Genetic and B Cell Functional Studies of X-linked Immunodeficiencies

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SUMMARY

Eight types of X-linked immunodeficiency diseases have been described. In this thesis, I will focus on four, viz X-linked agammaglobulinaemia (XLA), X-linked severe combined immunodeficiency (X-linked SCID), Wiskott-Aldrich syndrome (WAS) and X-linked hyperimmunoglobulinaemia M (X-linked hyperIgM). Their clinical features, treatment and prognosis as well as cellular and genetic aspects are reviewed in Chapter 1; followed by objectives of the studies presented in this thesis, viz localization of the gene loci of XLA and X-linked SCID, clinical application of the linked DNA probes in families with XLA and identification of the B cell defects in patients with XLA and WAS. The practical issues of collecting patients and families for linkage analysis, as well as their immunological profiles and pedigrees are given in Chapter 2. Various laboratory techniques employed in these studies are detailed in Chapter 3. There are five sections in Chapter 4, which is on the genetic studies of XLA. Section one reviews the principle of linkage analysis, genetic heterogeneity and restriction fragment length polymorphism (RFLP). Results of the genetic localization of XLA to Xq21.3-q22 are presented in section two. Evidence of non-allelic genetic heterogeneity in XLA is presented in section three, followed by the analysis of all the family data of XLA in the literature in order to estimate the proportion of families unlinked to Xq21.3-q22, which is probably 10-20%. The

posterior probability of each family being linked to Xq21.3-q22 is also estimated. Section four describes the clinical application of the two linked probes, S21 and pXG12, in the genetic counselling of thirteen families with XLA; as well as developing a method of risk calculation allowing for non-allelic genetic heterogeneity. Seven obligate carriers under the age of 45 can all be offered prenatal diagnosis. Of the thirty-four females at risk of being carriers, seventeen have their risks increased, fifteen decreased and two unchanged by the RFLP results. Eleven of the seventeen women whose risks were increased are under 45 years of age and seven of them can be offered prenatal diagnosis. Successful predictions have been made in a newborn male infant and a male fetus at risk of being affected with XLA. Section five presents the evidence that X-linked hyperIgM is not an allelic genetic disease with XLA. Chapter 5 presents the results of the genetic localization of X-linked SCID to Xq11-q13 and the clinical application of the linked probe, cpX73, in carrier detection. The results of the functional studies of Epstein-Barr virus (EBV) tranformed B cell lines from patients with XLA and WAS are presented in Chapter 6. B cell lines from patients with WAS did not differ from normal B cell lines in any of the functional assays I have used. However, differences were found in B cells from patients with XLA. EBV-transformed B cell lines from patients with XLA did not proliferate in response to KG1-a supernatant and they did not produce IgG in the presence or absence of various B cell growth and

differentiation factors. Finally, Chapter 7 summarises the two approaches of investigations adopted in this thesis, which are applicable in investigating any diseases of single gene defect; future directions are also speculated.

Chapter 1 INTRODUCTION

1.1 PRIMARY IMMUNODEFICIENCY DISEASES

Since the first recorded case of primary agammaglobulinaemia in a boy over 35 years ago by Bruton (1952), an ever increasing number of primary immunodeficiency diseases have been described, affecting nearly all aspects of immunity (Rosen et al 1983, 1984a, 1984b). Specific deficiencies in the B-cell (antibody) system, T-cell (cellular immune) system, phagocytic system or complement system have all been described (Rosen et al 1983) and the primary immunodeficiencies can be classified into these four types depending upon which arm of immunity is predominantly affected (Stiehm and Fulginiti 1980).

Antibody immunodeficiencies are the most common and comprise about 50 percent of the primary immunodeficiencies; cellular immunodeficiencies comprise about 40 percent and most of these patients also have associated antibody deficiencies. Phagocytic immunodeficiencies account for 6 percent and complement deficiencies 4 percent of the total primary immunodeficiencies (Stiehm and Fulginiti 1980).

1.1.1 INCIDENCE

The most prevalent immunodeficiency is selective IgA deficiency, with an average frequency of 1 in 568 among blood donors (Hanson et al 1983). It can occur in healthy individuals but may also be associated with recurrent

infections, atopy and autoimmunity. There is otherwise very little data on the incidence of primary immunodeficiencies. In countries where immunodeficiency registers were established (Sweden, Australia and Japan), the incidences of various primary immunodeficiencies have been estimated (Fasth 1982, Hayakawa et al 1981, Hosking and Roberton 1983). The overall incidence of significant symptomatic antibody deficiency was between 1 in 8,000 to 1 in 11,000 live births and that of severe combined immunodeficiency to be 1 in 70,000 live births (Hosking and Roberton 1983, Fasth 1982). In Sweden, the incidence of granulocyte deficiencies was estimated to be 1 in 15,000 live births (Fasth 1982). Apart from C9 deficiency, which is relatively common (0.1%) in Japanese people, other complement deficiencies are extremely rare (Rother 1986). C9 deficiency is not associated with clinical disease.

1.1.2 AGE AND SEX

The age and sex profile of the primary immunodeficiencies can be estimated from a survey of hypogammaglobulinaemia in 176 patients (MRC working party 1969). Although it is a study on hypogammaglobulinaemia only and may include some acquired immunodeficiency cases, it still reflects the overall impression of the age and sex pattern of primary immunodeficiencies; 17 percent of the diagnosed cases were in infants less than 1 year old and 41 percent were in children age 1 to 15 years old. Therefore, children accounted for 58 percent of the cases

and three-quarters of these were under age 5 years (MRC working party 1969). There was a predominance of males (83 percent) in these children under age 15 years while in adults over age 15, only 41 percent were male (MRC working party 1969). This probably reflects that late-onset common variable immunodeficiency is more prevalent in females (Stiehm and Fulginiti 1980).

1.1.3 BASIC DEFECTS

The basic defects in most of the primary immunodeficiencies are not known. The few diseases whose biochemical defects are well understood include adenosine deaminase deficiency (Cohen et al 1978a), purine nucleoside phosphorylase deficiency (Cohen et al 1978b), X-linked chronic granulomatous disease (Teahan et al 1987), bare lymphocyte syndrome (de Preval et al 1985, Lisowska-Grospierre et al 1985) and leucocyte adherance defect (Lisowska-Grospierre et al 1986). For the majority of the immunodeficiencies, identification of the defect is still limited to the cell types and function involved.

1.1.4 GENETIC INHERITANCE

Genetic factors are pre-eminent in many of the immunodeficiencies and genetic predictions are possible in some of the well-defined diseases (Lau and Levinsky 1988a). Several immunodeficiency diseases are inherited as single-gene defects, either in X-linked or autosomal inheritance (Table 1). There are others in which inheritance pattern is not clear but familial occurence is

Table 1

PRIMARY IMMUNODEFICIENCY DISEASES OF MENDELIAN INHERITANCE

AUTOSOMAL RECESSIVE INHERITANCE

Severe combined immunodeficiency due to:
1. Adenosine deaminase deficiency
2. Purine nucleoside phosphorylase deficiency
3. Absence of HLA class I and/or II (Bare lymphocyte syndrome)
4. Reticular dysgenesis

Leucocyte adhesion deficiency
Ataxia telangiectasia
Chronic granulomatous disease
Chediak-Higashi syndrome
Short-limbed dwarfism with severe combined immunodeficiency

X-LINKED INHERITANCE

X-linked agammaglobulinaemia
X-linked severe combined immunodeficiency
Wiskott-Aldrich syndrome
X-linked hyperimmunoglobulinaemia M
X-linked chronic granulomatous disease
X-linked lymphoproliferative disease
X-linked properdin deficiency
X-linked agammaglobulinaemia with growth hormone deficiency

evident, e.g. selective IgA deficiency (Nell et al 1972).

There are at least eight X-linked immunodeficiency diseases described (Table 1). Six of these result from some defects within the lymphoid system, viz X-linked agammaglobulinaemia, X-linked severe combined immunodeficiency, Wiskott-Aldrich syndrome, X-linked hyper-immunoglobulinaemia M, X-linked lymphoproliferative disease (Purtilo et al 1977,Hamilton et al 1980) and X-linked agammaglobulinaemia with growth hormone deficiency (Fleischer et al 1980). The remaining two, viz chronic granulomatous disease and properdin deficiency, involve the phagocytic and the complement systems respectively, and their basic biochemical defects have been elucidated recently (Teahan et al 1987, Dinauer et al 1987, Densen et al 1987).

1.1.5 X CHROMOSOME AND THE LYMPHOID SYSTEM

Since at least six immunodeficiency diseases involving the lymphoid system are X-linked and neither the immunoglobulin genes nor the T-cell receptor genes are themselves X-linked, it has been suggested the X-linked disease loci might include regulatory genes responsible for lymphocyte development. This hypothesis is supported by some work on a mouse model (CBA/N mice) for X-linked immunodeficiency (Cohen et al 1985a, 1985b). CBA/N mice have an X-linked mutation, xid, which renders them incapable of producing antibodies to soluble polysaccharide (Scher 1982). Cohen et al (1985a, 1985b) isolated a murine X-linked cDNA clone, termed XLR (X-

linked, lymphocyte-regulated) which is closely linked to the xid locus and is developmentally expressed in lymphocytes from normal mice but not from those carrying the xid mutation; XLR represents a family of about ten genes. Therefore, with a developmentally-regulated expression which is restricted to lymphocytes, the XLR genes may well be responsible for regulating lymphocyte development in the mouse. Recently, Siegel et al (1987) found a single major XLR transcript, termed pM1, expressed by both B and T lymphocytes by analysing XLR cDNA clones generated from B-lineage tumours and from thymic tissue. This XLR transcript codes for a protein of 24 kilodaltons and its predicted amino acid sequence displays homology to the nuclear envelope constituents lamins A and C. However, the gene as defined by pM1 is not closely linked to xid, in contrast to other members of the XLR gene family (Siegel et al 1987, Cohen et al 1985a). Moreover, a cDNA clone identical to pM1 was isolated from a cDNA library from splenic RNA from a mouse strain BXSB.xid with the disease xid, thereby ruling out the possibility that the xid mutation is associated with any direct change in the sequence of this major XLR transcript, pM1 (Siegel et al 1987). In humans with Wiskott-Aldrich syndrome, there is also a defect in producing antibodies to polysaccharide antigens (Blaese et al 1968) as with the CBA/N mice, but unlike the mouse disease, Wiskott-Aldrich syndrome affects T-cell and platelet function as well (Blaese et al 1968). Therefore, it is probable that there is also a series of

X-linked genes in the human responsible for regulating lymphocyte development, the abberations of which will lead to the various X-linked immunodeficiency diseases. In this thesis, I will focus on X-linked agammaglobulinaemia, X-linked severe combined immunodeficiency, Wiskott-Aldrich syndrome and X-linked hyperimmunoglobulinaemia M only.

1.2 X-LINKED AGAMMAGLOBULINAEMIA

1.2.1 DEFINITION

X-linked agammaglobulinaemia (XLA) is a syndrome in boys with serious and recurrent bacterial infections starting usually in the second half of first year of life, characterised by a markedly diminished level of all serum immunoglobulin isotypes, a lack of circulating B cells, inability to make antibodies even after stimulation with a potent antigen and absence of plasma cells in lymphoid tissue (Rosen et al 1984a, 1984b). Cellular immunity is intact and X-linked inheritance ascertained from pedigree analysis.

1.2.2 CLINICAL FEATURES

In 1952, Bruton described the first boy with agammaglobulinaemia. Lederman and Winkelstein (1985) then reviewed 96 patients with XLA. The patients usually present with recurrent bacterial infections between three and nine months of age as earlier onset is prevented by maternal antibodies which only decline to insignificant levels after first few months of life. The pyogenic infections include mainly recurrent sinopulmonary

infections, osteomyelitis, meningitis and sepsis; the organisms are usually encapsulated such as haemophilus influenzae, pneumococci and streptococci. Immunity to viruses is in general intact with the exception of persistent and usually fatal echovirus infection, especially of the central nervous system (Wilfert et al 1977, Bardelas et al 1977) and possibly to hepatitis B as well (Good and Page 1960, Thomas et al 1974). Resistance to fungal infection and to tuberculosis, which depends on cellular immunity, remains intact. Other infectious complications include diarrhoea and malabsorption secondary to giardiasis (Ochs et al 1972) and chronic arthritis secondary to ureaplasma urealyticum (Stuckey et al 1978). Inadvertent administration of live oral poliovirus vaccine has also led to paralytic poliomyelitis (Wright et al 1977) and therefore no live vaccine should be given to patients with XLA.

1.2.3 TREATMENT AND PROGNOSIS

The main stay of treatment is adequate immunoglobulin replacement therapy and aggressive treatment of any infection. This is in order to minimise end-organ damage such as bronchiectasis and cor pulmonale, which now accounts for the majority of morbidity and mortality (Lederman and Winkelstein 1985). Unlike certain other immunodeficiencies, such as Wiskott-Aldrich syndrome and ataxia-telangiectasia, there is only a minor association with malignancy. Only 2 cases of cancer were observed amongst 274 patients with XLA in the immunodeficiency-

cancer registry survey between 1975 and 1977 (Spector et al 1978).

1.2.4 CELLULAR DEFECTS

The primary defect in XLA is a lack of B lymphocytes in the circulation and lymphoid tissue (Siegal et al 1971) although pre-B cells are usually present in the bone marrow (Pearl et al 1978). It has been postulated that the underlying defect involves differentiation of B lymphocytes. However this defect is not complete as these patients do have small amount of serum immunoglobulins and very low number of circulating B cells (Conley 1985). Epstein-Barr virus transformed lymphoblastoid cell lines have been derived from the peripheral blood (Levitt et al 1984) and bone marrow (Fu et al 1980) of these patients and were shown to secrete IgM (Levitt et al 1984). Therefore, these patients do have small number of B lymphocytes which are capable of differentiating into antibody forming plasma cells.

The defect in B cell differentiation could be intrinsic or extrinsic to the B cells. In vitro study of peripheral blood mononuclear cells from patients has demonstrated a suppressor effect upon differentiation of normal lymphocytes to plasma cells (Siegal et al 1976, Herrod and Buckley 1979). However, this in-vitro suppression was not observed in all the patients studied and could be a secondary phenomenon. No in-vitro immunoglobulin synthesis or secretion was demonstrated from the patients' peripheral blood mononuclear cells,

whether unstimulated or stimulated with pokeweed mitogen (Choi et al 1972, Herrod and Buckley 1979). The functional in-vitro study has not been able to resolve the issue whether the primary defect involves the B cells intrinsically or whether the failure of B cell development is secondary to some non-B cell defect. However, it is now clear from X chromosome inactivation analysis of the carriers (see following section) that the primary defect of XLA does involve the B cells intrinsically.

Edwards et al (1978) reported deficiency of ecto-5'-nucleotidase in patients with congenital agamma-globulinaemia but it has been subsequently shown to be a secondary effect due to absence of B cells (Thompson et al 1979). B cells have at least three times the activity of ecto-5'-nucleotidase of T cells and hence the simplest explanation for reduced activity of ecto-5'-nucleotidase in peripheral blood mononuclear cells from patients with XLA is the absence in these patients of B cells (Thompson et al 1979, 1980). Ecto-5'-nucleotidase seems to be a marker of B cell maturation and its role in normal B cell function is unknown. Banks et al (1976) has reported absence of B lymphocytes in a horse with primary agammaglobulinaemia, which may be comparable to XLA in man.

1.2.5 PHENOTYPIC HETEROGENEITY WITHIN AND BETWEEN FAMILIES

In an extended pedigree of XLA with seven affected males, Leickley and Buckley (1986) demonstrated heterogeneity in terms of clinical presentation, serum

immunoglobulin concentrations (one had a normal IgA and tetanus antibodies) and B cell phenotypes (the two youngest patients had normal percentage of surface immunoglobulin positive cells). This suggests the primary defect in XLA may affect B cells at more than one stage of development rather than just at the level of pre-B cell.

There are reports of phenotypic heterogeneity between patients from different families as well (Landreth et al 1985a, Dobozy et al 1986, Golay and Webster 1986). Landreth et al (1985a) divided six boys with agammaglobulinaemia and lack of circulating B cells into two groups based on absence and presence of pre-B cells in the bone marrow. The group with normal percentage of pre-B cells in bone marrow is that of classical XLA and that without is a variant. Dobozy et al (1986) reported 4 boys with XLA in one family had high percentages of mouse erythrocytes rosette forming (MERF) cells in their peripheral blood while 2 other boys with XLA in another family had few MERF cells; hence suggesting B cell defects in XLA occur at different stages of differentiation in these two families. MERF cells represent a subpopulation of human B cells positive for both sIgM, sIgD and C3 receptors but largely negative for IgG Fc receptors (Lucivero et al 1981). Therefore, there may be genetic heterogeneity in XLA giving rise to differences in the phenotypes observed between families.

1.2.6 GENETIC ASPECTS

Early studies to demonstrate linkage between XLA and other X-linked phenotypic and protein polymorphisms, viz colour blindness and Xg blood group, have been unsuccessful (Sanger and Race 1963, Goldblum et al 1973, Mensink et al 1984). With the availability of X-chromosome specific DNA probes which detect restriction fragment length polymorphism (RFLP), linkage study of X-linked diseases have become more feasible (Drayna and White 1985). During the tenure of my research fellowship several studies have been reported concerning genetic linkage in XLA. Kwan et al (1986) demonstrated close linkage between XLA and 2 random DNA probes, 19.2 (DXS3) and S21 (DXS17), mapping the XLA locus to Xq21.3-q22. This finding was confirmed by Mensink et al (1986a) but the question of non-allelic genetic heterogeneity was raised. Our group (Chapter 4, this thesis) again confirmed the mapping of the XLA locus to Xq21.3-q22 and extended the linkage with another probe, pXG12 (DXS94) (Malcolm et al 1987).

The question of non-allelic genetic heterogeneity has complicated the clinical application of such linked DNA probes (Mensink et al 1987a) but a novel method of genetic risk calculation has been described to take into account the uncertainty due to non-allelic genetic heterogeneity (Chapter 4, this thesis, Lau et al 1988b). It will be essential to investigate the few XLA families which do show non-allelic genetic heterogeneity whether the

patients have other less apparent difference in their immunological defects from those with XLA which map to Xq21.3-q22.

The Lyon hypothesis states that permanent inactivation of one of the two X chromosomes occurs at random in every somatic cell in the female early in embryogenesis (Lyon 1961). The pattern of X chromosome inactivation is then transmitted in a stable fashion to all progeny cells. It seems the gene, the abberation of which leads to XLA, is essential for the development of B cells. Therefore there is only one population of mature B cells with the normal X chromosome active in female carrier of XLA who is immunologically indistinguishable from normal (Conley et at 1986, Fearon et al 1987). Conley et al (1986) established this by demonstrating XLA female carriers who were heterozygous for glucose-6-phosphate dehydrogenase (G6PD) and who only expressed one of the G6PD isoenzymes in their mature B cells while T cells and granulocytes expressed both isoenzymes. Using recombinant DNA probes which simultaneously detect RFLP and patterns of methylation of X chromosome genes, Fearon et al (1987) demonstrated the pattern of random X chromosome inactivation in T cells and granulocytes in female carriers of XLA, but not in B cells where only one of the two X chromosomes was preferentially active.

Furthermore, Schwaber et al (1983) reported Epstein-Barr virus (EBV) transformed pre-B cells from patients with XLA produced exclusively immunoglobulin u-heavy chain

constant (C_u) region without the normally associated heavy chain variable (V_H) region while only 5 percent of normal pre-B cells produced these incomplete *u*-heavy chains. It was postulated that the enzymes specific for translocation of variable genes or regulatory genes necessary for pre-B cells to differentiate to a stage utilizing these enzymes may be encoded on the XLA locus. However, EBV-transformed B cell lines from XLA patients were shown to be able to form complete V_HDJ_H rearrangements (Mensink et al 1986b). It was suggested that if the XLA gene is involved in IgH and/or IgL chain rearrangement, such rearrangements can still occur but at a reduced frequency (Mensink et al 1986b). It has been further postulated that the XLA gene may control the relative accessibility of the V_H locus to the common recombinase system which assemble the V-region genes of both the immunoglobulin and T-cell receptor of B and T cells respectively (Yancopoulos et at 1986, Mensink et al 1986b). Therefore, it seems the XLA defect is a relative but not an absolute block in B cell differentiation at the pre-B cell stage.

