. Variant in RGS2 moderates posttraumatic stress symptoms following potentially traumatic event exposure. J Anxiety Disord 2009. 23:369-373.

Amstadter AB, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, Gelernter J. NPY moderates the relation between hurricane exposure and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. Depress Anxiety 2010. 27:270-275.

Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 2002. 3:299-309.

Asada H, Kawamura Y, Maruyama K, Kume H, Ding RG, Kanbara N, Kuzume H, Sanbo M, Yagi T, Obata K. Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A 1997. 94:6496-6499.

Atack JR. GABAA receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. Curr Top Behav Neurosci 2010. 2:331-360.

Badia-Elder NE, Henderson AN, Bertholomey ML, Dodge NC, Stewart RB. The effects of neuropeptide S on ethanol drinking and other related behaviors in alcohol-preferring and -nonpreferring rats. Alcohol Clin Exp Res 2008. 32:1380-1387.

Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, Simerly RB, Susser ES, Nestler EJ. Early life programming and neurodevelopmental disorders. Biol Psychiatry 2010. 68:314-319.

Bandelow B, Zohar J, Hollander E, Kasper S, Moller HJ, WFSBP Task Force on Treatment Guidelines for Anxiety, Obsessive-Compulsive and Post-Traumatic Stress Disoders, Zohar J, Hollander E, Kasper S, Moller HJ, Bandelow B, Allgulander C, Ayuso-Gutierrez J, Baldwin DS, Buenvicius R, Cassano G, Fineberg N, Gabriels L, Hindmarch I, Kaiya H, Klein DF, Lader M, Lecrubier Y, Lepine JP, Liebowitz MR, Lopez-Ibor JJ, Marazziti D, Miguel EC, Oh KS, Preter M, Rupprecht R, Sato M, Starcevic V, Stein DJ, van Ameringen M, Vega J. World federation of societies of biological psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. World J Biol Psychiatry 2008. 9:248-312.

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005. 21:263-265.

Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M. Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. Proc Natl Acad Sci U S A 2007. 104:10164-10169.

Bergado-Acosta JR, Sangha S, Narayanan RT, Obata K, Pape HC, Stork O. Critical role of the 65-kDa isoform of glutamic acid decarboxylase in consolidation and generalization of pavlovian fear memory. Learn Mem 2008. 15:163-171.

Berglund MM, Hipskind PA, Gehlert DR. Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. Exp Biol Med (Maywood) 2003. 228:217-244.

Bernier V, Stocco R, Bogusky MJ, Joyce JG, Parachoniak C, Grenier K, Arget M, Mathieu MC, O'Neill GP, Slipetz D, Crackower MA, Tan CM, Therien AG. Structure-function relationships in the neuropeptide S receptor: Molecular consequences of the asthma-associated mutation N107I. J Biol Chem 2006. 281:24704-24712.

Berrios GE, Campbell C, Politynska BE. Autonomic failure, depression and anxiety in parkinson's disease. Br J Psychiatry 1995. 166:789-792.

Bespalov AY, van Gaalen MM, Gross G. Antidepressant treatment in anxiety disorders. Curr Top Behav Neurosci 2010. 2:361-390.

Bienvenu OJ, Hettema JM, Neale MC, Prescott CA, Kendler KS. Low extraversion and high neuroticism as indices of genetic and environmental risk for social phobia, agoraphobia, and animal phobia. Am J Psychiatry 2007. 164:1714-1721.

Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 2008. 299:1291-1305.

Björkqvist K. Social defeat as a stressor in humans. Physiol Behav 2001. 73:435-442.

Blechert J, Michael T, Grossman P, Lajtman M, Wilhelm FH. Autonomic and respiratory characteristics of posttraumatic stress disorder and panic disorder. Psychosom Med 2007a. 69:935-943.

Blechert J, Michael T, Vriends N, Margraf J, Wilhelm FH. Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. Behav Res Ther 2007b. 45:2019-2033.

Bloch MH, Landeros-Weisenberger A, Sen S, Dombrowski P, Kelmendi B, Coric V, Pittenger C, Leckman JF. Association of the serotonin transporter polymorphism and obsessive-compulsive disorder: Systematic review. Am J Med Genet B Neuropsychiatr Genet 2008. 147B:850-858.

Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet 2008. 40:695-701.

Bonferroni CE. Teoria statistica delle classi e calcolo delle probabilita. Pubblicazioni Del R Istituto Superiore Di Scienze Economiche e Commerciali Di Firenze 1932. 8:3-62.

Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. Introduction to meta-analysis. 2009. Chichester, UK: John Wiley & Sons, Ltd.

Bormann J. The 'ABC' of GABA receptors. Trends Pharmacol Sci 2000. 21:16-19.

Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980. 32:314-331.

Bradwejn J, Koszycki D, Shriqui C. Enhanced sensitivity to cholecystokinin tetrapeptide in panic disorder. clinical and behavioral findings. Arch Gen Psychiatry 1991. 48:603-610.

Brandes M, Bienvenu OJ. Personality and anxiety disorders. Curr Psychiatry Rep 2006. 8:263-269.

Buckland PR, Hoogendoorn B, Coleman SL, Guy CA, Smith SK, O'Donovan MC. Strong bias in the location of functional promoter polymorphisms. Hum Mutat 2005. 26:214-223.

Bundy H, Stahl D, MacCabe JH. A systematic review and meta-analysis of the fertility of patients with schizophrenia and their unaffected relatives. Acta Psychiatr Scand 2011. 123:98-106.

Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: Recent developments. Neurosci Biobehav Rev 2009. 33:1089-1098.

Calboli FC, Tozzi F, Galwey NW, Antoniades A, Mooser V, Preisig M, Vollenweider P, Waterworth D, Waeber G, Johnson MR, Muglia P, Balding DJ. A genome-wide association study of neuroticism in a population-based sample. PLoS One 2010. 5:e11504.

Cao J, de Lecea L, Ikemoto S. Intraventricular administration of neuropeptide S has reward-like effects. Eur J Pharmacol 2011. 658:16-21.

Cardon LR, Bell JI. Association study designs for complex diseases. Nat Rev Genet 2001. 2:91-99.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 2003. 301:386-389.

Chang L, Cloak CC, Ernst T. Magnetic resonance spectroscopy studies of GABA in neuropsychiatric disorders. J Clin Psychiatry 2003. 64 Suppl 3:7-14.

Childs E, Hohoff C, Deckert J, Xu K, Badner J, de Wit H. Association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. Neuropsychopharmacology 2008. 33:2791-2800.

Choi DC, Rothbaum BO, Gerardi M, Ressler KJ. Pharmacological enhancement of behavioral therapy: Focus on posttraumatic stress disorder. Curr Top Behav Neurosci 2010. 2:279-299.

Christiana JM, Gilman SE, Guardino M, Mickelson K, Morselli PL, Olfson M, Kessler RC. Duration between onset and time of obtaining initial treatment among people with anxiety and mood disorders: An international survey of members of mental health patient advocate groups. Psychol Med 2000. 30:693-703.

Clamp M, Fry B, Kamal M, Xie X, Cuff J, Lin MF, Kellis M, Lindblad-Toh K, Lander ES. Distinguishing protein-coding and noncoding genes in the human genome. Proc Natl Acad Sci U S A 2007. 104:19428-19433.

Cloninger CR. The temperament and character inventory (TCI): A guide to its development and use. 1994. St. Louis, MO: Center for Psychobiology of Personality, Washington University.

Cold Spring Harbor Laboratory Press. Electrophoretic mobility shift assays. Nature Methods 2005. 2:557-558.

Collins FS, Guyer MS, Charkravarti A. Variations on a theme: Cataloging human DNA sequence variation. Science 1997. 278:1580-1581.

Conrad DF, Keebler JE, DePristo MA, Lindsay SJ, Zhang Y, Casals F, Idaghdour Y, Hartl CL, Torroja C, Garimella KV, Zilversmit M, Cartwright R, Rouleau GA, Daly M, Stone EA, Hurles ME, Awadalla P, 1000 Genomes Project. Variation in genome-wide mutation rates within and between human families. Nat Genet 2011. 43:712-714.

Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J, Stephan DA. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset alzheimer's disease. J Clin Psychiatry 2007. 68:613-618.

Coric V, Feldman HH, Oren DA, Shekhar A, Pultz J, Dockens RC, Wu X, Gentile KA, Huang SP, Emison E, Delmonte T, D'Souza BB, Zimbroff DL, Grebb JA, Goddard AW, Stock EG. Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. Depress Anxiety 2010. 27:417-425.

Costa PT, McCrae RR. NEO PI-R professional manual. 1992. Odessa, FL: Psychological Assessment Resources.