1.3 X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

1.3.1 DEFINITION

X-linked severe combined immunodeficiency is diagnosed on the basis of a complete absence of mature T cells, lack of T cell proliferation whereas B cells are detected in normal or increased numbers, suggesting this disease results from a block in T cell differentiation (Griscelli et al 1978, Gelfand and Dosch 1982,1983). Patients have significant functional deficiency of both T and B cell systems, despite presence of B cells. Unequivocal X-linked inheritance can only be ascertained from pedigree analysis of disease segregation.

1.3.2 CLINICAL FEATURES

Patients most commonly present with a triad of failure to thrive, persistent oral candidiasis which is refractory to treatment, and recurrent diarrhoea, commonly secondary to enteric viruses (Gelfand and Dosch 1983). The age of presentation is usually within the first six months of age. Pulmonary infections with pneumocystis carinii, cytomegalovirus, measles virus or parainfluenza virus are usually fatal. Chronic hepatitis secondary to cytomegalovirus or other unknown agents is common and a variant of sclerosing cholangitis with focal disappearance of bile ducts is also recognised. Progressive multifocal leukoencephalopathy due to a papova-virus, JC, has also been described in a four year old with severe combined immunodeficiency (ZuRhein et al 1978).

1.3.3 TREATMENT AND PROGNOSIS

Patients usually die before the age of two unless bone marrow transplantation (BMT) is attempted. With human leucocyte antigen (HLA) matched BMT, the success rate is over 90 percent and with HLA mismatched BMT, only 50 percent (Fischer et al 1987). Various means of depleting T cells in the donor marrow in order to prevent graft-versus-host disease in HLA-mismatched BMT have been attempted (Reinherz et al 1982, Morgan et al 1986, Buckley et al 1986).

In the past when no HLA-matched donor has been available, several approaches have been followed. These include fetal liver transplantation (Buckley et al 1976), fetal thymus transplantation (Hitzig et al 1965) and cultured thymic epithelium transplantation (Hong et al 1976). These are now considered not to be an option since HLA-mismatched BMT for severe combined immunodeficiency has become a relatively successful treatment (Fischer et al 1987).

1.3.4 CELLULAR DEFECTS

Severe combined immunodeficiency (SCID) is a heterogenous group of diseases (Gelfand and Dosch 1983), of which the X-linked form is only one variety. The B lymphocytes from these patients have been shown to undergo terminal differentiation in vitro in the presence of normal T cells and pokeweed mitogen (Pahwa et al 1980), however it is not clear which type of SCID is actually involved. Another piece of evidence for the functional

integrity of SCID B cells is that following donor T-cell engraftment in bone marrow transplantation in two patients, one of which has the X-linked variety, host B cells have become functional (Griscelli et al 1978). The origin of the B cells was determined by sex chromosome analysis as the donor and the host were of opposite sexes.

The primary defect seems to be a block in the T-cell differentiation (Gelfand and Dosch 1982) and the thymus is also uniformly dysplastic with marked lymphocyte depletion, absence of corticomedullary demarcation and absence of Hassall corpuscles.

1.3.5 GENETIC ASPECTS

Female carriers of X-linked severe combined immunodeficiency are immunologically normal and there is no immunological means of detecting carrier status. However, there are now two approaches to resolve this.

Our group has mapped the gene of X-linked SCID to Xq11-q13 in a study of 9 families (Chapter 5 this thesis, de Saint Basile et al 1987). There is no evidence of non-allelic genetic heterogeneity so far and a lod score of 5.27 at θ=0.00 was obtained with the marker cpX73 (DXS 159), which can be used carefully for carrier detection and prenatal diagonsis.

Another approach is based on the effect of Lyonisation. Puck et al (1987) first demonstrated non-random X-chromosome inactivation pattern in the T cells of

three obligate female carriers of X-linked SCID by a combination of two techniques: first, human/rodent hybrids to separate and identify the active and inactive human X chromosomes, and second, Southern blot hybridization with X-linked restriction fragment length polymorphisms to distinguish the two X chromosomes. They then identified an at-risk female as a carrier and excluded two using this approach. Recently, our group has used gene probes from the 5' end of the hypoxanthine phosphoribosyltransferase (HPRT) and phosphoglycerate kinase (PGK) genes, which will detect both a restriction fragment length polymorphism and also a difference in methylation between the active and inactive X chromosome, to demonstrate non-random X-chromosome inactivation pattern in the T cells of obligate female carriers of X-SCID (Goodship et al 1988) and used this approach to ascertain carrier status.

1.4 WISKOTT-ALDRICH SYNDROME

1.4.1 DEFINITION

Wiskott-Aldrich Syndrome (WAS) is an X-linked recessive immunodeficiency characterised by the triad of thrombocytopaenia, eczema and recurrent infections involving all classes of microorganisms. Immunologically there is defective antibody response to a variety of bacterial, viral and protein antigens, especially to polysaccharide antigens. Blood group antibodies (IgM isohaemagglutinins) are usually absent. Delayed skin hypersensitivity responses are also impaired (Blaese et al 1968).

1.4.2 CLINICAL FEATURES

Wiskott first described the syndrome in 1937 (Stiehm and Fulginiti 1980) and Aldrich et al (1954) described another child in which the pedigree analysis suggested an X-linked inheritance, and it was confirmed rapidly by other workers (Wolff and Bertucio 1957, Krivit and Good 1959). Patients usually present with petechiae or a bleeding episode in the first six months of life. They soon develop eczema and recurrent infections with various bacterial, viral (herpes simplex and cytomegalovirus) and fungal (candida) agents (Cooper et al 1968). Severe reactions to immunization, particularly polysaccharide vaccines, may ensue with exacerbations of bleeding and eczema.

Variants of WAS have been described. Canales and Mauer (1967) described 7 male members of a 4-generation family with thrombocytopaenia and some with low isohaemagglutinins and isolated increases in IgA levels, but without recurrent infection or eczema. Evans and Holzel (1970) described a girl with features of WAS but no thrombocytopenia.

1.4.3 TREATMENT AND PROGNOSIS

Acute bleeding episodes usually respond to transfusion of fresh platelets which should be irradiated to prevent graft-versus-host reactions. Bleeding in WAS tends to decrease with advancing age and splenectomy should not be undertaken lightly as it results in

increased risk of fulminating septicaemia. Infections should be treated early and aggressively. However, patients still die of the consequences of bleeding, as intracranial haemorrhage, or infection, as disseminated herpes simplex virus infection in the first ten years of life (Cooper et al 1968). Later, there is a high risk of developing malignancies, particularly lymphomas and systemic reticuloendotheliosis (Spector et al 1978).

Bach et al (1968) achieved reconstitution in a 2 year old boy with WAS by histocompatible bone marrow transplantation following high-dose cyclophosphamide. A 15-year follow-up report of the same patient showed he has had full T cell, partial B cell and no haematopoietic engraftment (Meuwissen et al 1984). He has had no serious infections or bleeding episodes. With better results of bone marrow transplantation (Fischer et al 1987), it is a definite option to be considered seriously as most patients eventually develop malignancies (Spector et al 1978).

1.4.4 CELLULAR DEFECTS

Both T and B cell defects occur in the WAS, but the most consistent and characteristic defect is the inability to form antibody to polysaccharide antigens (Krivit and Good 1959, Cooper et al 1968, Blaese et al 1968). Patients do not form isohaemagglutinins or antibodies to such antigens as pneumococcal polysaccharide and lipopolysaccharide Vi from E. Coli. Although IgG

antibodies to polysaccharides are predominantly restricted to IgG2 in man, patients with WAS have been shown to have normal levels of the different IgG subclasses, including IgG2 (Nahm et al 1986). Blaese et al (1968) also demonstrated poor antibody responses to various bacterial and viral antigens.

T cell abnormality was demonstrated by decreased responsiveness to dinitrochlorobenzene sensitisation and skin-test antigens (Blaese et al 1968) and it has been shown there is a gradual loss of cellular elements in the thymus-dependent areas of the lymphoid organs (Cooper et al 1968).

Haematological abnormalities include thrombo-cytopaenia, lymphopaenia, eosinophilia and haemolytic anaemia. The platelets in the WAS are small when compared to normals or other acquired destructive thrombocytopaenia (Murphy et al 1972).

It is difficult to explain the above cellular defects with a unifying mechanism but Parkman et al have (1981) suggested an absence of a surface glycoprotein of molecular weight 115 kDa could be the primary defect in the WAS (Remold-O'Donnell et al 1984).

1.4.5 GENETIC ASPECTS

Female carriers of WAS are clinically normal and cannot be detected by immunological means. Shapiro et al (1978) has reported a method of carrier detection based on a stress test to demonstrate abnormal platelet

aggregation, but it has not been substantiated elsewhere. Later, Gealy et al (1980) described a WAS carrier who was also heterozygous for glucose-6-phosphate dehydrogenase (G6PD), and who only expressed one of the isoenzymes in platelets and T cells, but both isoenzymes in erythrocytes and neutrophils. It was soon substantiated by Prchal et al (1980) in another female who was doubly heterozygous for both WAS and G6PD. This WAS carrier only expressed one of the G6PD isoenzymes in her platelets, T cells, B cells, monocytes and granulocytes, while her fibroblasts expressed both isoenzymes. This method of carrier detection can only be applied to a minority who are heterozygous for G6PD.

Recently, the WAS gene locus has been mapped to the pericentric region using restriction fragment length polymorphism (Peacocke and Siminovitch 1987) and genetic prediction becomes possible by gene tracking . Kohn et al (1987) also demonstrated non-random X chromosome inactivation pattern in the T cells of obligate WAS carriers by using the same technique as Fearon et al (1987) for XLA and Goodship et al (1988) for X-linked SCID. This can be used to detect carriers who are heterozygous for the DNA probes from the 5' end of the genes of PGK and HPRT.

1.5 X-LINKED HYPERIMMUNOGLOBULINAEMIA M

1.5.1 DEFINITION

X-linked hyperimmunoglobulinaemia M (X-linked hyperIgM) is a syndrome in boys with recurrent bacterial infections, characterised by absent or decreased serum levels of IgG, IgA and IgE, but elevated or normal IgM. X-linked inheritance can only be ascertained from pedigree analysis of disease segregation. (Stiehm and Fulginiti 1980).

1.5.2 CLINICAL FEATURES

Rosen et al (1961) reported the first two cases in two separate families and the X-linked inheritance has since been established in several families. The patients usually present with recurrent bacterial sinopulmonary infections during the first or second year of life. Neutropaenia is a frequent concomitant feature and is associated with stomatitis and mouth ulcers. Pneumocystis carinii pneumonia in early infancy can be the presenting feature, suggesting abnormal T cell function (Marshall et al 1964). In addition to susceptibility to infections, some patients are prone to various manifestations of autoimmune process, such as thrombocytopaenia, haemolytic anaemia, nephritis and arthritis (Goldman et al 1967). This syndrome has been reported in a few females (Rosen and Bougas 1963), indicating clinical and genetic heterogeneity (Kyong et al 1978). Furthermore the syndrome can be secondary to infection as it may be seen

in children with congenital rubella (Schimke et al 1969, Geha et al 1979).

1.5.3 TREATMENT AND PROGNOSIS

Treatment is the same as for X-linked agammaglobulinaemia, viz adequate immunoglobulin replacement and aggressive treatment of any infection to minimise end-organ damage. A return of the IgM to normal levels usually indicates an adequate immunoglobulin replacement. Prognosis is generally better than in X-linked agammaglobulinaemia (Stiehm and Fulginiti 1980).

1.5.4 CELLULAR DEFECTS

The central defect of this syndrome is a failure of switch from IgM to IgG secretion, but whether it is due to an intrinsic B cell defect or lack of help from another cell type is not clear. Geha et al (1979) in a study of 9 such patients, reported normal T cell function and identified a unique subpopulation of large B cells which secrete IgM spontaneously in large amounts; they concluded that the defect is intrinsic to B cells, which fail to switch from IgM to IgG synthesis. However, Mayer et al (1986) demonstrated IgM to IgG switch in B cells of 8 out of 9 such patients by using T cells derived from a patient with a Sezary-like syndrome. They concluded a defect in "switch T cells" may be pathogenic in this syndrome.

1.5.5 GENETIC ASPECTS

This syndrome of immunodeficiency with hyperIgM can result from either genetic or environmental causes, such

as congenital rubella (Schimke et al 1969). The inheritance pattern is initially described of X-linkage but families with autosomal recessive inheritance have also been reported (Mayer et al 1986). Recently, the gene locus for X-linked hyperIgM has been mapped to Xq24-q27 by linkage with restriction fragment length polymorphism in one family (Mensink et al 1987b). We also analysed another family with X-linked hyperIgM in which at least two recombinations were observed with the DNA probes linked with XLA, viz 19-2 (DXS3), S21 (DXS17) and pXG12 (DXS94), thereby indicating a distinct localisation for the genetic defect in X-linked hyperIgM from that for XLA (Chapter 4, this thesis). Carrier detection and prenatal diagnosis should not be attempted by gene tracking in this disease until more such families are studied to see whether there is non-allelic genetic heterogeneity.

1.6 DECLARATION AND OBJECTIVES OF STUDIES PRESENTED

1.6.1 DECLARATION

When I started my research fellowship in the Institute of Child Health in January 1986, there was already an active research programme into X-linked immunodeficiencies as well as basic B cell physiology. My responsibility within this extensive research programme has been defined as mainly in the family linkage study of X-linked agammaglobulinaemia and X-linked severe combined immunodeficiency, subsequently the genetic counselling using the linked probes, as well as investigation into the in-vitro B cell function of patients with X-linked

agammaglobulinaemia and Wiskott-Aldrich syndrome. I was personally involved in recruiting all the families, except families 15 and 17, and collecting blood samples for DNA extraction and establishing B cell lines. I did all the Southern blotting in the linkage study of XLA using pXG12 (DXS94) and in that of X-linked SCID using cpX73 (DXS159) and pXG12 (DXS94). After establishing the linkage of XLA with probes from Xq21.3-q22, I was responsible in contacting potential female carriers in these families and providing genetic counselling with the use of probes S21 (DXS17) and pXG12 (DXS94), including doing the Southern blotting. I carried out the mathematical analysis of the RFLP data and was personally involved in developing the risk calculation which takes into account non-allelic genetic heterogeneity presented in this thesis. I also designed and performed all the B cell functional studies described in chapter 6.

1.6.2 LOCALIZATION OF THE GENE LOCI OF X-LINKED AGAMMAGLOBULINAEMIA AND X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

Human X chromosome accounts for about 6 percent of the total genomic DNA and its total genetic length was estimated to be about 200 centimorgans (Davies 1985). With the application of restriction fragment length polymorphisms (RFLP) as genetic markers (Botstein et al 1980), a map spanning the entire X chromosome is almost complete (Drayna and White 1985) and any X-linked disease locus could now be mapped within ten centimorgans of at least two markers.

Since the basic genetic defects of neither X-linked agammaglobulinaemia nor X-linked severe combined immunodeficiency is known, it would be desirable to map these disease loci using RFLPs which then open up the possibility of carrier detection and first trimester prenatal diagnosis. It may also lead to the accurate delineation of the underlying genetic defect, as has been achieved with X-linked chronic granulomatous disease (Royer-Pokora et al 1986, Dinauer et al 1987). We therefore carried out linkage analysis of families with these two X-linked immunodeficiency diseases (Chapter 4 and 5, this thesis).

1.6.3 CLINICAL APPLICATION OF THE LINKED DNA MARKERS IN FAMILIES WITH X-LINKED AGAMMAGLOBULINAEMIA

Using the strategy of gene tracking (Pembrey 1986), there is enormous potential for carrier detection and first trimester prenatal diagnosis in monogenic diseases. However, non-allelic genetic heterogeneity has complicated this approach in X-linked agammaglobulinaemia (Mensink et al 1987a). We have nevertheless evaluated the feasibility of this approach in genetic prediction in thirteen families and developed a novel method of genetic risk calculation which takes into account the uncertainty due to non-allelic genetic heterogeneity (Chapter 4, this thesis).

1.6.4 IN VITRO STUDY OF B CELL FUNCTIONS OF PATIENTS WITH X-LINKED AGAMMAGLOBULINAEMIA AND WISKOTT-ALDRICH SYNDROME

There is both immunological and genetic evidence to implicate that the B cells are primarily affected in both X-linked agammaglobulinaemia and Wiskott-Aldrich syndrome as reviewed above. Therefore, it will be essential to investigate these patients' B cell functions in detail and we have chosen the Epstein-Barr virus transformed B cell lines as an experimental model for evaluation of their responses to various B cell growth and differentiation factors in order to detect any possible defect (Chapter 6, this thesis).

Chapter 2 PATIENTS AND FAMILIES

2.1 INTRODUCTION

Several practical issues of linkage analysis arose from this study which are by no means unique. Since X-linked agammaglobulinaemia (XLA) and X-linked severe combined immunodeficiency (X-linked SCID) are relatively rare diseases, any one paediatric centre is not likely to have enough affected families for linkage study. Therefore over one hundred letters were initially sent to paediatricians, geneticists and immunologists in the United Kingdom just to ascertain such families suitable for linkage analysis. Obviously, families with sporadic cases or with only one surviving son, whether affected or unaffected will not be informative. The next step was to ensure the diagnosis was correct in the index patient. The clinical history should be compatible. Immunological investigations should also be characteristic of the disease concerned. No family refused to co-operate in the study after the nature and possible benefits of the project were explained to them. A detailed and extended family history was then taken. A decision was made which members would contribute to the lod score in the linkage analysis, and they were then approached for their blood to be taken.

Since there was no test available for female carriers of XLA or X-linked SCID, a woman was only considered to be a carrier if she has had two or more affected sons or she has had at least an affected brother and an affected son.

Such defined female carriers were then generally typed first for the DNA probes. Only if they were heterozygous would their offsprings be informative for linkage analysis and hence they were typed in turn. However in a multigenerational family, due to missing or partial information, the situation is not always that cut and dried. There is as yet no best schedule such that the number of individuals to be typed is minimized (Ott 1985).

Another assumption made for the linkage analysis was that XLA and X-linked SCID in a male should usually manifest some symptoms before one year of age. Therefore, any absolutely healthy male over one year of age could be classified as unaffected, without their immunological state being formally investigated. This classification criterion was applied to the healthy brothers of both affected boys and female carriers, as well as the men who married into the pedigree from the population.

In one family (family 7), a case of monozygotic male twins was encountered. Genetically, they have to be taken as a single individual whose genotype has been independently expressed twice. For linkage analysis, they have to be coded as one and the same individual. If they were dizygotic twins, they should obviously be coded as two separate individuals.

2.2 X-LINKED AGAMMAGLOBULINAEMIA

Index patients of families 1-14 (Figure 2-1) have all been investigated either in the Hospital for Sick Children and Northwick Park Hospital, London, Newcastle General Hospital or East Birmingham Hospital. They all fulfilled the clinical features and immunological profiles (Table 2) of X-linked agammaglobulinaemia (XLA). Parents of all index patients were contacted directly. Explanations regarding the study were given and consent to participate obtained before a detailed family history was taken. Blood samples from patients and other relevant family members were then collected in ethylenediamine tetraacetate (EDTA) directly or via their own family doctors in order to extract DNA. Attempts were also made to establish Epstein-Barr virus transformed B cell lines from separated mononuclear cells from the patients.

Family 15 (Figure 2-1) reported initially by Jamieson and Kerr (1962) was obtained through Dr. Donnai, Manchester. The only affected surviving male was reinvestigated by Dr. Roifman in Toronto, Canada and confirmed to have panhypogammaglobulinaemia and lack of peripheral B cells, the characteristic immunological profile of XLA.

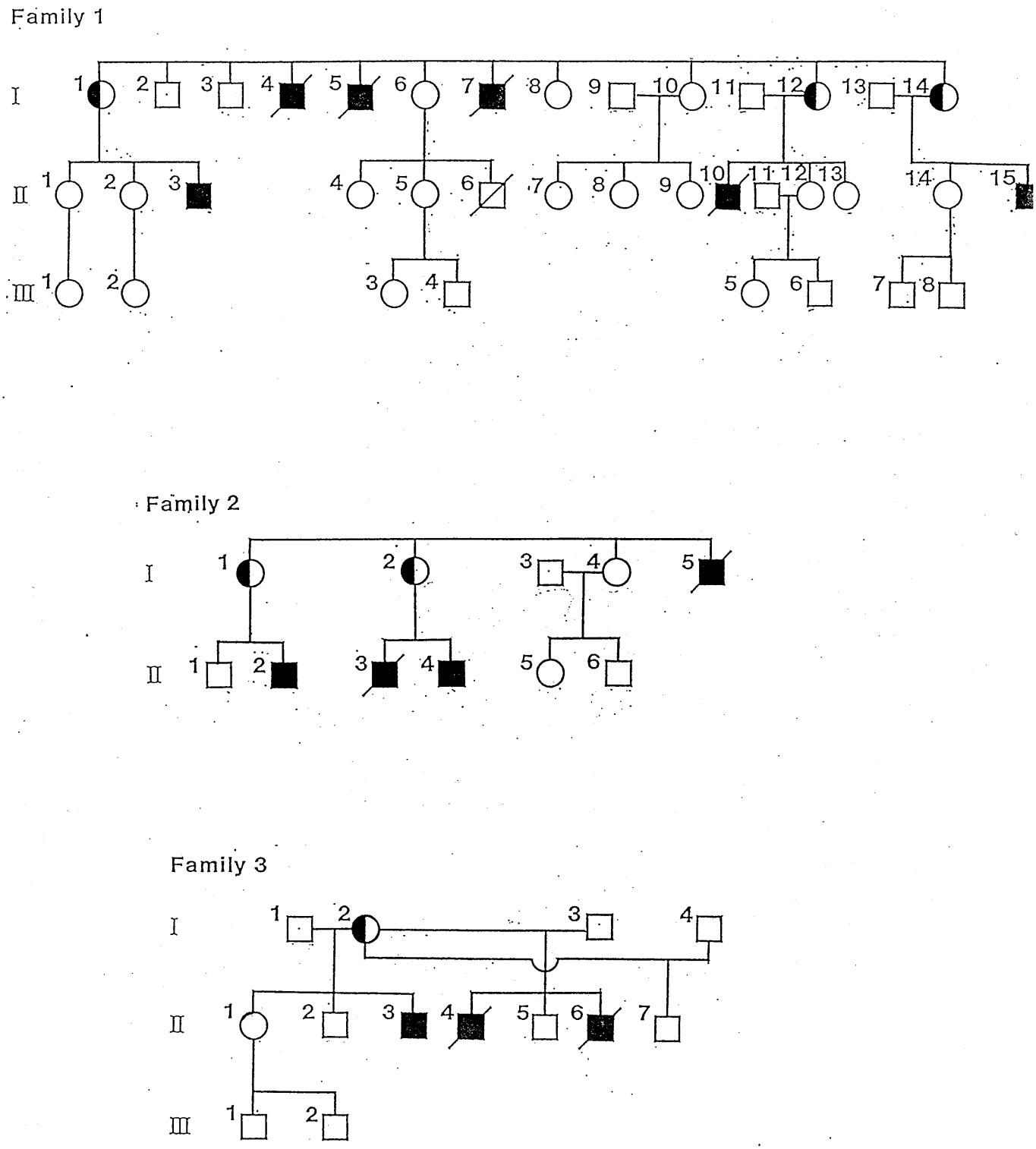
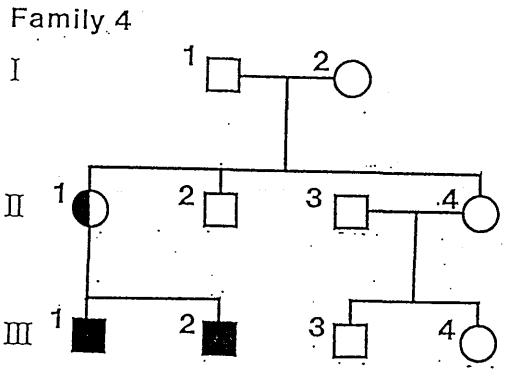
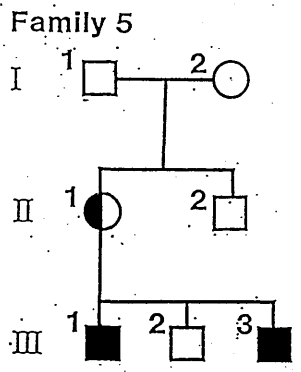
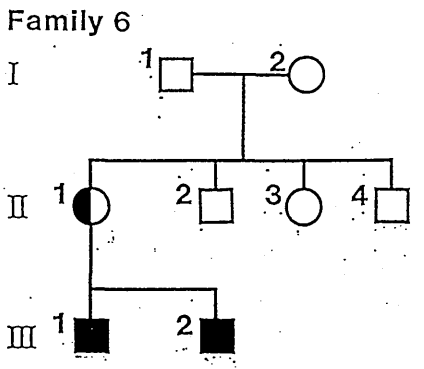


Figure 2-1 Pedigrees of fifteen families with X-linked agammaglobulinaemia (Families 1-15)







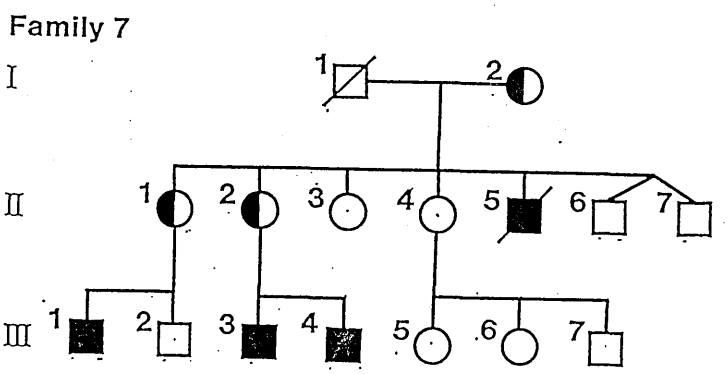


Figure 2-1 (continued)

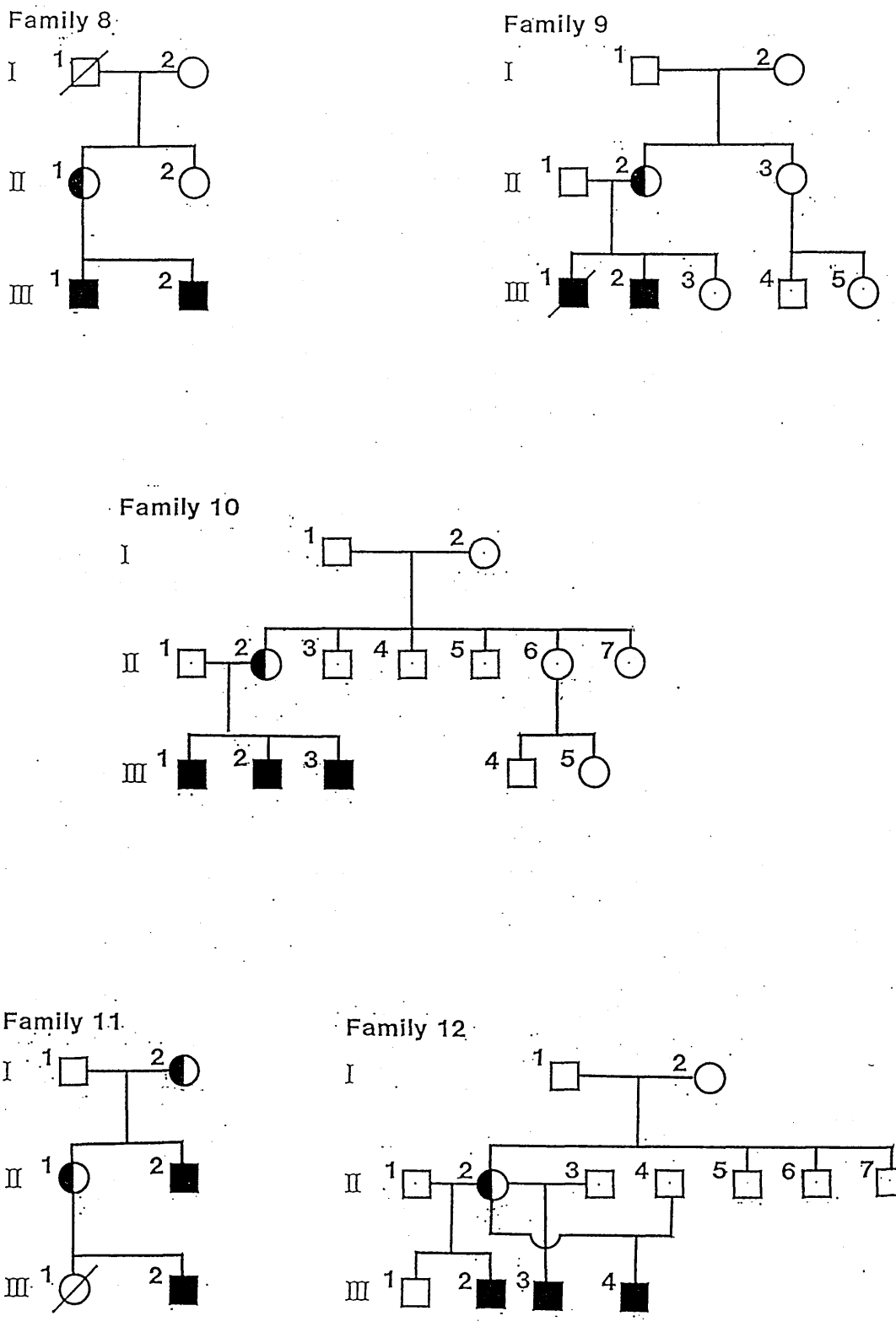
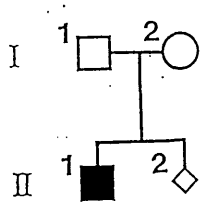
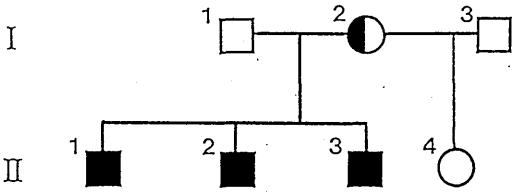


Figure 2-1 (continued)

Family 13



Family 14



Family 15

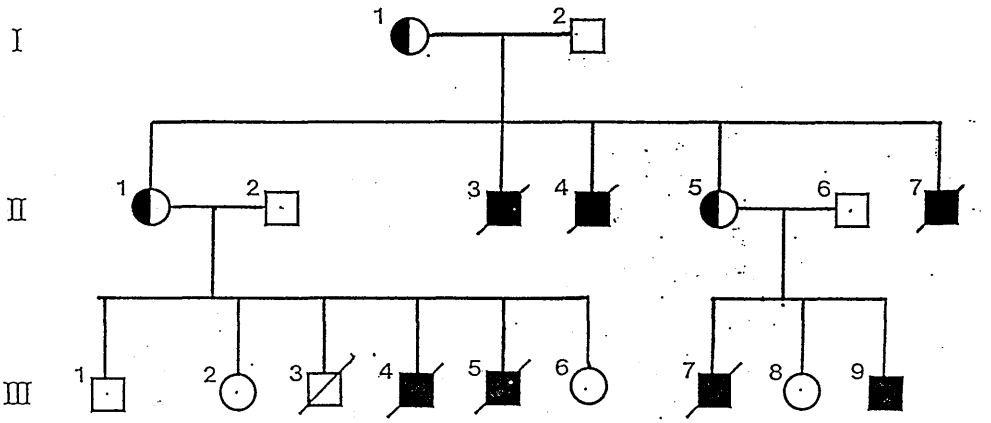


Figure 2-1 (continued)

Table 2

IMMUNOLOGICAL PROFILES OF INDEX PATIENTS WITH XLA

		(G/L)			%	
Family	Patient	IgG	IgA	IgM	T cells	B cells
1	II-3	*3.1	<0.1	<0.1	85	<1
	II-15	*1.9	<0.1	<0.1	nd	nd
2	II-2	*2.3	<0.1	<0.1	75	<1
	II-4	*1.9	<0.1	<0.1	60	nd
3	II-3	<0.6	<0.1	<0.1	normal	<1
4	III-1	0.45	0.05	0.45	normal	<1
	III-2	*1.9	0.02	0.07	normal	<1
5	III-1	<0.6	<0.1	<0.1	nd	nd
	III-3	0.55	<0.1	<0.1	nd	nd
6	III-1	<0.6	<0.1	<0.1	normal	<1
	III-2	<0.6	<0.1	<0.1	normal	<1
7	III-1	*1.85	0.05	0.02	nd	nd
	III-3	trace	not detected	trace	nd	nd
	III-4	*1.26	0.1	0.05	nd	nd
8	III-1	0.15	0.05	0.2	nd	nd
	III-2	0.01	0.05	0.05	75	<1
9	III-2	<0.6	<0.1	<0.1	normal	<1
10	III-1	<0.6	<0.1	<0.1	normal	<1
	III-2	<0.6	<0.1	<0.1	normal	<1
	III-3	<0.6	<0.1	<0.1	normal	<1

(continued next page)

Table 2 (continued)

		(G/L)			%	
Family	Patient	IgG	IgA	IgM	T cells	B cells
11	II-1	<1.0	<0.1	<0.1	nd	<1
	III-2	1.2	<0.1	<0.2	75	<1
12	III-2	0.95	<0.1	<0.1	nd	nd
	III-3	0.1	<0.1	<0.1	nd	nd
	III-4	0.8	<0.2	0.09	nd	nd
13	II-1	*2.0	<0.1	<0.1	normal	<1
14	II-1	<0.1	<0.1	<0.1	normal	<1

nd = not done
* = on immunoglobulin replacement

2.3 X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

Three families were collected (Figure 2-2) and the index patients all fulfilled the diagnostic criteria of the World Health Organization Committee on Immunodeficiency (Rosen et al 1983), viz absence of T lymphocytes and lack of T-lymphocyte proliferation with normal or increased numbers of B lymphocytes. One family was obtained through Dr. S. Strobel from Germany, one through Dr. T. Espanol from Spain and the last family has been under our care for years and is of Turkish-Cypriot origin from London. Similarly, blood samples were collected from family members for DNA analysis.

2.4 X-LINKED HYPERIMMUNOGLOBULINAEMIA M

One family was studied (Figure 2-3) and the index patient was diagnosed as having X-linked hyperIgM at the Hospital for Sick Children, London. He had abnormally high serum IgM levels, no serum IgG or IgA and his B cells which were present in normal numbers had only IgM and IgD on the cell surface.

2.5 WISKOTT-ALDRICH SYNDROME

Six patients, all from different families, were diagnosed as having Wiskott-Aldrich Syndrome (WAS) either in the Hospital for Sick Children, London or Booth Hall Hospital, Manchester. They again fulfilled the diagnostic criteria of WAS, viz thrombocytopaenia, eczema, lack of isohaemagglutinins and variable degree of T cell deficiency. Preservative-free heparinised blood were obtained from these six patients for establishing Epstein-

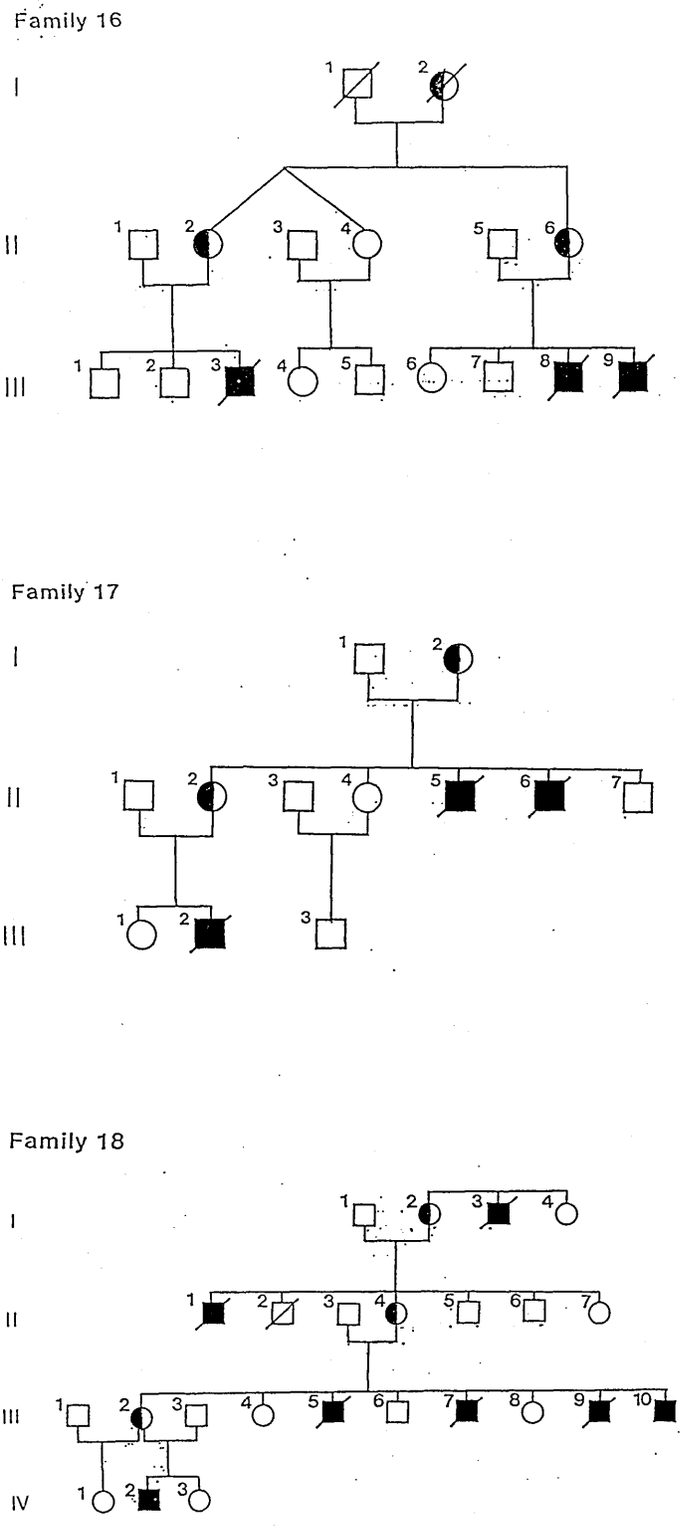


Figure 2-2 Pedigrees of three families with X-linked severe combined immunodeficiency (Families 16-18)

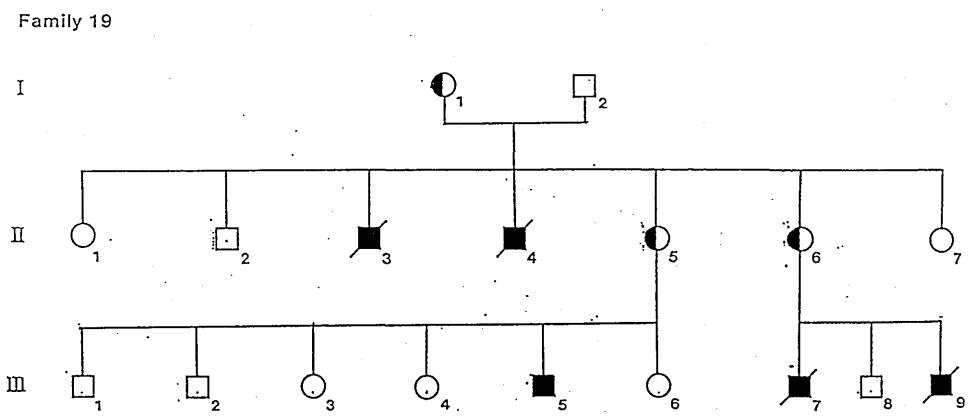


Figure 2-3 Pedigree of the family with X-linked hyperimmunoglobulinaemia M (Family 19)

Barr virus transformed B cell lines. Linkage study was not possible due to limitation of suitable families for linkage analysis.