Costas J, Gratacos M, Escaramis G, Martin-Santos R, de Diego Y, Baca-Garcia E, Canellas F, Estivill X, Guillamat R, Guitart M, Gutierrez-Zotes A, Garcia-Esteve L, Mayoral F, Molto MD, Phillips C, Roca M, Carracedo A, Vilella E, Sanjuan J. Association study of 44 candidate genes with depressive and anxiety symptoms in post-partum women. J Psychiatr Res 2010. 44:717-724.

Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 1980. 13:167-170.

Crowe RR, Goedken R, Samuelson S, Wilson R, Nelson J, Noyes R,Jr. Genomewide survey of panic disorder. Am J Med Genet 2001. 105:105-109.

Cuijpers P, Dekker J, Hollon SD, Andersson G. Adding psychotherapy to pharmacotherapy in the treatment of depressive disorders in adults: A meta-analysis. J Clin Psychiatry 2009. 70:1219-1229.

Czibere L, Baur LA, Wittmann A, Gemmeke K, Steiner A, Weber P, Putz B, Ahmad N, Bunck M, Graf C, Widner R, Kuhne C, Panhuysen M, Hambsch B, Rieder G, Reinheckel T, Peters C, Holsboer F, Landgraf R, Deussing JM. Profiling trait anxiety: Transcriptome analysis reveals cathepsin B (ctsb) as a novel candidate gene for emotionality in mice. PLoS One 2011. 6:e23604.

Dal Ben D, Antonini I, Buccioni M, Lambertucci C, Marucci G, Thomas A, Volpini R, Cristalli G. Neuropeptide S receptor: Recent updates on nonpeptide antagonist discovery. ChemMedChem 2011. 6:1163-1171.

Dannlowski U, Kugel H, Franke F, Stuhrmann A, Hohoff C, Zwanzger P, Lenzen T, Grotegerd D, Suslow T, Arolt V, Heindel W, Domschke K. Neuropeptide-S (NPS) receptor genotype modulates basolateral amygdala responsiveness to aversive stimuli. Neuropsychopharmacology 2011. 36:1879-1885.

Davis M. Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 2006. 61:741-756.

de Bakker PIW, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005. 37:1217-1223.

Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, Nothen MM, Maffei P, Franke P, Fritze J, Maier W, Propping P, Beckmann H, Bellodi L, Lesch KP. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 1999. 8:621-624.

Deckert J, Nothen MM, Franke P, Delmo C, Fritze J, Knapp M, Maier W, Beckmann H, Propping P. Systematic mutation screening and association study of the A1 and A2a adenosine receptor genes in panic disorder suggest a contribution of the A2a gene to the development of disease. Mol Psychiatry 1998. 3:81-85.

Dermitzakis ET, Reymond A, Antonarakis SE. Conserved non-genic sequences - an unexpected feature of mammalian genomes. Nat Rev Genet 2005. 6:151-157.

Dierssen M, Gratacos M, Sahun I, Martin M, Gallego X, Amador-Arjona A, Martinez de Lagran M, Murtra P, Marti E, Pujana MA, Ferrer I, Dalfo E, Martinez-Cue C, Florez J, Torres-Peraza JF, Alberch J, Maldonado R, Fillat C, Estivill X. Transgenic mice overexpressing the full-length neurotrophin receptor TrkC exhibit increased catecholaminergic neuron density in specific brain areas and increased anxiety-like behavior and panic reaction. Neurobiol Dis 2006. 24:403-418.

Dilger S, Straube T, Mentzel HJ, Fitzek C, Reichenbach JR, Hecht H, Krieschel S, Gutberlet I, Miltner WH. Brain activation to phobia-related pictures in spider phobic humans: An event-related functional magnetic resonance imaging study. Neurosci Lett 2003. 348:29-32.

Ditzen C, Varadarajulu J, Czibere L, Gonik M, Targosz BS, Hambsch B, Bettecken T, Kessler MS, Frank E, Bunck M, Teplytska L, Erhardt A, Holsboer F, Muller-Myhsok B, Landgraf R, Turck CW. Proteomic-based genotyping in a mouse model of trait anxiety exposes disease-relevant pathways. Mol Psychiatry 2010. 15:702-711.

Domschke K, Deckert J, O'donovan MC, Glatt SJ. Meta-analysis of COMT val158met in panic disorder: Ethnic heterogeneity and gender specificity. Am J Med Genet B Neuropsychiatr Genet 2007. 144B:667-673.

Domschke K, Hohoff C, Jacob C, Maier W, Fritze J, Bandelow B, Krakowitzky P, Kastner F, Rothermundt M, Arolt V, Deckert J. Chromosome 4q31-34 panic disorder risk locus: Association of neuropeptide Y Y5 receptor variants. Am J Med Genet B Neuropsychiatr Genet 2008. 147B:510-516.

Domschke K, Reif A, Weber H, Richter J, Hohoff C, Ohrmann P, Pedersen A, Bauer J, Suslow T, Kugel H, Heindel W, Baumann C, Klauke B, Jacob C, Maier W, Fritze J, Bandelow B, Krakowitzky P, Rothermundt M, Erhardt A, Binder EB, Holsboer F, Gerlach AL, Kircher T, Lang T, Alpers GW, Strohle A, Fehm L, Gloster AT, Wittchen HU, Arolt V, Pauli P, Hamm A, Deckert J. Neuropeptide S receptor gene -- converging evidence for a role in panic disorder. Mol Psychiatry 2011. 16:938-948.

Duangdao DM, Clark SD, Okamura N, Reinscheid RK. Behavioral phenotyping of neuropeptide S receptor knockout mice. Behav Brain Res 2009.

Dudbridge F. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered 2008. 66:87-98.

Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. Genet Epidemiol 2008. 32:227-234.

Durant C, Christmas D, Nutt D. The pharmacology of anxiety. Curr Top Behav Neurosci 2010. 2:303-330.

Eaton K, Sallee FR, Sah R. Relevance of neuropeptide Y (NPY) in psychiatry. Curr Top Med Chem 2007. 7:1645-1659.

Elston RC, Spence MA. Advances in statistical human genetics over the last 25 years. Stat Med 2006. 25:3049-3080.

Erhardt A, Czibere L, Roeske D, Lucae S, Unschuld PG, Ripke S, Specht M, Kohli MA, Kloiber S, Ising M, Heck A, Pfister H, Zimmermann P, Lieb R, Putz B, Uhr M, Weber P, Deussing JM, Gonik M, Bunck M, Kebler MS, Frank E, Hohoff C, Domschke K, Krakowitzky P, Maier W, Bandelow B, Jacob C, Deckert J, Schreiber S, Strohmaier J, Nothen M, Cichon S, Rietschel M, Bettecken T, Keck ME, Landgraf R, Muller-Myhsok B, Holsboer F, Binder EB. TMEM132D, a new candidate for anxiety phenotypes: Evidence from human and mouse studies. Mol Psychiatry 2011. 16:647-663.

Eysenck HJ, Eysenck SBG. Manual of the eysenck personality questionnaire. 1975. London: Hodder and Stoughton.

Feldblum S, Erlander MG, Tobin AJ. Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. J Neurosci Res 1993. 34:689-706.

Fendt M, Buchi M, Burki H, Imobersteg S, Ricoux B, Suply T, Sailer AW. Neuropeptide S receptor deficiency modulates spontaneous locomotor activity and the acoustic startle response. Behav Brain Res 2011. 217:1-9.

Fendt M, Imobersteg S, Burki H, McAllister KH, Sailer AW. Intra-amygdala injections of neuropeptide S block fear-potentiated startle. Neurosci Lett 2010. 474:154-157.

File SE, Hyde JR. Can social interaction be used to measure anxiety? Br J Pharmacol 1978. 62:19-24.

Filiou MD, Zhang Y, Teplytska L, Reckow S, Gormanns P, Maccarrone G, Frank E, Kessler MS, Hambsch B, Nussbaumer M, Bunck M, Ludwig T, Yassouridis A, Holsboer F, Landgraf R, Turck CW. Proteomics and metabolomics analysis of a trait anxiety mouse model reveals divergent mitochondrial pathways. Biol Psychiatry 2011. 70:1074-1082.

Finn DA, Rutledge-Gorman MT, Crabbe JC. Genetic animal models of anxiety. Neurogenetics 2003. 4:109-135.

Fisher RA. The correlation between relatives on the supposition of mendelian inheritance. T Roy Soc Edin 1918. 52:399-433.

Flint J, Corley R, DeFries JC, Fulker DW, Gray JA, Miller S, Collins AC. A simple genetic basis for a complex psychological trait in laboratory mice. Science 1995. 269:1432-1435.

Flint J, Valdar W, Shifman S, Mott R. Strategies for mapping and cloning quantitative trait genes in rodents. Nat Rev Genet 2005. 6:271-286.

Foa EB, Franklin ME, Moser J. Context in the clinic: How well do cognitive-behavioral therapies and medications work in combination? Biol Psychiatry 2002. 52:987-997.