Chapter 3 METHODOLOGY

During the tenure of my research fellowship, the following laboratory techniques have been used in the various projects. Materials used are detailed in appendix 1. The mathematical aspects of linkage analysis are given in Chapter 4.

3.1 PREPARATION OF DNA FROM VENOUS BLOOD

Fresh blood has to be collected in tubes containing the anticoagulants ethylenediamine tetraacetate (EDTA) or heparin, otherwise the sample will clot and provide little DNA. The sample may be kept at +4^{o} C for several days without deterioration, or frozen and kept indefinitely at -70^{o}C or up to six months at -20^{o}C. 30 ml of ice-cold distilled water was added to 10 ml of thawed venous blood. It was then spun at 2000 RPM, 4^{o}C for 20 minutes. Supernatant was poured off and the nuclear pellet at the bottom was retained. 25 ml of ice-cold 0.1% Nonidet P40 solution was added to the pellet. The pellet was re-suspended and was spun at 2000 RPM, 4^{o}C for 20 minutes; supernatant was decanted again. 2.5 ml of 75mM NaCl/25mM EDTA (pH8) was added to pellet and resuspended thoroughly. 125 microlitres of 10% SDS, and 1 mg proteinase K (10 mg/ml) were added in turn. It was then left at 37^{o}C overnight or 56^{o}C for 3 hours. 2.5 ml of 75mM NaCl/25mM EDTA (pH8) and 5 ml of phenol (saturated with 50mM Tris pH8.0) were added. It was mixed well and spun at 3000 RPM, 4^{o}C for 20 minutes. The top aqueous layer which contains DNA was retrieved. 2.5 ml phenol and 2.5 ml

chloroform were added, mixed well and spun at 3000 RPM, 4°C for 15 minutes. The top layer was taken again and 5 ml chloroform was added. It was then spun at 2000 RPM, 4°C for 5 minutes. The last step was repeated once. The top layer was taken. 0.5 ml 3M sodium acetate (pH 5.2) was added and topped up to 15 ml with ice-cold ethanol and mixed well. DNA would precipitate out as threads and could be fished out on glass rod. DNA was allowed to dry and put into 0.5 ml TE solution in eppendorf tube and spun at 4°C to dissolve overnight.

3.2 SOUTHERN BLOTTING

5 micrograms of DNA, approximately 15 microlitres of DNA as prepared in (3.1) were digested with 10 units of enzyme under the manufacturer's conditions. 1 microlitre of 1 mg/ml bovine serum albumin (BSA) and 1 microlitre of 0.1M spermidine were added as well in a final volume of 25 microlitres. Incubation was carried out for at least 4 hours. 5 microlitres of bromophenol blue/25% glycerol/EDTA was added as a loading dye to each sample of digest. 0.8% agarose gel plate was prepared by dissolving 2 grams of electrophoresis grade agarose powder in 250 ml Tris E buffer, then 12.5 microlitres ethidium bromide (10 mg/ml) was added. Each sample was loaded into a well. Hind III digested lambda marker was put onto the first left hand lane. The gel was run submerged in Tris E buffer for 16 hours at approximately 1.5V/cm or 50 mA. DNA migrates to the positive electrode. The gel was checked and photographed under ultra-violet light with a ruler by the

side. The gel was then soaked in denaturing solution for 1 hour, followed by one hour in neutralising solution, twice. The Southern blot was then set up for an overnight transfer of DNA to a nitrocellulose or nylon filter with 20xSSC (Southern 1975). The filter was rinsed with 2xSSC, and then baked at 80^{o}C for 3 hours. After baking, the filter was damped in 2xSSC and put into a plastic bag. 5 ml pre-hybridization solution for 125 ml gel was added into the plastic bag. All bubbles were squeezed out and the bag was double sealed. It was left at 65^{o}C (water bath) for 3 to 12 hours. The filter was retrieved from the plastic bag and put into a second bag with the radio-labelled probe (boiled for 5 minutes just prior to use) added into the 5 ml prehybridization solution. It was left at 65^{o}C (water bath) overnight. The filter was removed and washed three times with 3xSSC/0.1% SDS at room temperature for 20 minutes each. It was checked with beta-counter for background radioactivity and bands. If there was too much background, the filter was washed once with 0.5xSSC/0.1% SDS at 65^{o}C for 20 to 30 minutes. The lower the concentration of SSC and higher the temperature, the more strigent the wash is. The filter was dried and wrapped in cling film. The position of wells was marked and orientation of filter labelled with radioactive ink. The filter was then autoradiographed in cassettes with intensifying screens at -80^{o}C using Kodak XAR-5 film. It was left for varying periods of time depending on strength of signals. Examples of the autoradiographs using the

probes S21, 19-2, pXG12 and cpX73 are presented in figure 3-1.

3.3 OLIGOLABELLING OF DNA PROBES

DNA probe to be used was diluted into 50 ng/ml of distilled water. 1 microlitre of DNA probe was added to 8 microlitres of water, which was boiled for 2 minutes. The DNA probe was then added to 11.4 microlitres of LS and 1 microlitre of BSA solution on ice. 50 microCi of [^{32}P] dCTP (5 microlitres) was added, followed by 2.5 units of DNA polymerase I Klenow fragment. The solution was mixed gently and left at room temperature overnight. Two microlitres of loading dye was added to the 25 microlitres of radio-labelled DNA probe. It was made up to 100 microlitres with 3xSSC. The solution was put down a column of Sephadex G50 in 3xSSC and eluants at 100 microlitres aliquots were collected as successive 100 microlitres of 3xSSC were put down through the column. Aliquots of eluants were checked for peak level of radioactivity and the 4 maximum aliquots were pooled. The cpm of the pooled radiolabelled DNA probe was measured on a scintillation counter and it was used at 1x10^{6} cpm/ml of hybridization solution.

3.4 ESTABLISHING EPSTEIN-BARR VIRUS TRANSFORMED B CELL LINES FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

10 ml of venous blood was collected into a container wetted with preservative-free heparin. An equal volume of RPMI 1640 medium supplemented with 25mM Hepes was used to dilute the blood. It was then layered onto Ficoll-Hypaque

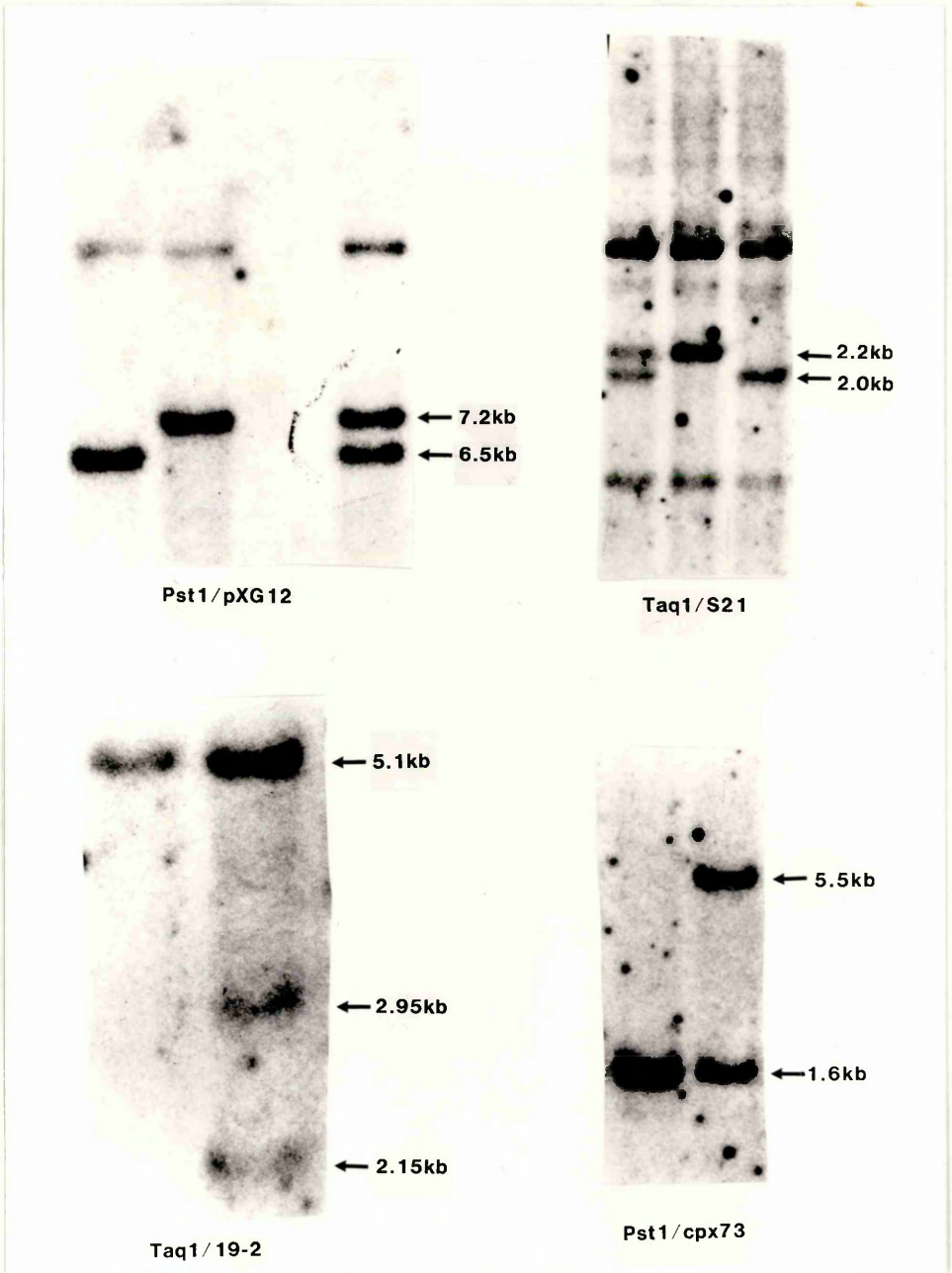


Figure 3-1 Autoradiographs showing the DNA polymorphism detected with the four X chromosome specific probes: S21, 19.2, pXG12 and cpX73.

(1.077 kg/l) (2 volumes of diluted blood to 1 volume of Ficoll-Hypaque) and spun at 1000 g for 20 minutes at room temperature. The centrifuge brake was off to prevent disturbance of the interface. Mononuclear cells were collected from the interface and washed twice with at least an equal volume of RPM1 1640 with 25mM Hepes; these were spun at 200g for 15 minutes. 1 ml of marmoset cell line B95-8 supernatant (Miller et al 1972) containing the Epstein-Barr virus was added to the cell pellet, which was resuspended gently and incubated at 37^{o}C for 1 hour. It was then washed and spun again. The mononuclear cells were then resuspended in RPM1 1640 with 25mM Hepes and 10% fetal calf serum (FCS) at a concentration of 0.5-1x10^{6} cells/ml. Cyclosporin A at a final concentration of 1 ng/ml was added. Aliquots of 2 ml were dispensed into each well of 24-well Costar plate which was then incubated at 37^{o}C, in an atmosphere of 5% CO_2 in air. Lymphoblastoid cell clumps were usually seen by the end of 2 weeks (Figure 3-2). When cell growth was vigorous, the cell line was expanded by transferring to an upright culture flask. Cell lines obtained from patients were either used for experiments immediately or frozen down in large number of aliquots for future experiments, in order to ensure the cell lines were still polyclonal.

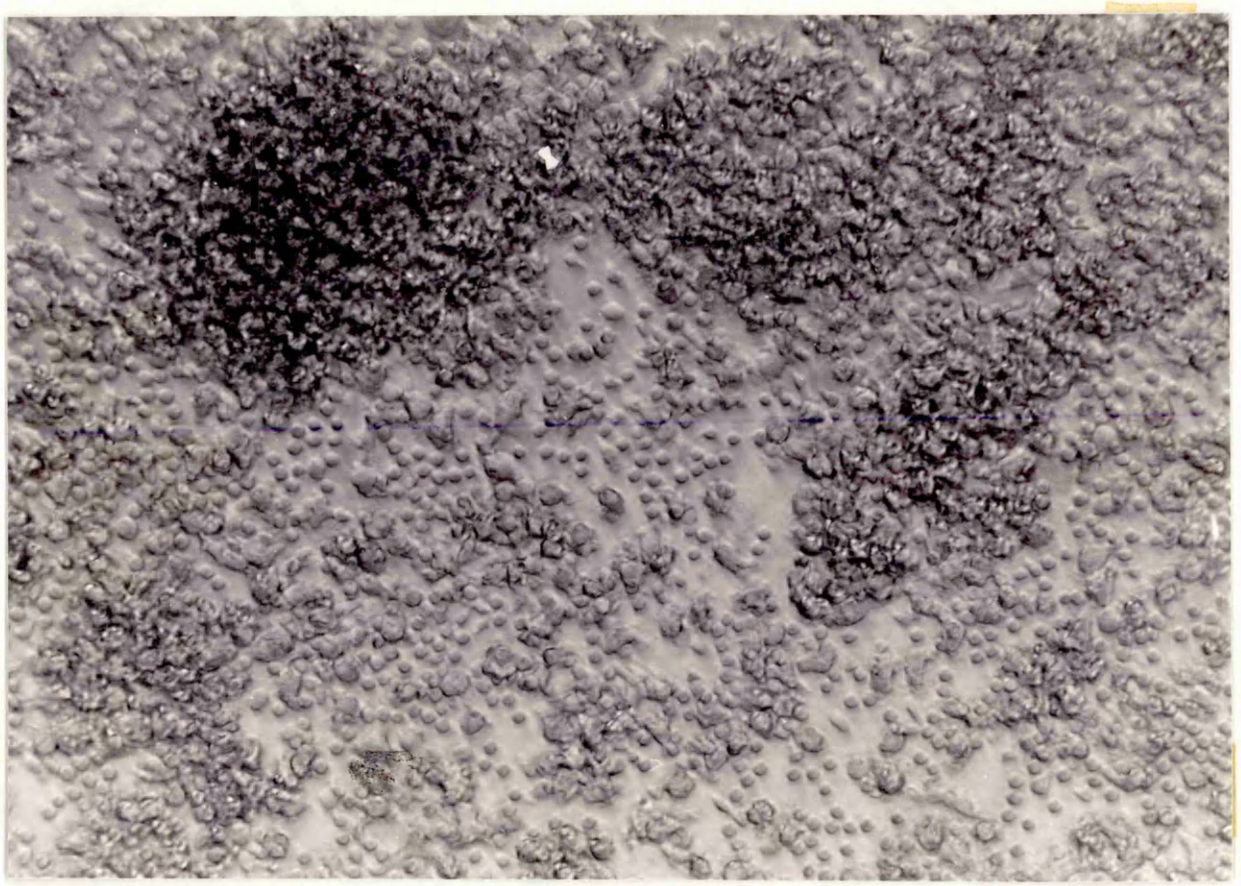


Figure 3-2 Micrograph showing Epstein-Barr virus transformed lymphoblastoid cell clumps.

3.5 MEASUREMENT OF IgG/IgM IN CULTURED CELL SUPERNATANT BY ENZYME-LINKED IMMUNOSORBENT ASSAY

80 microlitres of affinity purified goat anti-human IgG/IgM (Sigma), at a concentration of 0.5 microgram/ml (1 in 2000) in bicarbonate buffer pH9.6, were dispensed into each well of a 96-well Sterilin Cooke round-bottomed microtitre plate which was then incubated in a moist box at room temperature overnight. The plate was washed thrice with bicarbonate buffer. 100 microlitres of 4% normal goat serum (NGS) diluted in bicarbone buffer were added to each well and left at room temperature for one hour. The plate was washed thrice with 0.05% Tween 20 in normal saline. 80 microlitres of test supernatant, or standards diluted in normal saline with 0.05% Tween 20 and 4% NGS were added and incubated at room temperature for 60-90 minutes. An 11-point standard curve using double dilutions of either pooled normal human serum or partially purified IgG/IgM at 1000 nanogram/ml was set up in duplicate on each plate along with buffer only zero standard. An example of the standard curve is given in figure 3-3. The plate was washed five times with 0.05% Tween 20 in normal saline. 80 microlitres of horseradish peroxidase (HRP)-conjugated affinity purified goat anti-human IgG/IgM diluted to 1:1000 in normal saline containing 0.05% Tween 20 and 4% NGS were added to each well. The plate was washed five times with 0.05% Tween 20 in normal saline. 80 microlitres of o-phenylenediamine diluted to 0.5 mg/ml in phosphate/citrate buffer containing 0.015% hydrogen peroxide were added to each

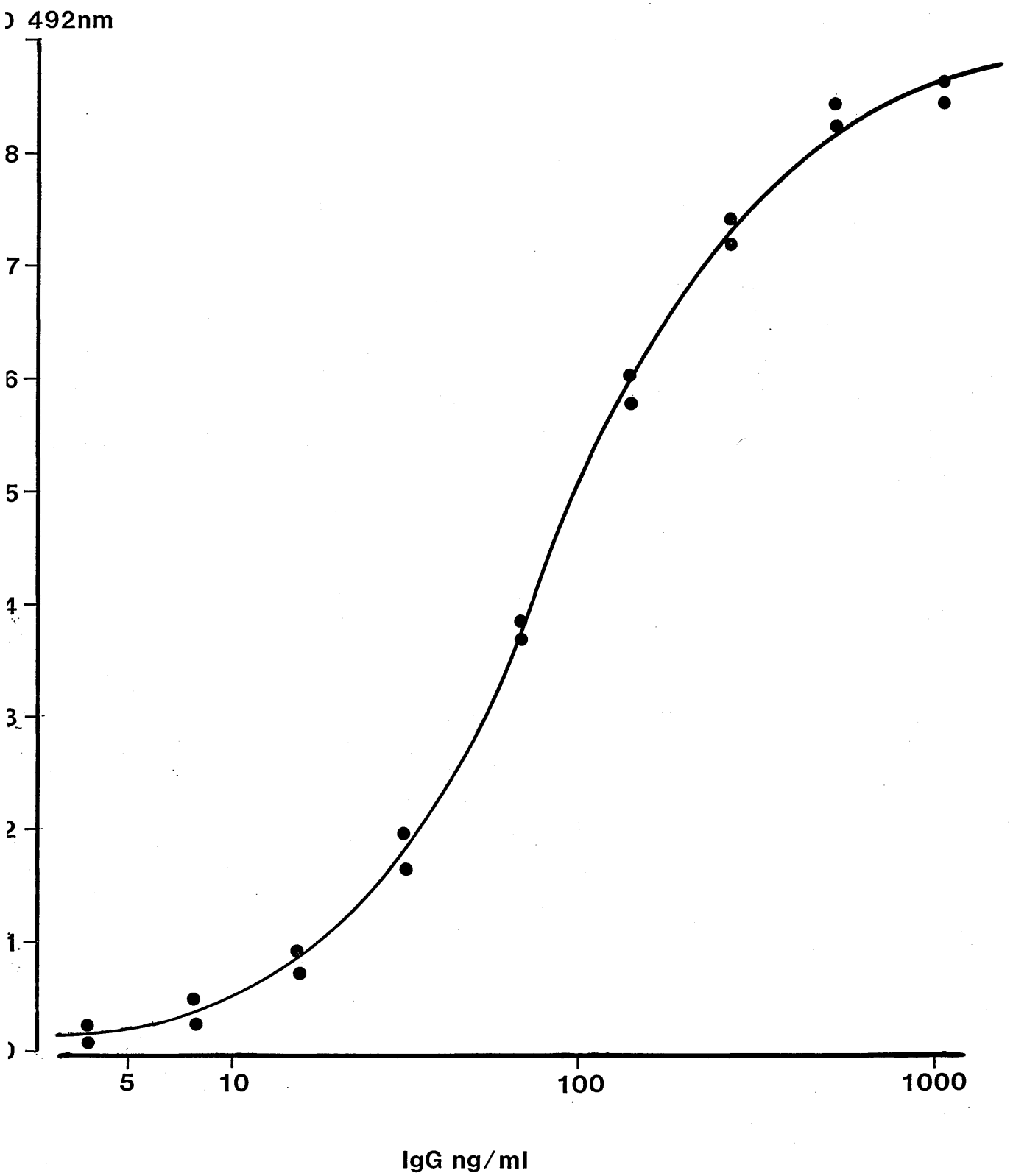


Figure 3-3 Standard curve of IgG measurement by enzyme linked immunosorbent assay

well as substrate. This reagent was made up just prior to use. The plate was then incubated in the dark at room temperature to allow colour development. For HRP, 15 minutes was usually sufficient. Further development was then stopped by adding 40 microlitres of 2M sulphuric acid to each well, and the absorbance read on an automatic ELISA plate reader at 492 nm. A standard curve was constructed, from which the value of test supernatants were read.