Frustaci A, Pozzi G, Gianfagna F, Manzoli L, Boccia S. Meta-analysis of the brain-derived neurotrophic factor gene (BDNF) Val66Met polymorphism in anxiety disorders and anxiety-related personality traits. Neuropsychobiology 2008. 58:163-170.

Fujimoto M, Uchida S, Watanuki T, Wakabayashi Y, Otsuki K, Matsubara T, Suetsugi M, Funato H, Watanabe Y. Reduced expression of glyoxalase-1 mRNA in mood disorder patients. Neurosci Lett 2008. 438:196-199.

Fullerton J, Cubin M, Tiwari H, Wang C, Bomhra A, Davidson S, Miller S, Fairburn C, Goodwin G, Neale MC, Fiddy S, Mott R, Allison DB, Flint J. Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative-trait loci that influence variation in the human personality trait neuroticism. Am J Hum Genet 2003. 72:879-890.

Fullerton JM, Willis-Owen SA, Shifman S, Copley RR, Miller SR, Bhomra A, Davidson S, Oliver PL, Mott R, Flint J. Human-mouse quantitative trait locus concordance and the dissection of a human neuroticism locus. Biol Psychiatry 2007.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science 2002. 296:2225-2229.

Garakani A, Murrough JW, Charney DS, Bremner D. 2009. The neurobiology of anxiety disorders. In: Charney DS, Nestler EJ, editors. Neurobiology of mental illness. Oxford, New York: Oxford University Press. p 655-690.

Geis C, Weishaupt A, Grunewald B, Wultsch T, Reif A, Gerlach M, Dirkx R, Solimena M, Perani D, Heckmann M, Toyka KV, Folli F, Sommer C. Human stiff-person syndrome IgG induces anxious behavior in rats. PLoS One 2011. 6:e16775.

Gelernter J, Page GP, Bonvicini K, Woods SW, Pauls DL, Kruger S. A chromosome 14 risk locus for simple phobia: Results from a genomewide linkage scan. Mol Psychiatry 2003. 8:71-82.

Gillespie CF, Phifer J, Bradley B, Ressler KJ. Risk and resilience: Genetic and environmental influences on development of the stress response. Depress Anxiety 2009. 26:984-992.

Goddard AW, Mason GF, Appel M, Rothman DL, Gueorguieva R, Behar KL, Krystal JH. Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. Am J Psychiatry 2004. 161:2186-2193.

Goodwin RD. Asthma and anxiety disorders. Adv Psychosom Med 2003. 24:51-71.

Goodwin RD, Faravelli C, Rosi S, Cosci F, Truglia E, de Graaf R, Wittchen HU. The epidemiology of panic disorder and agoraphobia in europe. Eur Neuropsychopharmacol 2005. 15:435-443.

Goodwin RD, Fergusson DM, Horwood LJ. Asthma and depressive and anxiety disorders among young persons in the community. Psychol Med 2004. 34:1465-1474.

Goodwin RD, Jacobi F, Thefeld W. Mental disorders and asthma in the community. Arch Gen Psychiatry 2003. 60:1125-1130.

Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. Am J Psychiatry 2000. 157:493-505.

Gormanns P, Mueller NS, Ditzen C, Wolf S, Holsboer F, Turck CW. Phenome-transcriptome correlation unravels anxiety and depression related pathways. J Psychiatr Res 2011. 45:973-979.

Green ED, Guyer MS, National Human Genome Research Institute. Charting a course for genomic medicine from base pairs to bedside. Nature 2011. 470:204-213.

Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. Arch Gen Psychiatry 2010. 67:113-123.

Guerrini R, Salvadori S, Rizzi A, Regoli D, Calo' G. Neurobiology, pharmacology, and medicinal chemistry of neuropeptide S and its receptor. Med Res Rev 2010. 30:751-777.

Hall CS, Ballachey EL. A study of the rat's behavior in a field: A contribution to method in comparative psychology. University of California Publications in Psychology 1932. 6:1-12.

Hall D, Dhilla A, Charalambous A, Gogos JA, Karayiorgou M. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. Am J Hum Genet 2003. 73:370-376.

Hamilton SP, Fyer AJ, Durner M, Heiman GA, Baisre de Leon A, Hodge SE, Knowles JA, Weissman MM. Further genetic evidence for a panic disorder syndrome mapping to chromosome 13q. Proc Natl Acad Sci U S A 2003. 100:2550-2555.

Hamilton SP, Slager SL, De Leon AB, Heiman GA, Klein DF, Hodge SE, Weissman MM, Fyer AJ, Knowles JA. Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder. Neuropsychopharmacology 2004. 29:558-565.

Hanna GL, Veenstra-Vanderweele J, Cox NJ, Van Etten M, Fischer DJ, Himle JA, Bivens NC, Wu X, Roe CA, Hennessy KA, Dickel DE, Leventhal BL, Cook EH,Jr. Evidence for a susceptibility locus on chromosome 10p15 in early-onset obsessive-compulsive disorder. Biol Psychiatry 2007. 62:856-862.

Haramati S, Navon I, Issler O, Ezra-Nevo G, Gil S, Zwang R, Hornstein E, Chen A. microRNA as repressors of stress-induced anxiety: The case of amygdalar miR-34. J Neurosci 2011. 31:14191-14203.

Harmer CJ. Serotonin and emotional processing: Does it help explain antidepressant drug action? Neuropharmacology 2008. 55:1023-1028.

Harter MC, Conway KP, Merikangas KR. Associations between anxiety disorders and physical illness. Eur Arch Psychiatry Clin Neurosci 2003. 253:313-320.

Heck A, Pfister H, Czamara D, Muller-Myhsok B, Putz B, Lucae S, Hennings J, Ising M. Evidence for associations between MDGA2 polymorphisms and harm avoidance: Replication and extension of a genome-wide association finding. Psychiatr Genet 2011. 21:257-260.

Heistaro S. Methodology report. health 2000 survey. Finnish National Public Health Institute 2008.

Henderson ND, Turri MG, DeFries JC, Flint J. QTL analysis of multiple behavioral measures of anxiety in mice. Behav Genet 2004. 34:267-293.

Henningsen P, Meinck HM. Specific phobia is a frequent non-motor feature in stiff man syndrome. J Neurol Neurosurg Psychiatry 2003. 74:462-465.

Hettema JM, An SS, Bukszar J, van den Oord EJ, Neale MC, Kendler KS, Chen X. Catechol-O-methyltransferase contributes to genetic susceptibility shared among anxiety spectrum phenotypes. Biol Psychiatry 2008. 64:302-310.

Hettema JM, An SS, Neale MC, Bukszar J, van den Oord EJ, Kendler KS, Chen X. Association between glutamic acid decarboxylase genes and anxiety disorders, major depression, and neuroticism. Mol Psychiatry 2006. 11:752-762.

Hettema JM, Neale MC, Kendler KS. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry 2001. 158:1568-1578.

Hettema JM, Prescott CA, Myers JM, Neale MC, Kendler KS. The structure of genetic and environmental risk factors for anxiety disorders in men and women. Arch Gen Psychiatry 2005. 62:182-189.

Hettema JM, Webb BT, Guo AY, Zhao Z, Maher BS, Chen X, An SS, Sun C, Aggen SH, Kendler KS, Kuo PH, Otowa T, Flint J, van den Oord EJ. Prioritization and association analysis of murine-derived candidate genes in anxiety-spectrum disorders. Biol Psychiatry 2011. 70:888-896.

Hettema JM, Neale MC, Myers JM, Prescott CA, Kendler KS. A population based twin study of the relationship between neuroticism and internalizing disorders. Am J Psychiatry 2006. 163:857-864.

Hiard S, Charlier C, Coppieters W, Georges M, Baurain D. Patrocles: A database of polymorphic miRNA-mediated gene regulation in vertebrates. Nucleic Acids Res 2010. 38:D640-51.

Hohoff C, Mullings EL, Heatherley SV, Freitag CM, Neumann LC, Domschke K, Krakowitzky P, Rothermundt M, Keck ME, Erhardt A, Unschuld PG, Jacob C, Fritze J, Bandelow B, Maier W, Holsboer F, Rogers PJ, Deckert J. Adenosine A(2A) receptor gene: Evidence for association of risk variants with panic disorder and anxious personality. J Psychiatr Res 2010. 44:930-937.

Hokfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M. Neuropeptides--an overview. Neuropharmacology 2000. 39:1337-1356.

Holmes A, Murphy DL, Crawley JN. Abnormal behavioral phenotypes of serotonin transporter knockout mice: Parallels with human anxiety and depression. Biol Psychiatry 2003. 54:953-959.

Hosing VG, Schirmacher A, Kuhlenbaumer G, Freitag C, Sand P, Schlesiger C, Jacob C, Fritze J, Franke P, Rietschel M, Garritsen H, Nothen MM, Fimmers R, Stogbauer F, Deckert J. Cholecystokinin- and cholecystokinin-B-receptor gene polymorphisms in panic disorder. J Neural Transm Suppl 2004. (68):147-156.

Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM, Ellison JA, Schadt EE, Verma IM, Lockhart DJ, Barlow C. Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature 2005. 438:662-666.

Inada Y, Yoneda H, Koh J, Sakai J, Himei A, Kinoshita Y, Akabame K, Hiraoka Y, Sakai T. Positive association between panic disorder and polymorphism of the serotonin 2A receptor gene. Psychiatry Res 2003. 118:25-31.

International HapMap 3 Consortium, Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Peltonen L, Dermitzakis E, Bonnen PE, Altshuler DM, Gibbs RA, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Yu F, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Gibbs RA, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Dermitzakis E, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Bonnen PE, Gibbs RA, Gonzaga-Jauregui C, Keinan A, Price AL, Yu F, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Schaffner SF, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Pollack S, Price AL, Schaffner SF, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE. Integrating common and rare genetic variation in diverse human populations. Nature 2010. 467:52-58.

Itokawa M, Arai M, Kato S, Ogata Y, Furukawa A, Haga S, Ujike H, Sora I, Ikeda K, Yoshikawa T. Association between a novel polymorphism in the promoter region of the neuropeptide Y gene and schizophrenia in humans. Neurosci Lett 2003. 347:202-204.

Jungling K, Seidenbecher T, Sosulina L, Lesting J, Sangha S, Clark SD, Okamura N, Duangdao DM, Xu YL, Reinscheid RK, Pape HC. Neuropeptide S-mediated control of fear expression and extinction: Role of intercalated GABAergic neurons in the amygdala. Neuron 2008. 59:298-310.

Kaabi B, Gelernter J, Woods SW, Goddard A, Page GP, Elston RC. Genome scan for loci predisposing to anxiety disorders using a novel multivariate approach: Strong evidence for a chromosome 4 risk locus. Am J Hum Genet 2006. 78:543-553.

Kaiya H, Namba M, Yoshida H, Nakamura S. Plasma glutamate decarboxylase activity in neuropsychiatry. Psychiatry Res 1982. 6:335-343.

Kananen L, Surakka I, Pirkola S, Suvisaari J, Lönnqvist J, Peltonen L, Ripatti S, Hovatta I. Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. PLoS One 2010. 5:e10826.

Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: Evidence of genetic moderation. Arch Gen Psychiatry 2011. 68:444-454.

Kash SF, Tecott LH, Hodge C, Baekkeskov S. Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci U S A 1999. 96:1698-1703.

Katon W, Lozano P, Russo J, McCauley E, Richardson L, Bush T. The prevalence of DSM-IV anxiety and depressive disorders in youth with asthma compared with controls. J Adolesc Health 2007. 41:455-463.

Katon WJ, Richardson L, Lozano P, McCauley E. The relationship of asthma and anxiety disorders. Psychosom Med 2004. 66:349-355.

Kaufman DL, Houser CR, Tobin AJ. Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. J Neurochem 1991. 56:720-723.

Kendler KS, Aggen SH, Knudsen GP, Roysamb E, Neale MC, Reichborn-Kjennerud T. The structure of genetic and environmental risk factors for syndromal and subsyndromal common DSM-IV axis I and all axis II disorders. Am J Psychiatry 2011. 168:29-39.

Kendler KS, Karkowski LM, Prescott CA. Fears and phobias: Reliability and heritability. Psychol Med 1999. 29:539-553.

Kennedy JL, Bradwejn J, Koszycki D, King N, Crowe R, Vincent J, Fourie O. Investigation of cholecystokinin system genes in panic disorder. Mol Psychiatry 1999. 4:284-285.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch Gen Psychiatry 2005a. 62:593-602.

Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity and comorbidity of 12-month DSM-IV disorders in the national comorbidity survey replication. Arch Gen Psychiatry 2005b. 62:617-627.

Kessler RC, Ruscio AM, Shear K, Wittchen HU. 2009. Epidemiology of anxiety disorders. In: Stein MB, Steckler T, editors. Behavioral neurobiology of anxiety and its treatment, Current topics in behavioral neurosciences. Heidelberg: Springer-Verlag Berlin.

Kim SJ, Kim YS, Kim SY, Lee HS, Kim CH. An association study of catechol-O-methyltransferase and monoamine oxidase A polymorphisms and personality traits in koreans. Neurosci Lett 2006. 401:154-158.

Knowles JA, Fyer AJ, Vieland VJ, Weissman MM, Hodge SE, Heiman GA, Haghighi F, de Jesus GM, Rassnick H, Preud'homme-Rivelli X, Austin T, Cunjak J, Mick S, Fine LD, Woodley KA, Das K, Maier W, Adams PB, Freimer NB, Klein DF, Gilliam TC. Results of a genome-wide genetic screen for panic disorder. Am J Med Genet 1998. 81:139-147.

Koenen KC, Amstadter AB, Nugent NR. Gene-environment interaction in posttraumatic stress disorder: An update. J Trauma Stress 2009. 22:416-426.

Kormann MS, Carr D, Klopp N, Illig T, Leupold W, Fritzsch C, Weiland SK, von Mutius E, Kabesch M. G-protein-coupled receptor polymorphisms are associated with asthma in a large german population. Am J Respir Crit Care Med 2005. 171:1358-1362.

Krömer SA, Keßler MS, Milfay D, Birg IN, Bunck M, Czibere L, Panhuysen M, Pütz B, Deussing JM, Holsboer F, Landgraf R, Turck CW. Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J Neurosci 2005. 25:4375-4384.

Kuo PH, Neale MC, Riley BP, Patterson DG, Walsh D, Prescott CA, Kendler KS. A genome-wide linkage analysis for the personality trait neuroticism in the irish affected sib-pair study of alcohol dependence. Am J Med Genet B Neuropsychiatr Genet 2007. 144B:463-468.

Kwan KY, Lam MM, Krsnik Z, Kawasawa YI, Lefebvre V, Sestan N. SOX5 postmitotically regulates migration, postmigratory differentiation, and projections of subplate and deep-layer neocortical neurons. Proc Natl Acad Sci U S A 2008. 105:16021-16026.

Lacroix JS, Ricchetti AP, Morel D, Mossimann B, Waeber B, Grouzmann E. Intranasal administration of neuropeptide Y in man: Systemic absorption and functional effects. Br J Pharmacol 1996. 118:2079-2084.

Laird NM, Lange C. Family-based designs in the age of large-scale gene-association studies. Nat Rev Genet 2006. 7:385-393.

Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, Mäkela S, Rehn M, Pirskanen A, Rautanen A, Zucchelli M, Gullsten H, Leino M, Alenius H, Petays T, Haahtela T, Laitinen A, Laprise C, Hudson TJ, Laitinen LA, Kere J. Characterization of a common susceptibility locus for asthma-related traits. Science 2004. 304:300-304.

Lander E, Kruglyak L. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat Genet 1995. 11:241-247.

Lander ES. Initial impact of the sequencing of the human genome. Nature 2011. 470:187-197.

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL,

Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ, International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001. 409:860-921.

Lavoie KL, Cartier A, Labrecque M, Bacon SL, Lemiere C, Malo JL, Lacoste G, Barone S, Verrier P, Ditto B. Are psychiatric disorders associated with worse asthma control and quality of life in asthma patients? Respir Med 2005. 99:1249-1257.

Lawford BR, Young R, Noble EP, Kann B, Ritchie T. The D2 dopamine receptor (DRD2) gene is associated with co-morbid depression, anxiety and social dysfunction in untreated veterans with post-traumatic stress disorder. Eur Psychiatry 2006. 21:180-185.

LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000. 23:155-184.

Le-Niculescu H, Balaraman Y, Patel SD, Ayalew M, Gupta J, Kuczenski R, Shekhar A, Schork N, Geyer MA, Niculescu AB. Convergent functional genomics of anxiety disorders: Translational identification of genes, biomarkers, pathways and mechanisms. Transl Psychiatry 2011. 1:e9.

Leppä E, Linden AM, Vekovischeva OY, Swinny JD, Rantanen V, Toppila E, Hoger H, Sieghart W, Wulff P, Wisden W, Korpi ER. Removal of GABA(A) receptor gamma2 subunits from parvalbumin neurons causes wide-ranging behavioral alterations. PLoS One 2011. 6:e24159.

Lindell SG, Schwandt ML, Sun H, Sparenborg JD, Bjork K, Kasckow JW, Sommer WH, Goldman D, Higley JD, Suomi SJ, Heilig M, Barr CS. Functional NPY variation as a factor in stress resilience and alcohol consumption in rhesus macaques. Arch Gen Psychiatry 2010. 67:423-431.