3.6 ASSAY FOR RESPONSES OF EPSTEIN-BARR VIRUS TRANSFORMED B CELL LINES AND INDICATOR B CELL LINES TO VARIOUS FACTORS

Cell lines were sub-cultured 24-48 hours prior to use in order to ensure vigorous log-phase growth. Cells were cultured in triplicate at concentrations between 1 and $20x10^3$ cells/well in 96-well flat-bottomed microtitre plates. The culture medium was RPMl 1640 supplemented with 2mM L-glutamine, 25mM Hepes and FCS (2% for growth and 5% for differentiation assay). Various factors were added to give final concentrations as indicated in each experiment. When assaying for proliferation, the cultures were incubated at 37^oC, 5% CO_2, for 3 days and pulsed with 1 microCi/well (37 kBq) of tritiated thymidine (3HTdR) for the last 8 hours. Cells were then harvested onto glass fibre filter discs using an automated cell harvester and incorporated 3HTdR determined on a liquid scintillation counter. When assaying for differentiation, the cultures were incubated for 5-6 days and the immunoglobulin levels in supernatant measured by enzyme-linked immunosorbent

assay (ELISA). The preliminary experiments to establish the above parameters, such as cell concentrations and length of culture, are detailed in Chapter 6.

3.7 INDIRECT IMMUNOFLUORESCENCE ANALYSIS OF EPSTEIN-BARR VIRUS TRANSFORMED B CELL LINES

$0.5x10^6$ cells from normal and XLA EBV-transformed B cell lines were incubated with each of the following monoclonal antibodies, 8EB1 (CD19), B1 (CD20), BL13/10B1 (CD21), MHM6 (CD23) and G28-10 (CD39), in 100 microlitres of holding medium containing 2% FCS and 0.01% sodium azide in LP3 tubes for 30 minutes on ice. Medium alone was used as the negative control. The cells were then washed twice in cold medium containing 2% FCS and 0.01% sodium azide. They were then incubated with 100 microlitres of pre-titrated fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin for a further 30 minutes on ice. they were washed twice and were then analysed on a fluorescent activated cell sorter.

3.8 COMPUTER PROGRAMMES USED IN LINKAGE ANALYSIS AND GENETIC RISK CALCULATION

LIPED (Ott 1974), a computer programme, has been used throughout to analyse the two-point linkage data in the family studies and to calculate genetic risk on an IBM-PC. HOMOG (Ott 1983), another computer programme, was used to assess the problem of non-allelic genetic heterogeneity in X-linked agammaglobulinaemia. The underlying concepts and mathematical aspects of linkage analysis and genetic heterogeneity are discussed in Chpater 4. The calculation

of the genetic risk depending on the proportion of unlinked families will also be explained fully in Chapter 4. A small computer programme has been written to help to do the calculation of the variation of the genetic risk of an individual as the proportion of unlinked families varies (appendix 2).

Chapter 4 GENETIC STUDIES OF X-LINKED AGAMMAGLOBULINAEMIA

4.1 INTRODUCTION

4.1.1 GENETIC LINKAGE

Linkage is the occurence of two loci on one chromosome sufficiently close together so that something less than completely independent assortment takes place (McKusick 1969). If two loci are on separate non-homologous chromosomes, then independent assortment occurs. Even if the two loci are on the same chromosome, if they are far apart, crossing over can still result in independent assortment. The proportion of such crossing-overs out of all opportunities for crossing-overs is called the recombination fraction, denoted by theta (θ). For linked gene loci, theta is less than 50% and for unlinked gene loci, theta is 50%. Linked loci are on the same chromosome and theta is a measure of genetic distance. For two linked loci, the smaller the theta, the smaller is the physical distance between them. However, there is no linear relationship between genetic and physical distance. A mapping function relating genetic to physical distance has been used to translate theta into map distance, measured in centimorgan (cM) (Emery 1986). For smaller map distance (less than 20 cM), one centimorgan means 1% recombination (Emery 1986). One centimorgan is approximately equivalent to one million base pairs in human (Barlow and Lehrach 1987). Recombination of X-linked genes can obviously only occur in females with two X chromosomes.

4.1.2 LINKAGE ANALYSIS

The objective of linkage analysis of two loci is to determine the value of theta and how significantly smaller it is than 0.5. The method most used as developed by Morton (1955) is nowadays called the "lod scores" method (Ott 1985). The principle of this maximum likelihood method of linkage analysis can be described as follows. The probability or likelihood, L(0.5), that the observed family data conform to the behaviour of two loci under full recombination without linkage is calculated. Similarly, the likelihood, L(θ), that the identical family data are the result of two linked loci under a specified recombination fraction, θ, is determined. The ratio of these two likelihoods, L(θ)/L(0.5), is called the odds for and against linkage, and expresses the odds for and against linkage. This odds ratio has to be determined for each family. In order to summate the linkage data for different families, it is much more convenient to express the odds ratio in decimal logarithm, the so-called lod or lod score (logarithm of the odds),

$$Z(\theta) = \log_{10}[L(\theta)/L(0.5)] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

The lod scores, Z(θ), for different families can simply be added together to give a combined score for a given θ value. To estimate the recombination fraction, one calculates Z(θ) for the sequence of θ values from 0 to 0.5. The value of θ at which Z(θ) is largest is the most likely value for the recombination fraction. If Z(θ) is

highest at $\theta=0.5$, negative evidence for linkage is obtained. By construction, $Z(\theta)=0$ in this situation. If $Z(\theta)$ is highest at $\theta<0.5$, evidence for linkage is obtained. This maximum value of lod score, $Z(\hat{\theta})$, at the most likely recombination fraction ,$\hat{\theta}$, is a statistical measure in the test of the null hypothesis of free recombination, $\theta=0.5$, against the alternative hypothesis of linkage, $\theta<0.5$. Linkage is considered significant when $Z(\hat{\theta})$ is equal to or greater than a value of 3, corresponding to odds for linkage of at least 1000 to 1. Likewise, values of θ with a lod score of $Z(\theta)<-2$ are considered very implausible and linkage is excluded at these values of θ.

It is important to construct confidence intervals for the maximum likelihood estimate of the recombination fraction by the lod score method described above. A simple method is recommended by the committee on Methods of Linkage Analysis and Reporting, Eighth Human Gene Mapping Workshop (Conneally et al 1985). A horizontal line is drawn at a distance of 1 lod score below the peak lod score. The two points of intersection between the straight line and the lod score curve mark the two end points of the confidence coefficient for θ. The associated confidence interval is at least one of 90% and better than 95% in large data sets. LIPED, a computer programme, has been written (Ott 1974) to compute the lod scores at various thetas for each family provided with the genotypes of the two loci tested. It takes into

consideration all the pedigree information and is used in most two-point linkage analysis.

4.1.3 HETEROGENEITY

Linkage analysis can reveal disease heterogeneity by demonstrating a difference in the recombination fraction between disease and marker loci in different families. One explanation for this is that the same disease phenotype can be casued by mutations at different gene loci in different families. Assume there are two types of families, those with linkage and those without and the disease phenotypes in these two types of families are impossible to differentiate between one another at the present time. One, therefore, cannot predivide these families into linked or unlinked types before performing the linkage analysis. Under such circumstances, the admixture test (A-test) is used to determine whether the data support presence of heterogeneity at a significant level, and to jointly estimate the proportion of linked families (a) and θ, the recombination fraction in these families (Conneally et al 1985, Ott 1983, 1985,1986a). The proportion of unlinked families will then be (1-a) with θ=0.5. Let Li(θ) be the likelihood of the ith family and Li(0.5)=1. The corresponding lod score is given by $Zi(\theta)=\log_{10}Li(\theta)$. With heterogeneity (Ott 1983), the lod score of the ith family becomes

$$Zi(a,\theta) = \log_{10} [aLi(\theta)+1-a] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where a denotes a sequence of a-values at which lod scores

are computed. The pair of values (a,θ) at which the total lod score

$$Z(a,\theta) = \sum \log_{10} [aLi(\theta)+1-a] \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

is highest, determines the maximum likelihood estimates of proportion of linked families ,$\hat{a}$, and recombination fraction of these families, $\hat{\theta}$. $Li(\hat{\theta})$ is then the conditional likelihood of the ith family, given that this is a linked family, while $\hat{a}$ is the prior probability that any family belongs to the linked group. By Bayes' formula, the posterior probability, wi, that the ith family be linked, is given by

$$wi = aLi(\theta)/[aLi(\theta)+1-a] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

When a and θ in (4) are replaced by their respective maximum likelihood estimates ($\hat{a}$ and $\hat{\theta}$) one obtains the estimated posterior probability, $\hat{wi}$, of linkage for the ith family. The input data for the A-test are the lod scores Zi(θ) at a sequence of θ-values for each individual family. A computer programme (HOMOG) has been written by J. Ott (1983, 1985) to carry out the computations. Approximate 95% confidence intervals for both $\hat{a}$ and $\hat{\theta}$ are also given by this programme. The number of families required to detect or exclude linkage heterogeneity has also been estimated (Ott 1986b).

Subsequently, families can be classified as being of the linked group by different rules (Ott 1983). The more reliable rule under most circumstances for a family to be

of the linked group is that wi>â. (Ott 1983, 1985).

4.1.4 RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Bacterial restriction endonucleases recognize specific sequences in DNA and catalyze endonucleolytic cleavages, yielding fragments of defined lengths. Throughout the human genome there are neutral base changes that may create or remove restriction endonuclease sites, leading to differences in the lengths of restriction fragments after digestion of genomic DNA by restriction endonucleases (Botstein et al 1980). These restriction fragment length polymorphisms (RFLPs) can also result from differing copy numbers of a simple tandemly repeating sequence within a fragment (Jeffreys et al 1985, Wong et al 1986). RFLPs are inherited as simple Mendelian co-dominant markers and provide a potentially large number of linkage markers for following disease genes through families (Botstein et al 1980). The technique to analyse the pattern of RFLPs is called Southern blotting (Southern 1975), described in Chapter 3, this thesis. The DNA probe used to reveal the RFLPs can either be the gene of interest or just recombinant DNA sequence of no function from various sources, such as human genomic or cDNA libraries (Botstein et al 1980), or X-chromosome library (Davies et al 1981). For monogenic diseases with unknown underlying defects, it is now possible to map the gene loci by linkage analysis as long as there are recombinant DNA probes which reveal RFLPs near enough to the gene loci. It has been calculated in order to provide an

adequate genetic map for the entire human genome of 33 Morgans in length, one would need at least 150 polymorphic DNA probes evenly spaced at about 0.2 Morgans (Botstein et al 1980). For the human X-chromosome, there are now more than 20 well characterised DNA marker loci with RFLPs spanning a genetic length of about 200 recombination units (Drayna and White 1985, Davies 1985). The distribution of these RFLP markers on the X-chromosome is such that any X-linked disease locus will map within ten recombination units of two markers provided enough informative families are available for linkage analysis.

One potential limitation of this approach is the degree of polymorphism of the RFLP marker loci, which should have at least two alleles with appreciable frequency. The maximum rate of heterozygosity of any two alleles RFLP marker is only 0.5 when the frequency of each allele being 0.5. The more polymorphic a locus is, the more useful it is for linkage analysis. One way to measure the degree of polymorphism is the polymorphism information content (PIC) (Botstein et al 1980). The PIC is the probability that an offspring of a random mating between a carrier of a rare dominant gene and a non-carrier is informative for linkage between the locus of the dominant gene and the marker locus. As more and more RFLP markers are defined, this aspect should not be a major limiting step in the linkage analysis. For rare genetic diseases, the availability of informative families is the major limitation. The number of families required

to establish linkage depends on the size and structure of the pedigrees as well as the degree of linkage between the disease locus and RFLP marker used. Multigenerational pedigrees are more useful than nuclear families and large families give considerably more information than smaller families (Botstein et al 1980). Less families are needed if the linkage between disease locus and the RFLP marker is tight.

4.2 LINKAGE ANALYSIS BETWEEN X-LINKED AGAMMAGLOBULINAEMIA AND RANDOM DNA FRAGMENTS FROM Xq21.3-q22

The underlying defect of X-linked agammaglobulinaemia (XLA) is still unclear and there is no immunological method of detecting carriers (Chapter 1, this thesis). Carrier detection is however feasible by demonstrating the pattern of non-random X-chromosome inactivation which is present in the B cells of female carriers (Fearon et al 1987) but the techniques are difficult. Prenatal exclusion based on B-cell enumeration has been performed but it is only limited to mid-trimester fetal blood samplying (Durandy et al 1986). Furthermore the heterogeneity with respect to the presence or absence of B cells in some XLA patients (Leickley and Buckley 1986) renders this approach unreliable for prenatal diagnosis. An alternative strategy of offering carrier detection and prenatal diagnosis in monogenic diseases of unknown aetiologies is to localise the defective gene by linkage analysis with DNA probes. Using the approach of gene tracking, such genetic prediction then becomes feasible

(Pembrey 1986). We therefore started a family linkage study of XLA two-and-a-half years ago with this objective in mind.

X-chromosomes of all mammals are thought to carry homologous genes as a special method of control is involved in X-chromosome inactivation which would make it difficult for genes to be exchanged between the X-chromosome and autosomes during evolution (Lyon 1988). Since the X-linked immunodeficiency disease (xid) gene in the mouse has been mapped between PGK (phosphoglycerate kinase) and Ags (alpha-galactosidase) (Berning et al 1980), it has been thought that the human X-linked immunodeficiency genes might also be mapped to a comparable location at the middle of the long arm of the human X chromosome. Indeed, two separate and parallel family studies of XLA (Kwan et al 1986, Mensink et al 1986a) were published during the tenure of my research fellowship demonstrating linkage of the XLA gene locus to DXS17 (S21) and DXS3 (19.2) in the region of Xq21.3-q22. By that time, we had collected 12 XLA families suitable for linkage analysis and in colloboration with a French group headed by Professor C. Griscelli in Paris and Dr. J. L. Mandel in Strasbourg who contributed data from 3 XLA families, we also mapped the XLA gene locus to Xq21.3-22 with probes S21 and 19.2; in addition we demonstrated close linkage with a further probe pXG12 (DXS94) (Malcolm et al 1987). The results of this linkage study and of the clinical application in genetic counselling in these

families are presented in this chapter.

4.2.1 LINKAGE OF 19.2, S21 AND pXG12 IN FAMILIES WITH XLA

DNA from members of the XLA families were analysed by Southern blotting for RFLPs using the probes 19.2, S21 and pXG12. The 15 XLA families which were used for the linkage analysis are families 1-8, 10-12 and 14 (Figure 2-1) plus the 3 French families. The RFLP data with the 3 probes, viz 19.2, S21 and pXG12 are shown in table 3 and the pedigrees with the RFLP data of S21, pXG12 and/or 19.2 are shown in figure 4-1. 19.2 detects a Taq 1 polymorphism with alleles of 5.0 kb or a doublet of 2.9 and 2.2 kb; S21 a Taq 1 polymorphism with alleles 2.2 kb and 2.0 kb; pXG12 a Pst 1 polymorphism with alleles 7.2 kb and 6.5 kb.

9 families were informative for linkage analysis with 19.2, 10 families with S21 and 11 families with pXG12. The RFLP data for each probe were analysed by the computer programme, LIPED (Ott 1974) to test for linkage with the XLA disease locus. For LIPED one has to specify the gene frequencies at each gene locus. Since XLA is a rare disease of unknown frequency, its gene frequency was arbitrarily set at 0.0001, which is the lowest allowed for in LIPED (Ott 1985). Hence the gene frequency of the

Table 3

RFLP DATA OF TNE 15 XLA FAMILIES AND ONE X-LINKED HYPERIgM FAMILY

Family	Individual	19.2	pXG12	S21
1	I-1 (O.C.)	1,2	1,2	1,2
	I-3	1	2	1
	I-6	nd	1,2	1,2
	I-9	nd	1	2
	I-10	nd	1,2	1,2
	I-11	1	1	1
	I-12 (O.C.)	1,2	1,2	1,2
	I-13	1	1	1
	I-14 (O.C.)	1,2	1,2	1,2
	II-1	1	2	1
	II-2	1	2	1
	II-3 (aff)	1	2	1
	II-5	nd	1,2	1,2
	II-7	nd	1,2	1,2
	II-8	nd	1,2	1,2
	II-11	1	2	1
	II-12	1,2	1	1,2
	II-13	1	1,2	1
	II-14	1,2	1	1,2
	II-15(aff)	1	2	1
	III-1	1,2	2	1

(continued next page)

Table 3 (continued)

Family	Individual	19.2	pXG12	S21
1	III-2	1	1,2	1
	III-4	nd	2	1
	III-5	1,2	1,2	1
	III-6	2	1	2
	III-8	nd	1	2
2	I-1 (O.C.)	1,2	2	1,2
	I-2 (O.C.)	1,2	2	1,2
	I-3	2	2	1
	I-4	1,2	2	1,2
	II-1	2	nd	2
	II-2 (aff)	1	nd	1
	II-4 (aff)	1	nd	1
	II-5	1,2	2	1
	II-6	2	nd	2
3	I-1	nd	1	1
	I-2 (O.C.)	2	1,2	1,2
	II-1	1,2	1	1,2
	II-2	2	2	1
	II-3 (aff)	2	1	2
	II-7	nd	2	1
	III-2	nd	1	1

(continued next page)

Table 3 (continued)

Family	Individual	19.2	pXG12	S21
4	I-1	nd	2	1
	I-2	nd	1,2	2
	II-1 (O.C.)	2	1,2	1,2
	II-2	nd	1	2
	II-3	nd	2	2
	II-4	nd	2	1,2
	III-1(aff)	nd	1	2
	III-2(aff)	nd	1	2
	III-3	nd	2	1
	III-4	nd	2	2
5	I-1	nd	2	1
	I-2	nd	1	nd
	II-1 (O.C.)	1,2	1,2	1
	II-2	nd	1	1
	III-1(aff)	2	2	nd
	III-2	1	1	nd
	III-3(aff)	2	2	nd
6	I-1	nd	1	nd
	I-2	nd	1,2	1
	II-1(O.C.)	1,2	1,2	1
	II-2	nd	1	nd
	II-3	nd	1,2	1
	II-4	nd	1	nd
	III-1(aff)	1	2	nd
	III-2(aff)	1	2	1

(continued next page)

Table3 (continued)

Family	Individual	19.2	pXG12	S21
7	I-2 (O.C.)	2	1,2	1,2
	II-1 (O.C.)	2	2	1
	II-2 (O.C.)	2	2	1
	II-3	nd	2	1
	II-4	nd	2	1
	II-6	nd	1	2
	II-7	nd	1	2
	III-1(aff)	nd	2	1
	III-2	nd	2	1
	III-3(aff)	2	2	1
	III-4(aff)	2	2	1
8	I-2	nd	1,2	1,2
	II-1 (O.C.)	2	2	1,2
	II-2	nd	1,2	1
	III-1(aff)	2	nd	2
	III-2(aff)	2	nd	2
9	I-1	nd	2	1
	I-2	nd	2	1,2
	II-1	nd	nd	2
	II-2 (O.C.)	nd	2	1,2
	II-3	nd	2	1,2
	III-2(aff)	nd	2	1
	III-3	nd	2	1,2

(continued next page)

Table 3 (continued)

Family	Individual	19.2	pXG12	S21
10	I-1	2	1	nd
	I-2	1	1,2	nd
	II-2 (O.C.)	1,2	1,2	1
	II-3	1	2	nd
	II-6	1,2	1,2	nd
	II-7	1,2	1	nd
	III-1(aff)	2	1	nd
	III-2(aff)	2	1	nd
	III-3(aff)	2	1	nd
11	I-1	nd	1	nd
	I-2 (O.C.)	1	1,2	1
	II-1 (O.C.)	1	1,2	1
	III-2(aff)	1	2	1
12	I-2	1,2	1,2	1,2
	II-2 (O.C.)	2	2	1,2
	II-5	1	1	1
	II-6	1	nd	1
	III-1	nd	nd	1
	III-2(aff)	nd	nd	2
	III-3(aff)	nd	nd	2
	III-4(aff)	nd	nd	2

(continued next page)

Table 3 (continued)

Family	Individual	19.2	pXG12	S21
13	I-1	nd	nd	1
	I-2	nd	nd	1,2
	II-1 (aff)	nd	nd	1
	II-2	nd	nd	2
14	I-2 (O.C.)	1,2	1	1
	II-1 (aff)	1	1	nd
	II-2 (aff)	1	1	nd
	II-3 (aff)	2	1	nd
15	I-1 (O.C.)	2	1,2	1,2
	I-2	1	1	1
	II-1 (O.C.)	1,2	1,2	1,2
	II-5 (O.C.)	1,2	1	1
	III-1	2	2	2
	III-9	2	nd	nd
19 X-linked hyperIgM	I-1 (O.C.)	1,2	2	1,2
	II-2	2	2	1
	II-5 (O.C.)	1,2	1,2	1
	II-6 (O.C.)	1,2	1,2	1
	III-1	1	1	1
	III-2	2	2	1
	III-5(aff)	2	2	1
	III-8	1	1	1

O.C. = obligate carrier
aff. = affected
nd = not done
allele 1 = larger fragment
allele 2 = smaller fragment(s)

Family 1

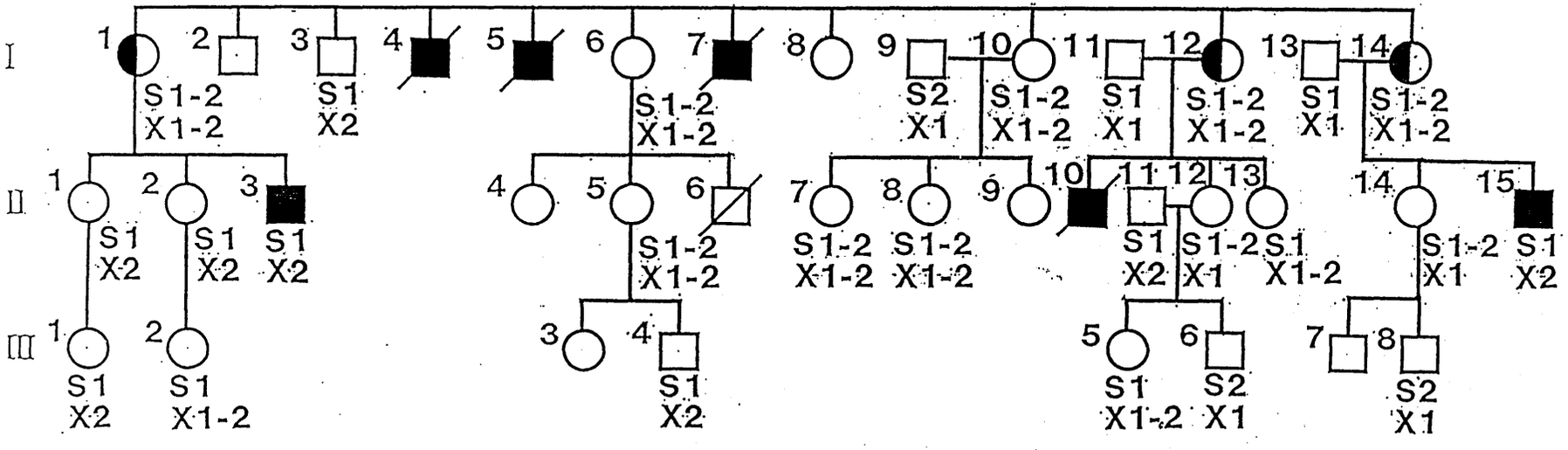


Figure 4-1 Pedigrees with RFLP data of the 15 families with X-linked agammaglobulinaemia.
S21 Taq 1 polymorphism (S1 is 2.2kb, S2 is 2.0kb).
pXG12 Pst 1 polymorphism (X1 is 7.2kb, X2 is 6.5kb).
19.2 Taq 1 polymorphism (P1 is 5.1kb, P2 is 2.95/2.15kb).

Family 2

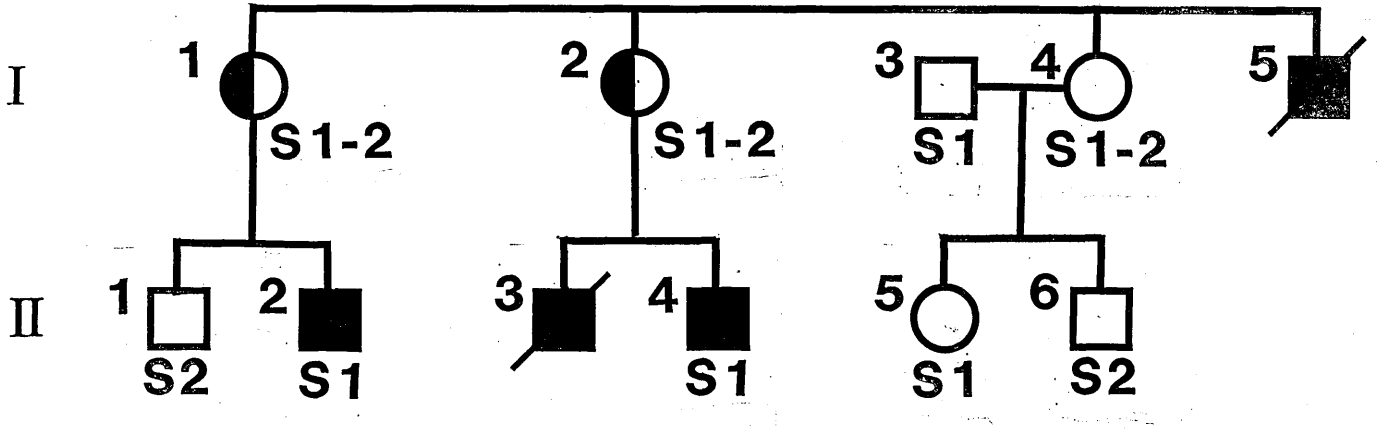


Figure 4-1 (continued)

Family 3

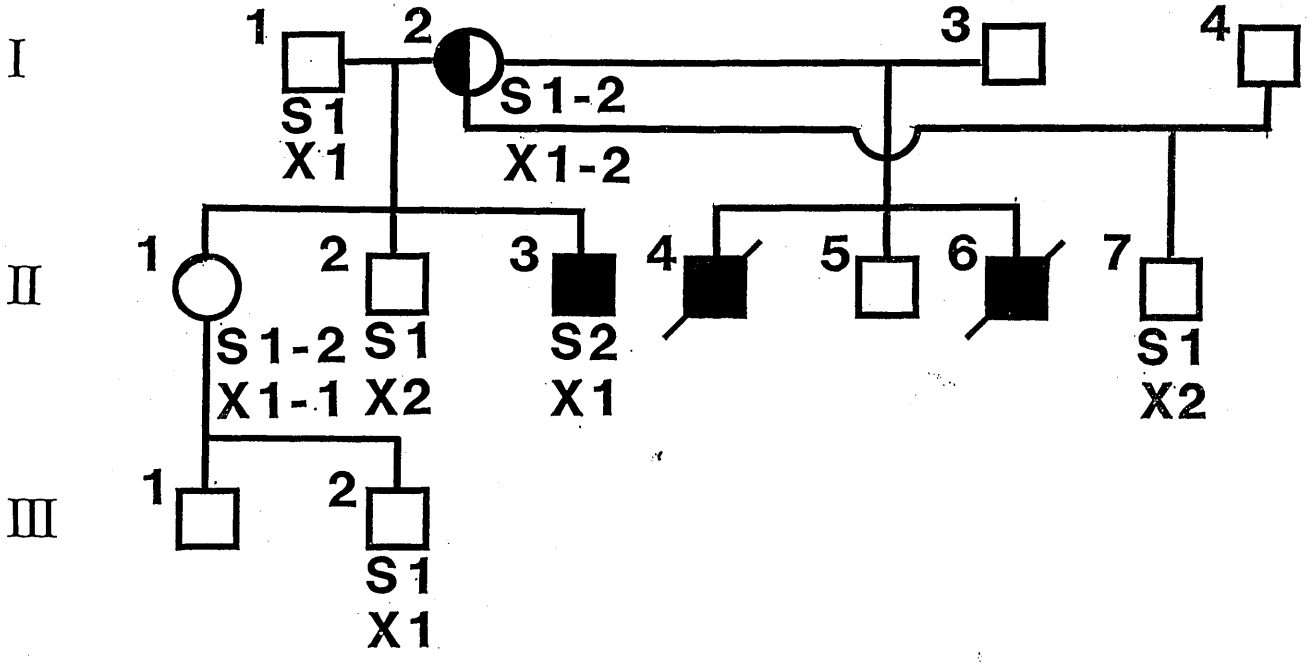


Figure 4-1 (continued)

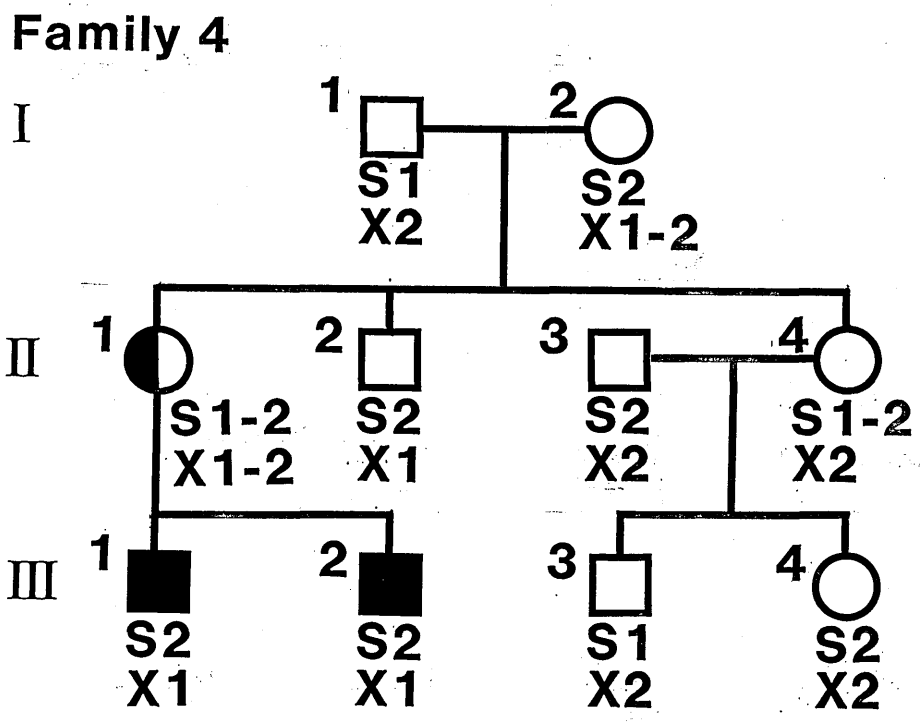


Figure 4-1 (continued)

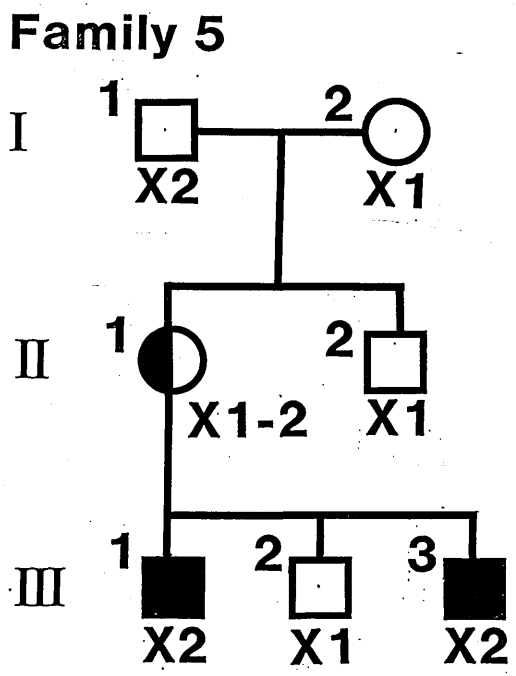


Figure 4-1 (continued)

Family 6

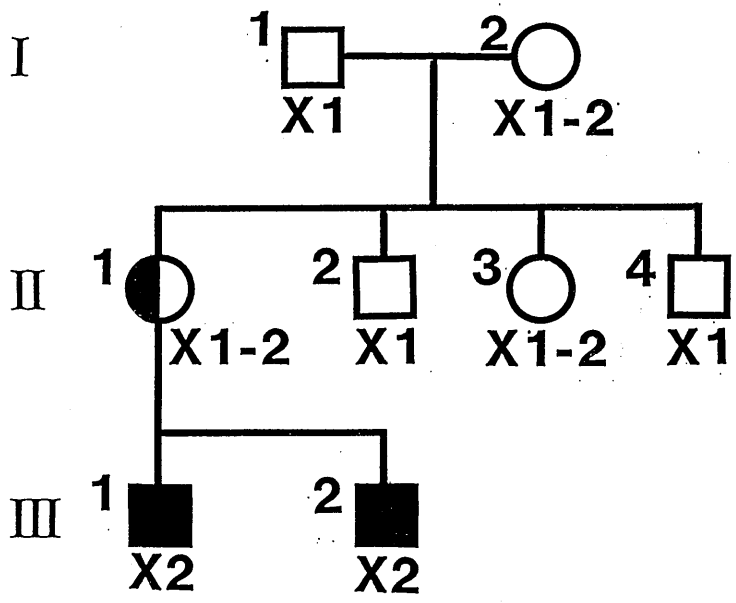


Figure 4-1 (continued)

Family 7

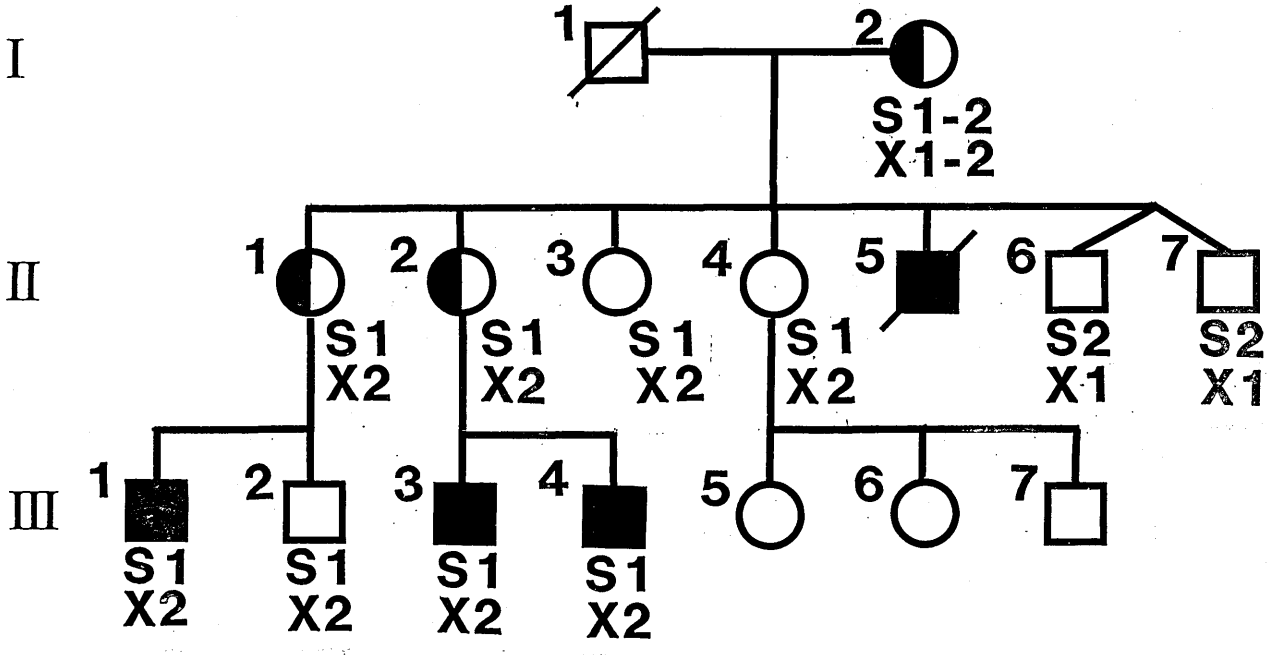


Figure 4-1 (continued)

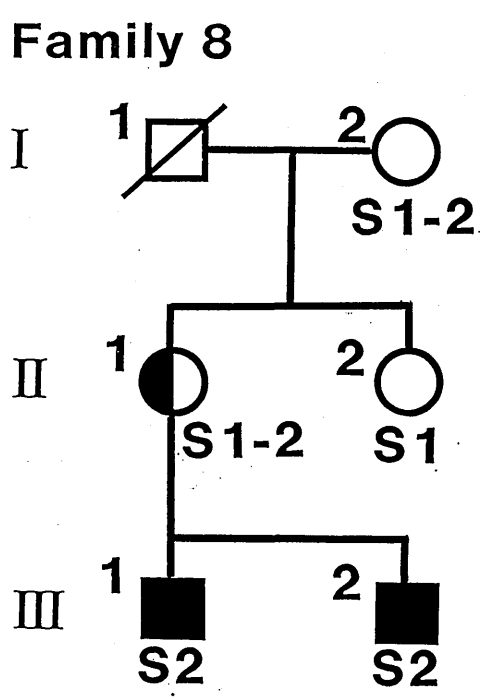


Figure 4-1 (continued)

Family 9

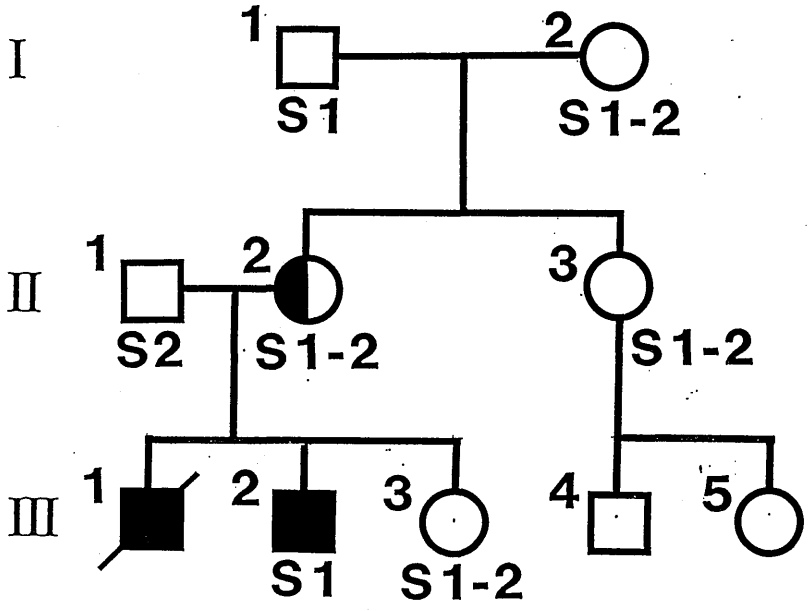


Figure 4-1 (continued)