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 1987. 92:180-185.

Logue MW, Durner M, Heiman GA, Hodge SE, Hamilton SP, Knowles JA, Fyer AJ, Weissman MM. A linkage search for joint panic disorder/bipolar genes. Am J Med Genet B Neuropsychiatr Genet 2009. 150B:1139-1146.

Luis CA, Mittenberg W. Mood and anxiety disorders following pediatric traumatic brain injury: A prospective study. J Clin Exp Neuropsychol 2002. 24:270-279.

Lukas M, Neumann ID. Nasal application of neuropeptide S reduces anxiety and prolongs memory in rats: Social versus non-social effects. Neuropharmacology 2012. 62:398-405.

Madaan V, Wilson DR. Neuropeptides: Relevance in treatment of depression and anxiety disorders. Drug News Perspect 2009. 22:319-324.

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. Nature 2009. 461:747-753.

Maren S. Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: Cautions and caveats. Eur J Neurosci 2008. 28:1661-1666.

Maron E, Kallassalu K, Tammiste A, Kolde R, Vilo J, Toru I, Vasar V, Shlik J, Metspalu A. Peripheral gene expression profiling of CCK-4-induced panic in healthy subjects. Am J Med Genet B Neuropsychiatr Genet 2010. 153B:269-274.

Maron E, Nikopensius T, Koks S, Altmae S, Heinaste E, Vabrit K, Tammekivi V, Hallast P, Koido K, Kurg A, Metspalu A, Vasar E, Vasar V, Shlik J. Association study of 90 candidate gene polymorphisms in panic disorder. Psychiatr Genet 2005. 15:17-24.

McAdams DP, Pals JL. A new big five: Fundamental principles for an integrative science of personality. Am Psychol 2006. 61:204-217.

McCauley E, Katon W, Russo J, Richardson L, Lozano P. Impact of anxiety and depression on functional impairment in adolescents with asthma. Gen Hosp Psychiatry 2007. 29:214-222.

McGrath M, Kawachi I, Ascherio A, Colditz GA, Hunter DJ, De Vivo I. Association between catechol-O-methyltransferase and phobic anxiety. Am J Psychiatry 2004. 161:1703-1705.

McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication II: Associations with persistence of DSM-IV disorders. Arch Gen Psychiatry 2010. 67:124-132.

McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res 2011. 45:1027-1035.

Meira-Lima I, Shavitt RG, Miguita K, Ikenaga E, Miguel EC, Vallada H. Association analysis of the catechol-o-methyltransferase (COMT), serotonin transporter (5-HTT) and serotonin 2A receptor (5HT2A) gene polymorphisms with obsessive-compulsive disorder. Genes Brain Behav 2004. 3:75-79.

Meis S, Bergado-Acosta JR, Yanagawa Y, Obata K, Stork O, Munsch T. Identification of a neuropeptide S responsive circuitry shaping amygdala activity via the endopiriform nucleus. PLoS ONE 2008. 3:e2695.

Melen E, Bruce S, Doekes G, Kabesch M, Laitinen T, Lauener R, Lindgren CM, Riedler J, Scheynius A, van Hage-Hamsten M, Kere J, Pershagen G, Wickman M, Nyberg F, PARSIFAL Genetics Study Group. Haplotypes of G protein-coupled receptor 154 are associated with childhood allergy and asthma. Am J Respir Crit Care Med 2005. 171:1089-1095.

Merikangas KR, Swanson SA. 2009. Comorbidity in anxiety disorders. In: Stein MB, Steckler T, editors.
Behavioral neurobiology of anxiety and its treatment, current topics in behavioral neurosciences. Heidelberg: Springer-Verlag Berin.

Middeldorp CM, Cath DC, Van Dyck R, Boomsma DI. The co-morbidity of anxiety and depression in the perspective of genetic epidemiology. A review of twin and family studies. Psychol Med 2005. 35:611-624.

Middeldorp CM, Hottenga JJ, Slagboom PE, Sullivan PF, de Geus EJ, Posthuma D, Willemsen G, Boomsma DI. Linkage on chromosome 14 in a genome-wide linkage study of a broad anxiety phenotype. Mol Psychiatry 2008. 13:84-89.

Montag C, Basten U, Stelzel C, Fiebach CJ, Reuter M. The BDNF Val66Met polymorphism and anxiety: Support for animal knock-in studies from a genetic association study in humans. Psychiatry Res 2010. 179:86-90.

Morgan CA,3rd, Rasmusson AM, Wang S, Hoyt G, Hauger RL, Hazlett G. Neuropeptide-Y, cortisol, and subjective distress in humans exposed to acute stress: Replication and extension of previous report. Biol Psychiatry 2002. 52:136-142.

Morgane PJ, Galler JR, Mokler DJ. A review of systems and networks of the limbic forebrain/limbic midbrain. Prog Neurobiol 2005. 75:143-160.

Morton NE. Sequential tests for the detection of linkage. Am J Hum Genet 1955. 7:277-318.

Munafo MR, Durrant C, Lewis G, Flint J. Gene X environment interactions at the serotonin transporter locus. Biol Psychiatry 2009a. 65:211-219.

Munafo MR, Freimer NB, Ng W, Ophoff R, Veijola J, Miettunen J, Jarvelin MR, Taanila A, Flint J. 5-HTTLPR genotype and anxiety-related personality traits: A meta-analysis and new data. Am J Med Genet B Neuropsychiatr Genet 2009b. 150B:271-281.

Nutt D. GABAA receptors: Subtypes, regional distribution, and function. J Clin Sleep Med 2006. 2:S7-11.

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004. 74:765-769.

O'Connell JR, Weeks DE. PedCheck: A program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998. 63:259-266.

Oeth P, Beaulieu M, Park C, Kosman D, del Mistro G, van den Boom D, Jurinke C. iPLEX™ assay: Increased plexing efficiency and flexibility for MassARRAY® system through single base primer extension with mass-modified terminators. Sequenom® Application Note 2005.

Okamura N, Garau C, Duangdao DM, Clark SD, Jungling K, Pape HC, Reinscheid RK. Neuropeptide S enhances memory during the consolidation phase and interacts with noradrenergic systems in the brain. Neuropsychopharmacology 2011. 36:744-752.

Ostblom E, Lilja G, Pershagen G, van Hage M, Wickman M. Phenotypes of food hypersensitivity and development of allergic diseases during the first 8 years of life. Clin Exp Allergy 2008. 38:1325-1332.

Otowa T, Shimada T, Kawamura Y, Sugaya N, Yoshida E, Inoue K, Yasuda S, Liu X, Minato T, Tochigi M, Umekage T, Kasai K, Tanii H, Okazaki Y, Kaiya H, Sasaki T. Association of RGS2 variants with panic disorder in a japanese population. Am J Med Genet B Neuropsychiatr Genet 2011. 156B:430-434.

Otowa T, Yoshida E, Sugaya N, Yasuda S, Nishimura Y, Inoue K, Tochigi M, Umekage T, Miyagawa T, Nishida N, Tokunaga K, Tanii H, Sasaki T, Kaiya H, Okazaki Y. Genome-wide association study of panic disorder in the japanese population. J Hum Genet 2009. 54:122-126.

Otto MW, Basden SL, Leyro TM, McHugh RK, Hofmann SG. Clinical perspectives on the combination of D-cycloserine and cognitive-behavioral therapy for the treatment of anxiety disorders. CNS Spectr 2007. 12:51-6, 59-61.

Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR, Chen J. Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. J Neurosci 2008. 28:8709-8723.

Pape HC, Jungling K, Seidenbecher T, Lesting J, Reinscheid RK. Neuropeptide S: A transmitter system in the brain regulating fear and anxiety. Neuropharmacology 2010. 58:29-34.

Parsons MJ, Grimm CH, Paya-Cano JL, Sugden K, Nietfeld W, Lehrach H, Schalkwyk LC. Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function. Mamm Genome 2008. 19:552-560.

Pavlov IP. Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. 1927. London: Oxford University Press.

Pearson K. On the correlation of characters not quantitatively measurable. Phil Trans R Soc Lond A 1900. 195:1-47.

Pego JM, Sousa JC, Almeida OF, Sousa N. Stress and the neuroendocrinology of anxiety disorders. Curr Top Behav Neurosci 2010. 2:97-117.

Peltonen L, Perola M, Naukkarinen J, Palotie A. Lessons from studying monogenic disease for common disease. Hum Mol Genet 2006. 15 Spec No 1:R67-74.

Perroud N, Guipponi M, Pertusa A, Fullana MA, Iervolino AC, Cherkas L, Spector T, Collier D, Mataix-Cols D. Genome-wide association study of hoarding traits. Am J Med Genet B Neuropsychiatr Genet 2011. 156:240-242.