Family 10

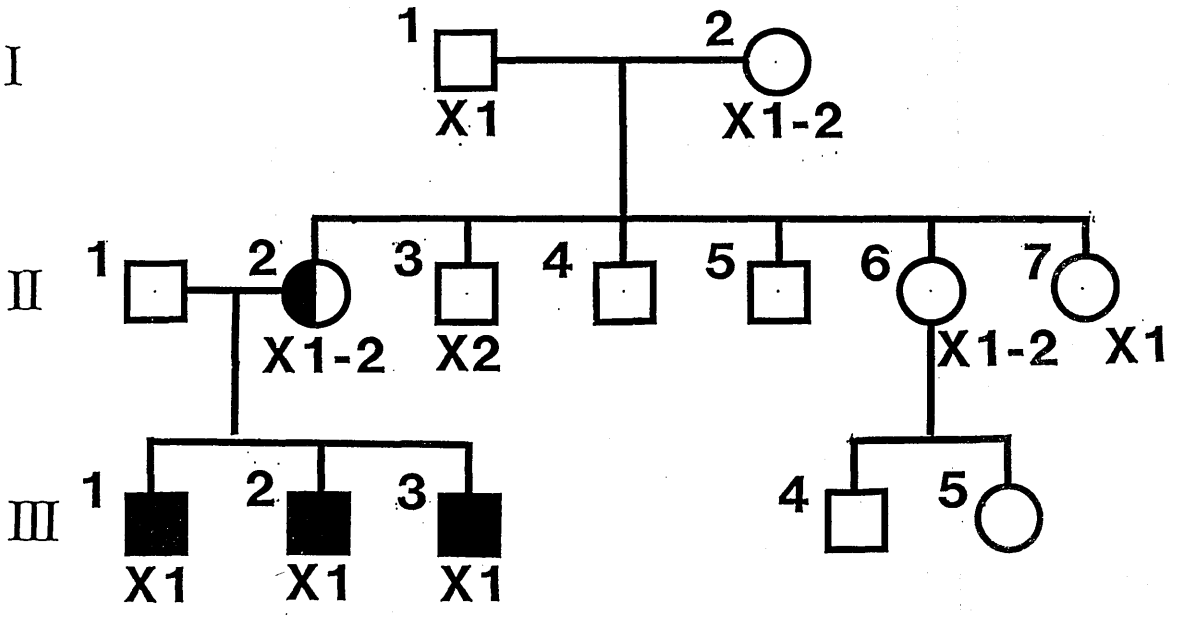


Figure 4-1 (continued)

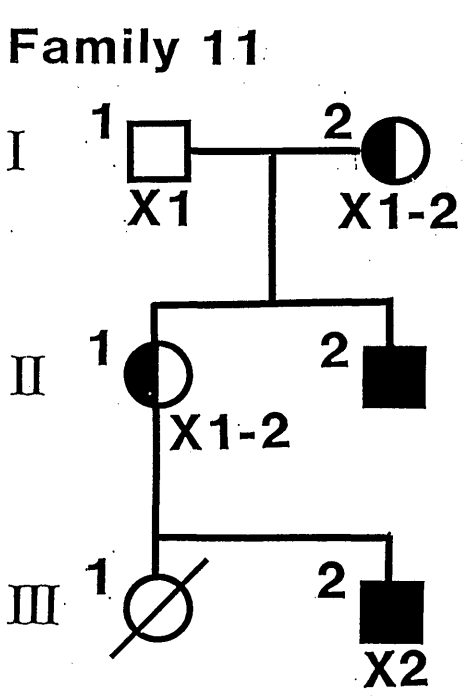


Figure 4-1 (continued)

Family 12

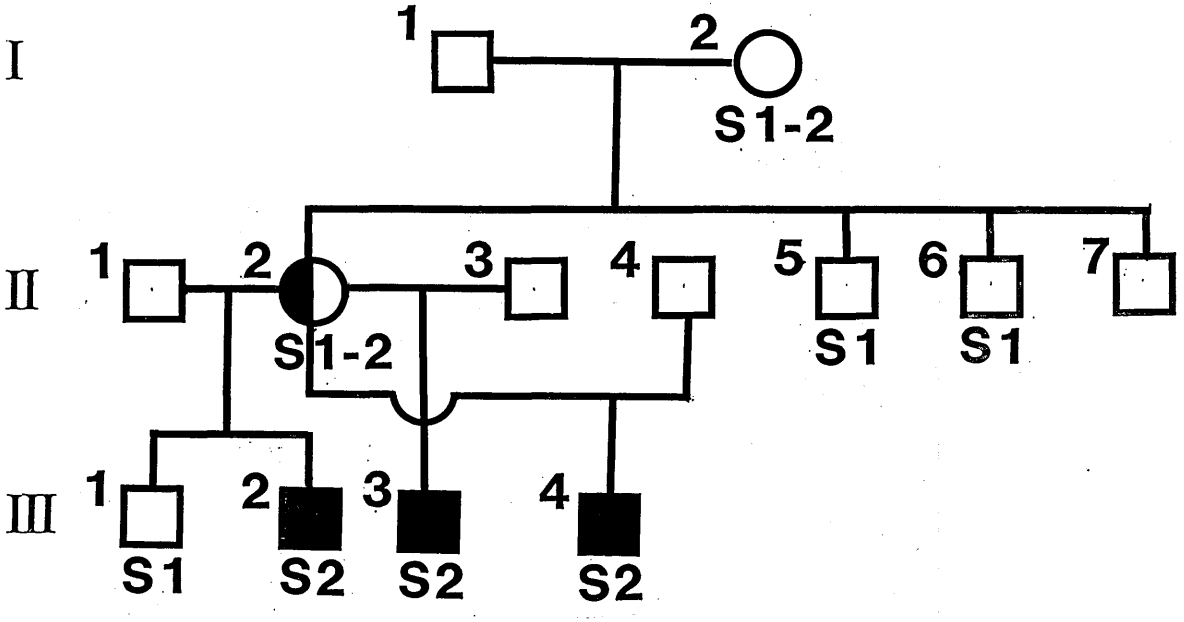


Figure 4-1 (continued)

Family 13

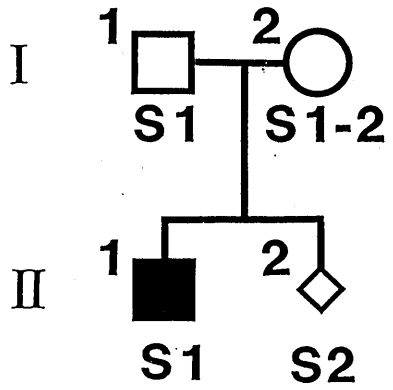


Figure 4-1 (continued)

Family 14

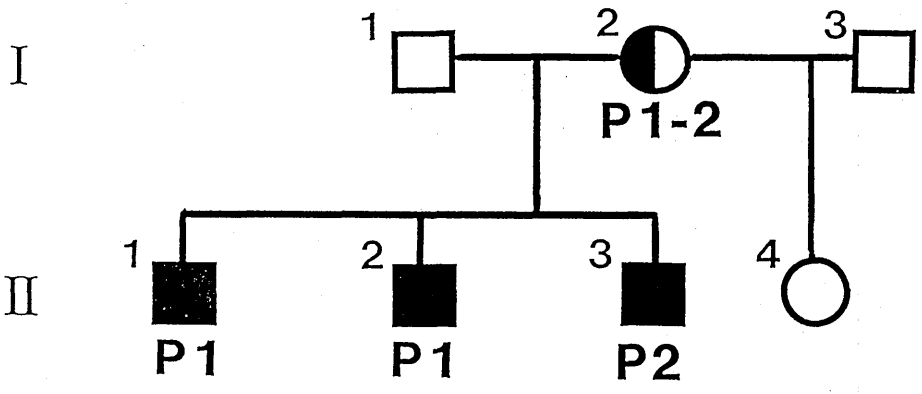


Figure 4-1 (continued)

Family 15

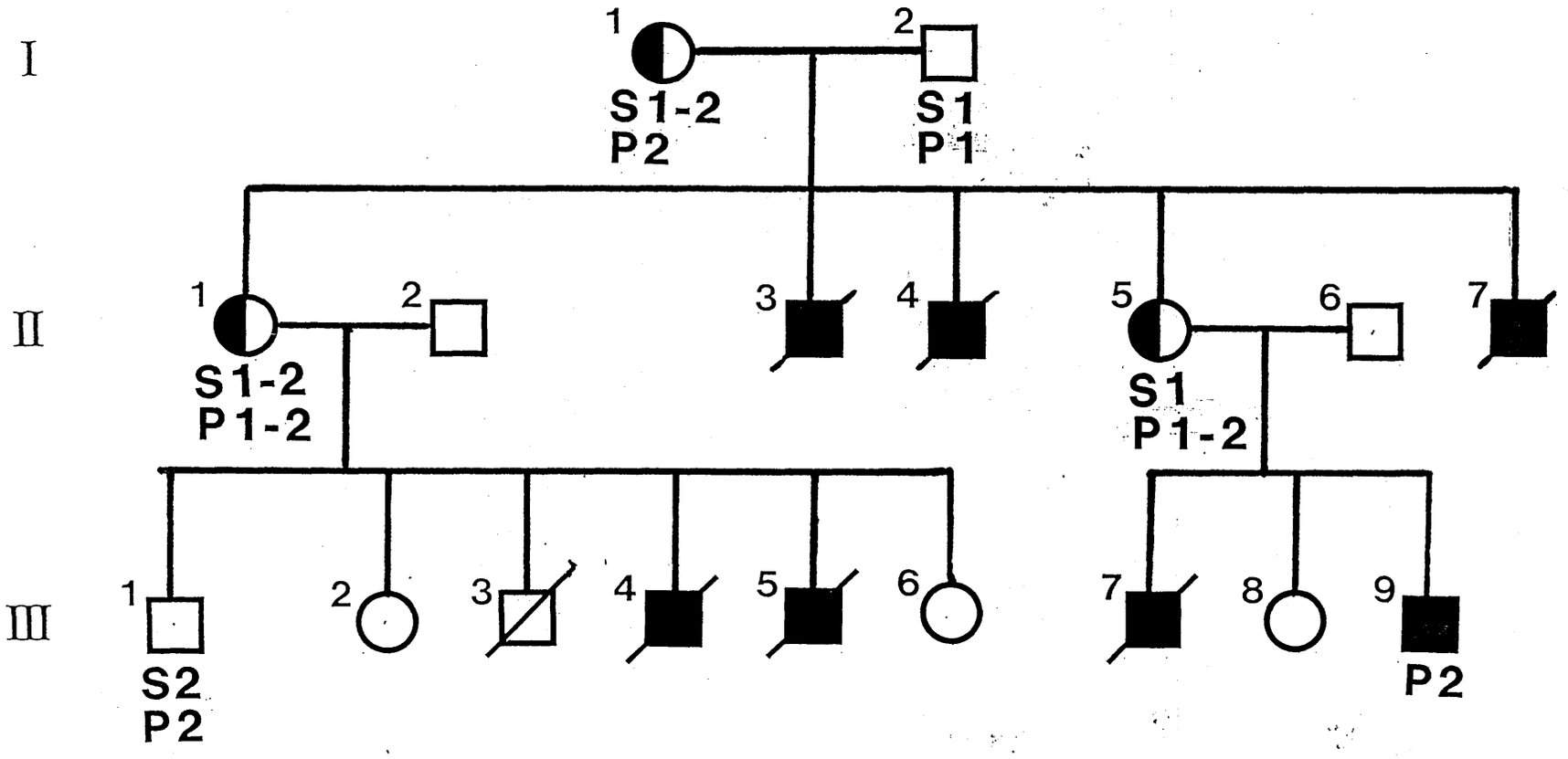


Figure 4-1 (continued)

normal allele at the XLA gene locus was set at 0.9999. The gene frequencies of the two alleles of each probe were set at the known values. The input and output files of LIPED for each of the three probes are included in appendix 3. The summation of lod scores at various theta values for the three probes against the XLA locus are given in table 4. No recombination was found with using S21 or pXG12. One cross-over was observed with 19.2 in a family which was non-informative for either S21 or pXG12 (family 14).

4.2.2 MAPPING OF PXG12

pXG12 was originally mapped to the proximal part of the long arm of the X chromosome (Davatelis et al 1985, Goodfellow et al 1985). Two lines of evidence now suggest it lies between 19.2 and S21. In a man with choroideraemia but normal immunity, high resolution chromosome studies showed that the band Xq21 was about half its normal size, probably due to a deletion of sub-band Xq21.1 (Hodgson et al 1987). DNA studies from him showed both the X loci of DXYS1 (pDP34) and DXS3 (19.2) were deleted but not the locus of DXS17 (S21). A clear 6.5kb allele of pXG12 was however observed (Figure 4-2), showing that pXG12 lies outside the DXYS1-DXS3 segment. Evidence that it falls below 19.2 rather than above pDP34 comes from a multipoint linkage analysis of 44 families, which suggests the order being centromere-pDP34-19.2-pXG12-S21 (Arveiler et al 1987a).

Table 4

LOD SCORES FOR FAMILIES WITH XLA

	LOD SCORES		
LOCUS Probe	DXS 3 19.2	DXS 94 pXG12	DXS 17 S21
θ=0.00	0.49	6.75	4.90
θ=0.01	3.39	6.62	4.79
θ=0.05	3.68	6.10	4.35
θ=0.10	3.46	5.41	3.78
θ=0.15	3.09	4.71	3.21
θ=0.20	2.66	3.98	2.63
θ=0.25	2.20	3.23	2.06
θ=0.30	1.71	2.48	1.51
θ=0.35	1.23	1.75	1.01
θ=0.40	0.78	1.06	0.56
θ=0.45	0.36	0.46	0.22

Combined lod scores for various recombination fractions (θ) between XLA locus and the listed loci in 15 XLA families (families 1-8, 10-12, 14 and three French families).

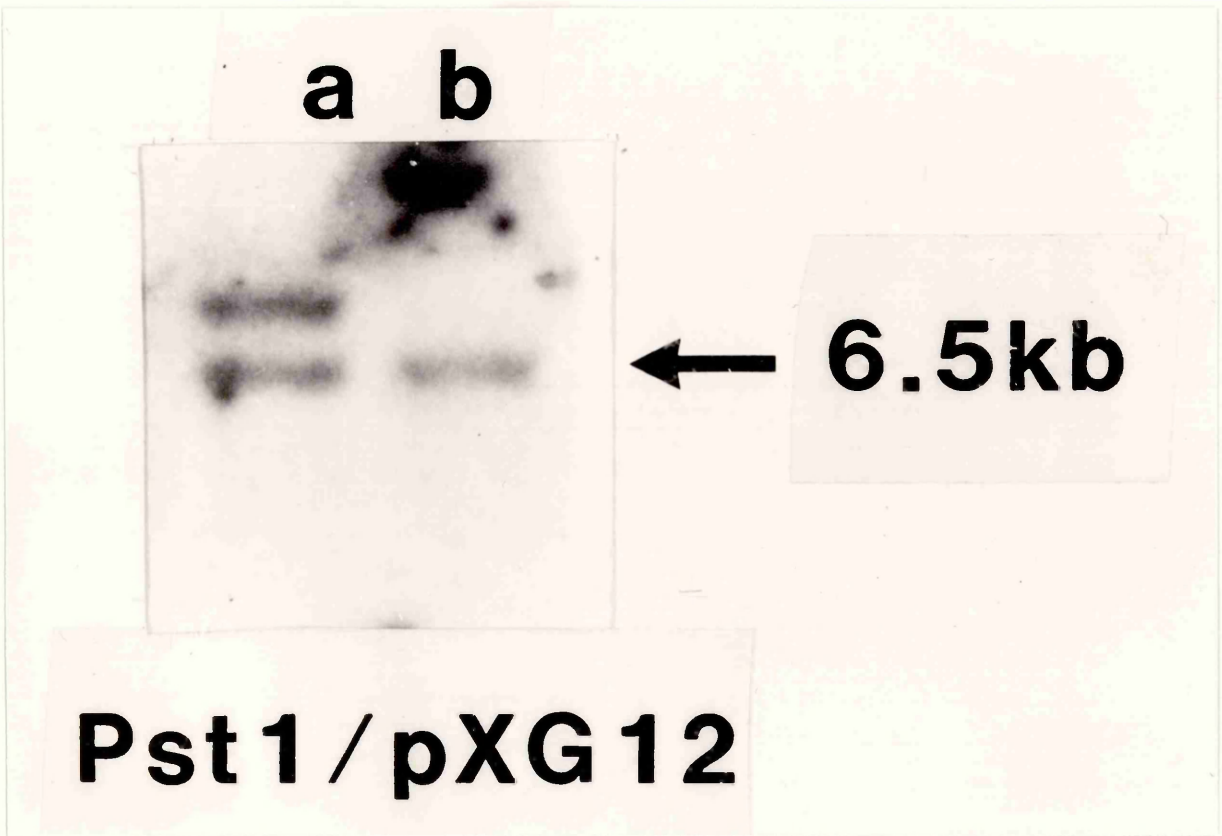


Figure 4-2 Autoradiograph showing the 6.5kb allele of pXG12 in a man with choroideraemia and deletion of sub-band Xq21.1 (lane b), normal female (lane a).

4.2.3 DISCUSSION

Although XLA has now been mapped to Xq21.3-q22 (Kwan et al 1986, Mensink et al 1986a, Malcolm et al 1987), a comparable region of the X chromosome as the xid gene locus in the mouse (Berning et al 1980, Lyon 1988), the two diseases (XLA and xid) are not strictly analogous in their phenotypes. XLA results in decreased immunoglobulin of all isotypes in man while xid results in impairment of antibody response to polysaccharide antigens in mouse (Scher 1982). Moreover, several human X-linked immunodeficiencies such as Wiskott-Aldrich syndrome and X-linked severe combined immunodeficiency have been mapped to outside this homologous region (Peacocke and Siminovitch 1986, de Saint Basile et al 1987). Hence, there does not seem to be a family of genes in this region of the human X chromosome which is analogous to the family of XLR genes which are linked to the xid in mouse (Cohen et al 1985 a&b).

Kwan et al (1986) was the first to find linkage of XLA with 19.2 at $\hat{\theta}$=0.04 with a lod score of 3.65 and with S21 at $\hat{\theta}$=0 with a lod score of 2.17. Mensink et al (1986a) soon confirmed the linkage of XLA with 19.2 in a large pedigree, 66-8, with a lod score of 3.3 at $\hat{\theta}$=0.06, but presented a family, Z, with indistinguishable phenotypes from XLA and which had a lod score of -3.14 at $\hat{\theta}$=0.06. Using the same set of nine families, Ott et al (1986) demonstrated two families, Z and EsB, have negative lod scores at the location of the S21 locus by multi-point

linkage analysis and hence appear not to be linked to the region of the S21 locus. The other seven families show linkage of XLA to S21. Hence the hypothesis of heterogeneity with one linked and one unlinked type is much more favoured than that of homogeneity (Ott et al 1986). Our data certainly confirmed the linkage of XLA with 19.2 at $\hat{\theta}$=0.04 with a lod score of 3.69 and with S21 at $\hat{\theta}$=0 with a lod score of 4.90 (Table 4). We also extended the linkage of XLA with another proble pXG12 with a lod score of 6.75 at $\hat{\theta}$=0 (Table 4). In this linkage study of 15 families (3 families from the French group), we have no evidence for genetic heterogeneity. However, it does not prove there is no heterogeneity as the number of families required to detect heterogeneity can be quite large depending on the proportion of linked families (a), the value of theta between the disease and marker as well as the structure of the families (Ott 1986b). Generally, the lower the proportion of linked families and the higher the θ, the more families one would need to detect heterogeneity at a significant level. Therefore, we may simply not have enough families to detect heterogeneity.

On the other hand, there are alternative explanations for the observed heterogeneity in families Z and EsB as reported by Mensink et al (1986a) and Ott et al (1986). The data from family EsB is not totally reliable as one female (II-1) was assigned as being a carrier on the basis that one of her sons born before 1950 died between 2 months and 8 years of age (Mensink et al 1986a). For

family Z, Ott et al (1986) has suggested a possible autosomal dominant and male limited inheritance, but no affected male has been reported to produce an affected son. This hypothesis can be tested by examining the B cells of the female carriers in this family for pattern of X-chromosome inactivation (Fearon et al 1987, Conley et al 1986). If the inheritance in family Z is autosomal, the pattern of X-chromosome inactivation should be random. Another explanation has been proposed by Mensink et al (1987a) that grandfather (I-1) in family Z could be a mosaic in his germ line, in which case grandmother (I-2) is not a carrier and no recombination need to be postulated between XLA and S21. This phenomenon of germ line mosaicism has been demonstrated in Duchenne's muscular dystrophy (Darras and Francke 1987) with the partial deletion of the muscular dystrophy gene transmitted twice by an unaffected male.