Philibert RA, Crowe R, Ryu GY, Yoon JG, Secrest D, Sandhu H, Madan A. Transcriptional profiling of lymphoblast lines from subjects with panic disorder. Am J Med Genet B Neuropsychiatr Genet 2007. 144B:674-682.

Pillay NS, Stein DJ. Emerging anxiolytics. Expert Opin Emerg Drugs 2007. 12:541-554.

Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bolte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Goldberg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI,Jr, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010. 466:368-372.

Pirkola S, Isometsä E, Aro H, Kestilä L, Hämäläinen J, Veijola J, Kiviruusu O, Lönnqvist J. Childhood adversities as risk factors for adult mental disorders: Results from the health 2000 study. Soc Psychiatry Psychiatr Epidemiol 2005a. 40:769-777.

Pirkola SP, Isometsä E, Suvisaari J, Aro H, Joukamaa M, Poikolainen K, Koskinen S, Aromaa A, Lönnqvist JK. DSM-IV mood-, anxiety- and alcohol use disorders and their comorbidity in the finnish general population. Soc Psychiatry Psychiatr Epidemiol 2005b. 40:1-10.

Pollack MH, Jensen JE, Simon NM, Kaufman RE, Renshaw PF. High-field MRS study of GABA, glutamate and glutamine in social anxiety disorder: Response to treatment with levetiracetam. Prog Neuropsychopharmacol Biol Psychiatry 2008. 32:739-743.

Pooley EC, Fineberg N, Harrison PJ. The met(158) allele of catechol-O-methyltransferase (COMT) is associated with obsessive-compulsive disorder in men: Case-control study and meta-analysis. Mol Psychiatry 2007. 12:556-561.

Pritchard JK, Cox NJ. The allelic architecture of human disease genes: Common disease-common variant...or not? Hum Mol Genet 2002. 11:2417-2423.

Purcell S, Cherny SS, Sham PC. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003. 19:149-150.

Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 2008. 33:56-72.

Raczka KA, Gartmann N, Mechias ML, Reif A, Buchel C, Deckert J, Kalisch R. A neuropeptide S receptor variant associated with overinterpretation of fear reactions: A potential neurogenetic basis for catastrophizing. Mol Psychiatry 2010. 15:1045, 1067-74.

Reichenberg A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S, Rabinowitz J, Shulman C, Malaspina D, Lubin G, Knobler HY, Davidson M, Susser E. Advancing paternal age and autism. Arch Gen Psychiatry 2006. 63:1026-1032.

Reinscheid RK. Neuropeptide S: Anatomy, pharmacology, genetics and physiological functions. Results Probl Cell Differ 2008. 46:145-158.

Reinscheid RK, Xu YL, Okamura N, Zeng J, Chung S, Pai R, Wang Z, Civelli O. Pharmacological characterization of human and murine neuropeptide s receptor variants. J Pharmacol Exp Ther 2005. 315:1338-1345.

Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M. Cognitive enhancers as adjuncts to psychotherapy: Use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry 2004. 61:1136-1144.

Richardson LP, Lozano P, Russo J, McCauley E, Bush T, Katon W. Asthma symptom burden: Relationship to asthma severity and anxiety and depression symptoms. Pediatrics 2006. 118:1042-1051.

Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, Merikangas KR. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. JAMA 2009. 301:2462-2471.

Risch NJ. Searching for genetic determinants in the new millennium. Nature 2000. 405:847-856.

Risch NJ, Merikangas K. The future of genetic studies of complex human diseases. Science 1996. 273:1516-1517.

Riva A, Kohane IS. SNPper: Retrieval and analysis of human SNPs. Bioinformatics 2002. 18:1681-1685.

Robinson J, Sareen J, Cox BJ, Bolton JM. Role of self-medication in the development of comorbid anxiety and substance use disorders: A longitudinal investigation. Arch Gen Psychiatry 2011. 68:800-807.

Ross J, Badner J, Garrido H, Sheppard B, Chavira DA, Grados M, Woo JM, Doo P, Umana P, Fournier E, Murray SS, Mathews CA. Genomewide linkage analysis in costa rican families implicates chromosome 15q14 as a candidate region for OCD. Hum Genet 2011. 130:795-805.

Rothe C, Koszycki D, Bradwejn J, King N, Deluca V, Tharmalingam S, Macciardi F, Deckert J, Kennedy JL. Association of the Val158Met catechol O-methyltransferase genetic polymorphism with panic disorder. Neuropsychopharmacology 2006. 31:2237-2242.

Rowe DC, Stever C, Gard JM, Cleveland HH, Sanders ML, Abramowitz A, Kozol ST, Mohr JH, Sherman SL, Waldman ID. The relation of the dopamine transporter gene (DAT1) to symptoms of internalizing disorders in children. Behav Genet 1998. 28:215-225.

Roy-Byrne PP, Davidson KW, Kessler RC, Asmundson GJ, Goodwin RD, Kubzansky L, Lydiard RB, Massie MJ, Katon W, Laden SK, Stein MB. Anxiety disorders and comorbid medical illness. Gen Hosp Psychiatry 2008. 30:208-225.

Salim S, Asghar M, Taneja M, Hovatta I, Wu YL, Saha K, Sarraj N, Hite B. Novel role of RGS2 in regulation of antioxidant homeostasis in neuronal cells. FEBS Lett 2011. 585:1375-1381.

Samochowiec J, Syrek S, Michal P, Ryzewska-Wodecka A, Samochowiec A, Horodnicki J, Zakrzewska M, Kucharska-Mazur J. Polymorphisms in the serotonin transporter and monoamine oxidase A genes and their relationship to personality traits measured by the temperament and character inventory and NEO five-factor inventory in healthy volunteers. Neuropsychobiology 2004. 50:174-181.

Samuels J, Shugart YY, Grados MA, Willour VL, Bienvenu OJ, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Wang Y, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Cullen B, Rasmussen SA, Hoehn-Saric R, Valle D, Liang KY, Riddle MA, Nestadt G. Significant linkage to compulsive hoarding on chromosome 14 in families with obsessive-compulsive disorder: Results from the OCD collaborative genetics study. Am J Psychiatry 2007. 164:493-499.

Sangha S, Narayanan RT, Bergado-Acosta JR, Stork O, Seidenbecher T, Pape HC. Deficiency of the 65 kDa isoform of glutamic acid decarboxylase impairs extinction of cued but not contextual fear memory. J Neurosci 2009. 29:15713-15720.

Sarapas C, Cai G, Bierer LM, Golier JA, Galea S, Ising M, Rein T, Schmeidler J, Muller-Myhsok B, Uhr M, Holsboer F, Buxbaum JD, Yehuda R. Genetic markers for PTSD risk and resilience among survivors of the world trade center attacks. Dis Markers 2011. 30:101-110.

Sareen J, Cox BJ, Clara I, Asmundson GJ. The relationship between anxiety disorders and physical disorders in the U.S. national comorbidity survey. Depress Anxiety 2005. 21:193-202.

Schork NJ, Fallin D, Lanchbury JS. Single nucleotide polymorphisms and the future of genetic epidemiology. Clin Genet 2000. 58:250-264.

Segman RH, Cooper-Kazaz R, Macciardi F, Goltser T, Halfon Y, Dobroborski T, Shalev AY. Association between the dopamine transporter gene and posttraumatic stress disorder. Mol Psychiatry 2002. 7:903-907.

Segman RH, Shefi N, Goltser-Dubner T, Friedman N, Kaminski N, Shalev AY. Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. Mol Psychiatry 2005. 10:500-13, 425.

Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. Am J Med Genet B Neuropsychiatr Genet 2004. 127B:85-89.

Shah SH, Freedman NJ, Zhang L, Crosslin DR, Stone DH, Haynes C, Johnson J, Nelson S, Wang L, Connelly JJ, Muehlbauer M, Ginsburg GS, Crossman DC, Jones CJ, Vance J, Sketch MH, Granger CB, Newgard CB, Gregory SG, Goldschmidt-Clermont PJ, Kraus WE, Hauser ER. Neuropeptide Y gene polymorphisms confer risk of early-onset atherosclerosis. PLoS Genet 2009. 5:e1000318.

Shifman S, Bhomra A, Smiley S, Wray NR, James MR, Martin NG, Hettema JM, An SS, Neale MC, van den Oord EJ, Kendler KS, Chen X, Boomsma DI, Middeldorp CM, Hottenga JJ, Slagboom PE, Flint J. A whole genome association study of neuroticism using DNA pooling. Mol Psychiatry 2008. 13:302-312.

Shin LM, Kosslyn SM, McNally RJ, Alpert NM, Thompson WL, Rauch SL, Macklin ML, Pitman RK. Visual imagery and perception in posttraumatic stress disorder. A positron emission tomographic investigation. Arch Gen Psychiatry 1997. 54:233-241.

Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology 2010. 35:169-191.

Shugart YY, Samuels J, Willour VL, Grados MA, Greenberg BD, Knowles JA, McCracken JT, Rauch SL, Murphy DL, Wang Y, Pinto A, Fyer AJ, Piacentini J, Pauls DL, Cullen B, Page J, Rasmussen SA, Bienvenu OJ, Hoehn-Saric R, Valle D, Liang KY, Riddle MA, Nestadt G. Genomewide linkage scan for obsessive-compulsive disorder: Evidence for susceptibility loci on chromosomes 3q, 7p, 1q, 15q, and 6q. Mol Psychiatry 2006. 11:763-770.

Sillanpää M, Andlin-Sobocki P, Lönnqvist J. Costs of brain disorders in finland. Acta Neurol Scand 2008. 117:167-172.

Sipilä T, Kananen L, Greco D, Donner J, Silander K, Terwilliger JD, Auvinen P, Peltonen L, Lönnqvist J, Pirkola S, Partonen T, Hovatta I. An association analysis of circadian genes in anxiety disorders. Biol Psychiatry 2010. 67:1163-1170.

Smith KL, Patterson M, Dhillo WS, Patel SR, Semjonous NM, Gardiner JV, Ghatei MA, Bloom SR. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology 2006. 147:3510-3518.

Smoller JW. Who's afraid of anxiety genetics? Biol Psychiatry 2011. 69:506-507.

Smoller JW, Block SR, Young MM. Genetics of anxiety disorders: The complex road from DSM to DNA. Depress Anxiety 2009. 26:965-975.

Smoller JW, Gardner-Schuster E, Misiaszek M. Genetics of anxiety: Would the genome recognize the DSM? Depress Anxiety 2008a. 25:368-377.

Smoller JW, Paulus MP, Fagerness JA, Purcell S, Yamaki LH, Hirshfeld-Becker D, Biederman J, Rosenbaum JF, Gelernter J, Stein MB. Influence of RGS2 on anxiety-related temperament, personality, and brain function. Arch Gen Psychiatry 2008b. 65:298-308.

Smoller JW, Rosenbaum JF, Biederman J, Kennedy J, Dai D, Racette SR, Laird NM, Kagan J, Snidman N, Hirshfeld-Becker D, Tsuang MT, Sklar PB, Slaugenhaupt SA. Association of a genetic marker at the corticotropin-releasing hormone locus with behavioral inhibition. Biol Psychiatry 2003. 54:1376-1381.

Smoller JW, Rosenbaum JF, Biederman J, Susswein LS, Kennedy J, Kagan J, Snidman N, Laird N, Tsuang MT, Faraone SV, Schwarz A, Slaugenhaupt SA. Genetic association analysis of behavioral inhibition using candidate loci from mouse models. Am J Med Genet 2001. 105:226-235.

Smoller JW, Yamaki LH, Fagerness JA, Biederman J, Racette S, Laird NM, Kagan J, Snidman N, Faraone SV, Hirshfeld-Becker D, Tsuang MT, Slaugenhaupt SA, Rosenbaum JF, Sklar PB. The corticotropin-releasing hormone gene and behavioral inhibition in children at risk for panic disorder. Biol Psychiatry 2005. 57:1485-1492.

Smoller JW, Acierno JS, Rosenbaum JF, Biederman J, Pollack MH, Meminger S, Pava JA, Chadwick LH, White C, Bulzacchelli M, Slaugenhaupt SA. Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions. Am J Med Genet 2001. 105:195-206.

Somers JM, Goldner EM, Waraich P, Hsu L. Prevalence and incidence studies of anxiety disorders: A systematic review of the literature. Can J Psychiatry 2006. 51:100-113.

Sommer WH, Lidstrom J, Sun H, Passer D, Eskay R, Parker SC, Witt SH, Zimmermann US, Nieratschker V, Rietschel M, Margulies EH, Palkovits M, Laucht M, Heilig M. Human NPY promoter variation rs16147:T>C as a moderator of prefrontal NPY gene expression and negative affect. Hum Mutat 2010. 31:E1594-608.

Sourander A, Jensen P, Davies M, Niemelä S, Elonheimo H, Ristkari T, Helenius H, Sillanmäki L, Piha J, Kumpulainen K, Tamminen T, Moilanen I, Almqvist F. Who is at greatest risk of adverse long-term outcomes? the finnish from a boy to a man study. J Am Acad Child Adolesc Psychiatry 2007. 46:1148-1161.

Stein MB, Schork NJ, Gelernter J. Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. Neuropsychopharmacology 2008. 33:312-319.

Stone MH. 2009. History of anxiety disorders. In: Textbook of anxiety disorders. Arlington, VA: American Psychiatric Publishing. p 3-15.

Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003. 100:9440-9445.

Stork O, Ji FY, Kaneko K, Stork S, Yoshinobu Y, Moriya T, Shibata S, Obata K. Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. Brain Res 2000. 865:45-58.

Stork O, Yamanaka H, Stork S, Kume N, Obata K. Altered conditioned fear behavior in glutamate decarboxylase 65 null mutant mice. Genes Brain Behav 2003. 2:65-70.

Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR. Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry 2007. 12:854-869.

Strug LJ, Suresh R, Fyer AJ, Talati A, Adams PB, Li W, Hodge SE, Gilliam TC, Weissman MM. Panic disorder is associated with the serotonin transporter gene (SLC6A4) but not the promoter region (5-HTTLPR). Mol Psychiatry 2010. 15:166-176.

Suvisaari J, Aalto-Setala T, Tuulio-Henriksson A, Harkanen T, Saarni SI, Perala J, Schreck M, Castaneda A, Hintikka J, Kestila L, Lahteenmaki S, Latvala A, Koskinen S, Marttunen M, Aro H, Lonnqvist J. Mental disorders in young adulthood. Psychol Med 2009. 39:287-299.

Szego EM, Janaky T, Szabo Z, Csorba A, Kompagne H, Muller G, Levay G, Simor A, Juhasz G, Kekesi KA. A mouse model of anxiety molecularly characterized by altered protein networks in the brain proteome. Eur Neuropsychopharmacol 2010. 20:96-111.

Tadic A, Rujescu D, Szegedi A, Giegling I, Singer P, Moller HJ, Dahmen N. Association of a MAOA gene variant with generalized anxiety disorder, but not with panic disorder or major depression. Am J Med Genet B Neuropsychiatr Genet 2003. 117B:1-6.

Talbot CJ, Nicod A, Cherny SS, Fulker DW, Collins AC, Flint J. High-resolution mapping of quantitative trait loci in outbred mice. Nat Genet 1999. 21:305-308.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007. 24:1596-1599.

Tasan RO, Bukovac A, Peterschmitt YN, Sartori SB, Landgraf R, Singewald N, Sperk G. Altered GABA transmission in a mouse model of increased trait anxiety. Neuroscience 2011. 183:71-80.

Terracciano A, Sanna S, Uda M, Deiana B, Usala G, Busonero F, Maschio A, Scally M, Patriciu N, Chen WM, Distel MA, Slagboom EP, Boomsma DI, Villafuerte S, Sliwerska E, Burmeister M, Amin N, Janssens AC, van Duijn CM, Schlessinger D, Abecasis GR, Costa PT,Jr. Genome-wide association scan for five major dimensions of personality. Mol Psychiatry 2010. 15:647-656.

Terwilliger JD, Ott J. Handbook of human genetic linkage. 1994. Baltimore, MD: Johns Hopkins University Press.

The EuroQol Group. EuroQol--a new facility for the measurement of health-related quality of life. Health Policy 1990. 16:199-208.

The International HapMap Consortium. The international HapMap project. Nature 2003. 426:789-796.

Thorgeirsson TE, Oskarsson H, Desnica N, Kostic JP, Stefansson JG, Kolbeinsson H, Lindal E, Gagunashvili N, Frigge ML, Kong A, Stefansson K, Gulcher JR. Anxiety with panic disorder linked to chromosome 9q in iceland. Am J Hum Genet 2003. 72:1221-1230.

Thorsell A. Central neuropeptide Y in anxiety- and stress-related behavior and in ethanol intake. Ann N Y Acad Sci 2008. 1148:136-140.

Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, Mathe AA, Heilig M. Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. Proc Natl Acad Sci U S A 2000. 97:12852-12857.

Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: Population substructure and genome-wide association studies. Hum Mol Genet 2008. 17:R143-50.

Tiihonen J, Kuikka J, Bergström K, Lepola U, Koponen H, Leinonen E. Dopamine reuptake site densities in patients with social phobia. Am J Psychiatry 1997. 154:239-242.

Tillfors M, Furmark T, Marteinsdottir I, Fischer H, Pissiota A, Langstrom B, Fredrikson M. Cerebral blood flow in subjects with social phobia during stressful speaking tasks: A PET study. Am J Psychiatry 2001. 158:1220-1226.

Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. Biol Psychiatry 2005. 57:252-260.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M, STAR*D Study Team. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. Am J Psychiatry 2006. 163:28-40.

Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 2002. 53:865-871.

Turri MG, Datta SR, DeFries J, Henderson ND, Flint J. QTL analysis identifies multiple behavioral dimensions in ethological tests of anxiety in laboratory mice. Curr Biol 2001. 11:725-734.

Uusi-Oukari M, Korpi ER. Regulation of GABA(A) receptor subunit expression by pharmacological agents. Pharmacol Rev 2010. 62:97-135.

van den Oord EJ, Kuo PH, Hartmann AM, Webb BT, Moller HJ, Hettema JM, Giegling I, Bukszar J, Rujescu D. Genomewide association analysis followed by a replication study implicates a novel candidate gene for neuroticism. Arch Gen Psychiatry 2008. 65:1062-1071.

Vendelin J, Bruce S, Holopainen P, Pulkkinen V, Rytila P, Pirskanen A, Rehn M, Laitinen T, Laitinen LA, Haahtela T, Saarialho-Kere U, Laitinen A, Kere J. Downstream target genes of the neuropeptide S-NPSR1 pathway. Hum Mol Genet 2006. 15:2923-2935.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di Francesco V, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferriera S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, Nodell M, Pan S, Peck J, Peterson M, Rowe W, Sanders R, Scott J, Simpson M, Smith T, Sprague A, Stockwell T, Turner R, Venter E, Wang M, Wen M, Wu D, Wu M, Xia A, Zandieh A, Zhu X. The sequence of the human genome. Science 2001. 291:1304-1351.

Virok DP, Kis Z, Szegedi V, Juhasz G, Zvara A,Jr, Muller G, Levay G, Harsing LG, Rajko R, Penke B, Janka Z, Janaky T, Puskas LG. Functional changes in transcriptomes of the prefrontal cortex and hippocampus in a mouse model of anxiety. Pharmacol Rep 2011. 63:348-361.

Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. Nat Rev Genet 2003. 4:587-597.

Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989. 44:388-396.

Weiss KM, Terwilliger JD. How many diseases does it take to map a gene with SNPs? Nat Genet 2000. 26:151-157.

Weissman MM, Fyer AJ, Haghighi F, Heiman G, Deng Z, Hen R, Hodge SE, Knowles JA. Potential panic disorder syndrome: Clinical and genetic linkage evidence. Am J Med Genet 2000. 96:24-35.

Wermter AK, Laucht M, Schimmelmann BG, Banaschweski T, Sonuga-Barke EJ, Rietschel M, Becker K. From nature versus nurture, via nature and nurture, to gene x environment interaction in mental disorders. Eur Child Adolesc Psychiatry 2010. 19:199-210.

Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: Presentation of a prospective longitudinal birth cohort study. Pediatr Allergy Immunol 2002. 13 Suppl 15:11-13.

Williams R,4th, Lim JE, Harr B, Wing C, Walters R, Distler MG, Teschke M, Wu C, Wiltshire T, Su AI, Sokoloff G, Tarantino LM, Borevitz JO, Palmer AA. A common and unstable copy number variant is associated with differences in Glo1 expression and anxiety-like behavior. PLoS One 2009. 4:e4649.

Witt SH, Buchmann AF, Blomeyer D, Nieratschker V, Treutlein J, Esser G, Schmidt MH, Bidlingmaier M, Wiedemann K, Rietschel M, Laucht M, Wust S, Zimmermann US. An interaction between a neuropeptide Y gene polymorphism and early adversity modulates endocrine stress responses. Psychoneuroendocrinology 2011.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-

Carulla L, Simon R, Steinhausen HC. The size and burden of mental disorders and other disorders of the brain in europe 2010. Eur Neuropsychopharmacol 2011. 21:655-679.

World Health Organization. The ICD-10 classification of mental and behavioural disorders: Diagnostic criteria for research. 1993. Geneva: World Health Organization.

Wray NR, James MR, Gordon SD, Dumenil T, Ryan L, Coventry WL, Statham DJ, Pergadia ML, Madden PA, Heath AC, Montgomery GW, Martin NG. Accurate, large-scale genotyping of 5HTTLPR and flanking single nucleotide polymorphisms in an association study of depression, anxiety, and personality measures. Biol Psychiatry 2009. 66:468-476.

Wray NR, James MR, Mah SP, Nelson M, Andrews G, Sullivan PF, Montgomery GW, Birley AJ, Braun A, Martin NG. Anxiety and comorbid measures associated with PLXNA2. Arch Gen Psychiatry 2007. 64:318-326.

Wray NR, Middeldorp CM, Birley AJ, Gordon SD, Sullivan PF, Visscher PM, Nyholt DR, Willemsen G, de Geus EJ, Slagboom PE, Montgomery GW, Martin NG, Boomsma DI. Genome-wide linkage analysis of multiple measures of neuroticism of 2 large cohorts from australia and the netherlands. Arch Gen Psychiatry 2008. 65:649-658.

Wright MW, Bruford EA. Naming 'junk': Human non-protein coding RNA (ncRNA) gene nomenclature. Hum Genomics 2011. 5:90-98.

Wu G, Feder A, Wegener G, Bailey C, Saxena S, Charney D, Mathe AA. Central functions of neuropeptide Y in mood and anxiety disorders. Expert Opin Ther Targets 2011. 15:1317-1331.

Xie B, Wang B, Suo P, Kou C, Wang J, Meng X, Cheng L, Ma X, Yu Y. Genetic association between BDNF gene polymorphisms and phobic disorders: A case-control study among mainland han chinese. J Affect Disord 2011. 132:239-242.

Xie P, Kranzler HR, Poling J, Stein MB, Anton RF, Brady K, Weiss RD, Farrer L, Gelernter J. Interactive effect of stressful life events and the serotonin transporter 5-HTTLPR genotype on posttraumatic stress disorder diagnosis in 2 independent populations. Arch Gen Psychiatry 2009. 66:1201-1209.

Xie P, Kranzler HR, Poling J, Stein MB, Anton RF, Farrer LA, Gelernter J. Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. Neuropsychopharmacology 2010. 35:1684-1692.

Xu YL, Gall CM, Jackson VR, Civelli O, Reinscheid RK. Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. J Comp Neurol 2007. 500:84-102.

Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, Brucher FA, Zeng J, Ly NK, Henriksen SJ, de Lecea L, Civelli O. Neuropeptide S: A neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004. 43:487-497.

Yalcin B, Willis-Owen SA, Fullerton J, Meesaq A, Deacon RM, Rawlins JN, Copley RR, Morris AP, Flint J, Mott R. Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. Nat Genet 2004. 36:1197-1202.

Yehuda R, Kahana B, Binder-Brynes K, Southwick SM, Mason JW, Giller EL. Low urinary cortisol excretion in holocaust survivors with posttraumatic stress disorder. Am J Psychiatry 1995. 152:982-986.

Young RM, Lawford BR, Noble EP, Kann B, Wilkie A, Ritchie T, Arnold L, Shadforth S. Harmful drinking in military veterans with post-traumatic stress disorder: Association with the D2 dopamine receptor A1 allele. Alcohol Alcohol 2002. 37:451-456.

Zaubler TS, Katon W. Panic disorder and medical comorbidity: A review of the medical and psychiatric literature. Bull Menninger Clin 1996. 60:A12-38.

Zhang TY, Hellstrom IC, Bagot RC, Wen X, Diorio J, Meaney MJ. Maternal care and DNA methylation of a glutamic acid decarboxylase 1 promoter in rat hippocampus. J Neurosci 2010. 30:13130-13137.

Zhou Z, Zhu G, Hariri AR, Enoch MA, Scott D, Sinha R, Virkkunen M, Mash DC, Lipsky RH, Hu XZ, Hodgkinson CA, Xu K, Buzas B, Yuan Q, Shen PH, Ferrell RE, Manuck SB, Brown SM, Hauger RL, Stohler CS, Zubieta JK, Goldman D. Genetic variation in human NPY expression affects stress response and emotion. Nature 2008. 452:997-1001.

Zhu H, Perkins C, Mingler MK, Finkelman FD, Rothenberg ME. The role of neuropeptide S and neuropeptide S receptor 1 in regulation of respiratory function in mice. Peptides 2011. 32:818-825.

Zhu M, Zhao S. Candidate gene identification approach: Progress and challenges. Int J Biol Sci 2007. 3:420-427.

Zieker J, Zieker D, Jatzko A, Dietzsch J, Nieselt K, Schmitt A, Bertsch T, Fassbender K, Spanagel R, Northoff H, Gebicke-Haerter PJ. Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. Mol Psychiatry 2007. 12:116-118.