S21 and 19.2 were placed within bands Xq21.3-q22 (Goodfellow et al 1985). The recombination fraction between 19.2 and S21 was estimated to be 0.10, that between 19.2 and pXG12 0.04 and that between pXG12 and S21 0.02 (Arveiler et al 1987a). The same study suggested on multipoint linkage that the order is centromere-19.2-pXG12-S21. Our maximum likelihood estimates of theta of 0.04 for 19.2 and of 0 for both pXG12 and S21 in these families agree reasonably well with these map distances. However due to lack of recombination events in these families, we are not able to place XLA exactly within the

cluster of centromere-19.2-pXG12-S21 (Arveiler et al 1987). The XLA locus should nevertheless be nearer to pXG12-S21 than 19.2, based on the fact that recombination was found with 19.2 but not with pXG12/S21 as well as that 19.2 was deleted in a man with choroideraemia and normal immunity, but not pXG12.

The issue of non-allelic genetic heterogeneity has certainly complicated the use of linked RFLPs in carrier detection and prenatal diagnosis of XLA. In the following section, further evidence of heterogeneity from another family (family 15) will be presented and the published data reviewed together with our own data to ascertain the extent of heterogeneity.

4.3 NON-ALLELIC GENETIC HETEROGENEITY

Similar disease phenotypes which cannot be easily distinguished clinically can be the result of mutations at different gene loci. This phenomenon is called non-allelic genetic heterogeneity. Linkage analysis has the potential to reveal such genetic heterogeneity, such as that in insulin-dependent diabetes mellitus (Harris et al 1985). Another X-linked immunodeficiency disease, X-linked lymphoproliferative disease (XLP), has been mapped to Xq24-qter by linkage with the probe p43-15 (DXS42) in one large pedigree (Skare et al 1987). However, in a study of 3 XLP families, Harris et al (1988) could find no evidence for linkage of the XLP locus to probes in the region Xq24-qter and their data suggested the XLP locus is

unlikely to be within Xq21.3-qter region. This may be due to the small numbers of individuals analysed in their study. Alternatively, the single family reported by Skare et al (1987) may represent a different genetic disease but with similar phenotype. For X-linked agammaglobulinaemia (XLA), there is also evidence of non-allelic genetic heterogeneity (Ott et al 1986).

4.3.1 FAMILY 15

Subsequent to our original linkage study of XLA as described above, another XLA family (family 15) was made available for linkage analysis through Dr. Donnai, Manchester. This family was first reported by Jamieson and Kerr in 1962. The only affected surviving male is in Canada and was reinvestigated recently by Dr Roifman, Toronto; he was found to have the characteristic immunological profile of XLA. The pedigree of family 15 is shown in figure 4-1 together with the S21 and 19.2 RFLP data; the RFLP data are also shown in table 3. There were at least two recombinations between the disease locus and that defined by S21 out of the three observed meioses; there were no recombinations among the three probes. LIPED was used to test for linkage of the XLA gene locus in this family with S21 and 19.2. The input and output files are included in appendix 4. Negative lod scores were obtained at all theta values indicating no evidence of linkage was present. Moreover, the lod scores for the S21 data at theta=0.03 is more negative than -2.0, hence linkage is excluded at theta values under 0.03. Germline

mosaicism cannot explain the two recombinations observed in family 15. Together with family Z from Ott et al (1986), there are now at least two XLA families which appear not to be linked with S21.

4.3.2 ADMIXTURE TEST

To assess the extent of non-allelic genetic heterogeneity in XLA, as much linkage data as possible was obtained from the published literature (Kwan et al 1986, Mensink et al 1986a, 1987a, Ott et al 1986) which was then combined with our own data for the admixture test (Ott 1983). It was analysed with respect to both S21 and 19.2 only because no other data with pXG12 have been published other than our own (Malcolm et al 1987). For 19.2, the lod scores for each individual family at various theta values were obtained by analysing the raw RFLP data of Mensink et al (1986a) using LIPED (8 families) and by assuming the 10 families of Kwan et al (1986) contributed evenly to the total lod score published. Together with our 10 families which are informative for 19.2, there are 28 families available for the admixture test. Similarly for S21, the lod scores for each individual family at various theta values were obtained by analysing the raw RFLP data of family Z (Mensink et al 1987a) using LIPED, by plotting the lod score versus theta curve for family 66.8 (Mensink et al 1987a) and by assuming the 4 families of Kwan et al (1986) contributed evenly to the total lod score. Together with our 11 families which are informative for S21, there are 17 families available for

the admixture test.

For input data to run the HOMOG, a computer programme which executes the computation of the admixture test (Ott 1983), the lod scores of each individual family at the desired theta values are required. Several runs of the HOMOG were initially executed to determine the range and interval of the desired theta values for 19.2 and S21 separately. It was then run at interval of 0.01 for the proportion of linked families (a). The output files for both 19.2 and S21 are included in appendix 5.

For 19.2, the estimated proportion of linked familes ($\hat{a}$) is 0.87 with a 95% confidence intervals of 0.52 to 1. The estimated recombination fraction ($\hat{\theta}$) of the linked families is 0.06 with a 95% confidence intervals of 0.04 to 0.16. For S21 with a smaller set of families to analyse, the corresponding values of $\hat{a}$ is 0.83 (95% confidence interval being 0.46 to 0.98) and $\hat{\theta}$ is 0.00 (95% confidence interval being 0.00 to 0.02). The HOMOG also tested the hypothesis of heterogeneity with one linked and one unlinked family type (H2) versus the hypothesis of homogeneity of linkage (H1) as well as versus the hypothesis of no linkage (HO). The components of chi-square in approximate chi-square tests (df=degree of freedom) are given for both 19.2 and S21 as follows.

Components	df	19.2 Chi-square	19.2 p-value	S21 Chi-square	S21 p-value
H2 VS HI	1	2.7	0.0515	12.7	0.0002
H1 VS HO	1	42.2	<0.0001	27.1	<0.0001
H2 VS HO	2	44.8	<0.0001	39.7	<0.0001

Both 19.2 and S21 data support the hypothesis of heterogeneity more than that of homogeneity, with S21 at a much more significant level than 19.2, because S21 is much more closely linked with the disease locus (θ=0.00 to 0.02) than 19.2 (θ=0.04 to 0.16).

The posterior probability of the ith family being the linked type (wi) is also computed with confidence limits corresponding to the calculated 95% confidence intervals of both the proportion of the linked families (a) and theta. The value of wi takes into account of the linkage data of all other families as well as that of the ith family. The calculated values of wi and the 95% confidence limits for our families and families Z and 66-8 of Mensink et al (1987a) from the analysis of both S21 and 19.2 data by the admixture test are shown in table 5.

All our families with S21 linkage data apart from family 15, have a probability (wi) of over 80% of

Table 5

POSTERIOR PROBABILITY OF LINKAGE OF FAMILIES WITH XLA

Family name	Family	POSTERIOR PROBABILITY OF LINKAGE (wi) S21	19-2
Pomfrett	1	0.95 (0.77-0.99)	0.96 (0.79-1.00)
Lucken	2	0.96 (0.82-1.00)	0.97 (0.82-1.00)
Rushin	3	0.95 (0.77-0.99)	ni
Kirsopp	4	0.91 (0.63-0.99)	ni
Barton	5	ni	0.96 (0.79-1.00)
Williams	6	ni	0.92 (0.66-1.00)
Pattersen	7	0.95 (0.77-0.99)	ni
Cox	8	0.91 (0.63-0.99)	ni
Crabbe	9*	0.83 (0.46-0.98)	0.87 (0.52-1.00)
Parry	10	ni	0.96 (0.79-1.00)
Berryman/Fox	11	ni	ni
Gerrard	12	0.98 (0.87-1.00)	ni
Lindridge	13*	0.83 (0.46-0.98)	0.87 (0.52-1.00)
Mann	14	ni	0.60 (0.15-1.00)
	15	0.00 (0.00-0.04)	0.60 (0.15-1.00)
	Z	0.00 (0.00-0.07)	0.00 (0.00-1.00)
	66-8	1.00 (1.00-1.00)	1.00 (1.00-1.00)

Posterior probability of linkage of each XLA family using RFLP data from S21 or 19-2 in the Admixture test; 95% confidence limits shown in brackets.

* = families with no linkage data
ni = not informative for RFLP tested

belonging to the linked group of families. The value of wi and its 95% confidence limits for each individual family depend on the maximum lod score, $Zi(\hat{\theta})$ of an individual family. The higher the value of $Zi(\hat{\theta})$ of an individual family, the higher the value of wi is and the tighter the 95% confidence limits of wi will be. When $Zi(\hat{\theta})$ is greater than 0.3, wi is greater than 0.9. As $Zi(\hat{\theta})$ rises above 0.9, wi becomes greater than 0.97. In a family with no linkage data of S21, i.e. $Zi(\hat{\theta})=0$, wi is the same as (a), the estimated proportion of linked families, which is 0.83. For families Z and 15 which appear unlinked to the locus of S21, wi is zero with the upper confidence limits less than 8%.

Similar observations are made from the analysis of the 19.2 linkage data by the admixture test, although the estimated proportion of the linked families (0.87) is slightly greater than that from S21 data (0.83). It is because there are more families with 19.2 data available.

4.3.3 DISCUSSION

With the knowledge of the likely proportion of linked families ($\hat{a}$) and the posterior probability of each family of being the linked type (wi), it may be feasible to use the linked probes for genetic counselling in the families classified as of being the linked group by $wi>\hat{a}$ (Ott 1983, 1985). This aspect is explored fully in the next section.

The identification of a subgroup of XLA families unlinked to Xq21.3-22 will be important in refining the

classification of XLA so as to see whether there is any difference in clinical features and prognosis between the two groups. It may also facilitate the discovery of immunological differences between the subgroups of families.

In diseases with non-allelic genetic heterogeneity, detecting linkage by traditional 2-point linkage analysis is much less effective in circumstances where the proportion of linked families (a) is small even though the recombination fraction, θ, of the linked probe used is small. It is because in the overall population, the chance that the probe will fail to cosegregate with the disease through a meiosis is the "apparent" recombination fraction $\theta'=\theta a+1/2(1-a)$ (Lander and Botstein 1986). Therefore if one neglects to take account of even a modest degree of heterogeneity, linkage can be missed entirely. In the case of XLA, it is fortunate that the proportion of linked families is greater than 0.8 and theta for S21 is still zero, otherwise linkage with Xq21.3-q22 could be easily missed by the 2-point linkage analysis. Realising the weakness of 2-point linkage analysis in mapping heterogenous genetic traits, Lander and Botstein (1986) proposed two strategies, viz interval mapping and simultaneous search, which utilize a complete RFLP linkage map of the human genome. The construction of such a map has already been attempted (Donis-Keller et al 1987). Interval mapping involves testing whether a putative locus lies in an interval of known size between two adjacent

markers. This is a more demanding hypothesis, and hence one easier to test, than whether the locus is linked to a single marker at an unknown distance. Simultaneous search involves testing several trait-causing loci simultaneouly, therefore in a heterogenous disease, at least one of the loci will appear to cosegregate with the disease in every family. By using these strategies, it is estimated only 1/3 as many affected families are needed to map a heterogeneous trait and only 1/5 to 1/50 as many are needed to detect that genetic heterogeneity is present. (Lander and Botstein 1986).

4.4 GENETIC PREDICTION IN X-LINKED AGAMMAGLOBULINAEMIA USING S21 AND PXG12

Excluding the two unlinked families Z and 15, the combined maximum lod score for S21 versus XLA locus in the 15 linked families published (4 families from Kwan et al 1986, family 66-8 from Mensink et at 1987a, 10 families from Malcolm et al 1987) is 10.5 at a recombination fraction θ of 0 with 90-95% confidence intervals 0 to 0.04 (Lau et al 1988b) as defined by taking the range of θ at one less than the maximum lod score (Conneally et al 1985). Since our original publications (Malcolm et al 1987, Lau et al 1988b), the maximum lod score for S21 for the 15 linked families above has been increased to 10.97 at θ=0 with a 90-95% confidence intervals 0 to 0.02, due to increase in lod score in one of our families. The lod score value of 10.97 has not included the lod scores of several families from Ott et al (1986) which were shown to be linked to the locus of S21 by multi-point linkage

analysis. In these 15 linked families, 40 informative meioses were observed with no recombination between the XLA locus and S21. 22 informative meioses were observed with no recombination with pXG12 in 11 XLA families in our original linkage study (Malcolm et al 1987). Since the recombination fraction between pXG12 and S21 was estimated to be 0.02 (Arveiler et al 1987a), we decided to use S21 and pXG12 in genetic counselling of XLA families, quoting a recombination fraction of 0.04 (Lau et al 1988b). A method of risk calculation has also been developed to allow for non-allelic genetic heterogeneity.

4.4.1 METHOD OF ALLOWING FOR NON-ALLELIC GENETIC HETEROGENEITY

To allow for non-allelic genetic heterogeneity, a method of risk calculation has been developed which will incorporate the probability of linkage within each individual family as well as a specified proportion of unlinked families (Lau et al 1988b). As estimated by the admixture test above the proportion of unlinked families is likely to be between 10% and 20% on the present published evidence. The true figure for the proportion of unlinked families is uncertain but one can show what effect using figures between 10% and 30% have on final prediction in any particular family.

The formal calculations are as follows. For any individual the probaility of a specific XLA genotype, given the pedigree and marker (RELP) data, can be expressed as:

$$\Pr(\text{genotype}|\text{pedigree}) = \frac{\Pr(\text{genotype},\text{pedigree})}{\Pr(\text{pedigree})} \quad ..(5)$$

If there is genetic heterogeneity the numerator and denominator in this expression (5) will be made up of two parts, depending on whether the pedigree is linked or unlinked, for example the numerator will be

$$\Pr(\text{linked}).\Pr(\text{genotype},\text{pedigree}|\text{linked}) + \Pr(\text{unlinked}).\Pr(\text{genotype},\text{pedigree}|\text{unlinked}) \quad ... \quad (6)$$

In the first part of the above expression (6), the prior probability that the disease segregating in that pedigree is in fact linked with the marker locus is multiplied by the probability of the specific genotype in the individual in question and the pedigree data given that the disease is linked to the marker (θ=0.04). In the second part of the expression (6), it is assumed that the disease is not linked to the marker (i.e. θ=0.5). Probability (5) can therefore be expressed as:

$$\frac{\Pr(\text{linked}).\Pr(\text{genotype},\text{pedigree}|\text{linked}) + \Pr(\text{unlinked}).\Pr(\text{genotype, pedigree}|\text{unlinked})}{\Pr(\text{linked}).\Pr(\text{pedigree}|\text{linked}) + \Pr(\text{unlinked}).\Pr(\text{pedigree}|\text{unlinked})} \quad ... \quad (7)$$

Pr(pedigree|linked) is the probability of the pedigree assuming the XLA genotype of the individual at risk is unknown and given that the disease is linked to the marker. These probabilities, together with their equivalents where the disease is unlinked to the marker, can be obtained from the computer programme LIPED (Ott

1974), in which recent versions give specific instructions for risk calculation. Pr(unlinked) can be varied from 0 to 1 to see its effect on final genetic risk of an individual in any particular family. A computer programme has been written to calculate the risk of an individual of being a specified genotype as Pr(unlinked) varies in expression (7) (Appendix 2). The input data for this programme are the values of the probabilities of the pedigrees in expression (7) which can be obtained from LIPED.

4.4.2 CARRIER DETECTION AND PREDICTION IN MALES

Out of 13 XLA families counselled (families 1-13), 11 were studied previously (Malcolm et al 1987) and demonstrated linkage between the XLA locus and probes S21, pXG12 and 19-2. One of the other two families has only one affected male living, with an affected brother who died. One family (family 13) contains a single affected male with the mother originally planning for abortion of any male fetus but opted for prenatal exclusion when she learnt that it was feasible. The pedigrees and the segregation of the S21 and/or pXG12 alleles of these 13 XLA families (families 1-13) are shown in figure 4-1. Table 6 lists all obligate and potential female carriers as well as a newborn male and a male fetus at risk from the 13 families, giving risk estimates and the possibility of prenatal diagnosis. The final risks combining pedigree

Table 6

PREDICTION OF GENETIC RISKS IN XLA FAMILIES

Subjects	Pedigree risk (%)	Pedigree + DNA risk (%) when proportion of unlinked families is: 0	0.1	0.3	Prenatal prediction with S=S21,X=XG12
Family 1					
I-1	100 (O.C.)				>45 years
I-12	100 (O.C.)				>45 years
I-14	100 (O.C.)				>45 years
I-6	27.27	24.17	24.23	24.38	>45 years
I-10	50	50	50	50	>45 years
II-1	50	95.98	95.08	92.71	ni
II-2	50	95.98	95.08	92.71	ni
II-5	9.09	1.48	1.62	2.01	
II-7	25	48.13	47.69	46.53	S+X
II-8	25	48.13	47.69	46.53	S+X
II-12	33.33	2.05	2.64	4.22	
II-13	50	95.99	95.12	92.8	X
II-14	20	1.04	1.34	2.16	
III-1	25	48	47.57	46.4	ni
III-2	25	48	47.57	46.4	X
III-5	16.67	1.03	1.32	2.11	

(continued next page)

Table 6 (continued)

Subjects	Pedigree risk (%)	Pedigree + DNA risk (%) when proportion of unlinked families is: 0	0.1	0.3	Prenatal prediction with S=S21,X=XG12
Family 2					
I-1	100 (O.C.)				>45 years
I-2	100 (O.C.)				>45 years
I-4	33.35	58.15	57.7	56.49	>45 years
II-5	16.68	55.82	55.11	53.21	ni
Family 3					
I-2	100 (O.C.)				>45 years
II-1	20	92	89.29	82.55	S
III-2*	16.67	3.69	4.25	5.62	
Family 4					
I-2	27.28	10.32	11.5	14.1	>45 years
II-1	100 (O.C.)				S+X
II-4	9.1	3.46	3.85	4.71	
III-4	4.57	1.75	1.94	2.38	
Family 5					
I-2	33.34	0.02	1.52	5.14	>45 years
II-2	100 (O.C.)				X

(continued next page)

Table 6 (continued)

Subjects	Pedigree risk (%)	Pedigree + DNA risk (%) when proportion of unlinked families is: 0	0.1	0.3	Prenatal prediction with S=S21,X=XG12
Family 6					
I-2	20	63.86	62.7	59.69	>45 years
II-1	100 (O.C.)				X
II-3	10	61.3	59.94	56.43	X
Family 7					
II-1	100 (O.C.)				>45 years
II-2	100 (O.C.)				>45 years
II-3	50	96	94.94	92.16	>45 years
II-4	33.33	92.31	90.95	87.39	>45 years
Family 8					
I-2	50	66.63	65.98	64.4	>45 years
II-1	100 (O.C.)				S
II-2	25	5.15	5.92	7.82	
Family 9					
I-2	42.86	4.16	10.24	20.35	>45 years
II-2	100 (O.C.)				S
II-3	14.3	3.56	5.25	8.05	
III-3	50	88.65	82.58	72.49	S

(continued next page)

Table 6 (continued)

Subjects	Pedigree risk (%)	Pedigree + DNA risk (%) when proportion of unlinked families is: 0	0.1	0.3	Prenatal prediction with S=S21,X=XG12
Family 10					
I-2	8.57	0.00	0.29	1.01	
II-2	100 (O.C.)				X
II-6	2.87	0.01	0.11	0.34	
II-7	4.31	0.02	0.17	0.53	
Family 11					
I-2	100 (O.C.)				>45 years
II-1	100 (O.C.)				X
Family 12					
I-2	11.11	36.52	36.23	35.42	>45 years
II-2	100 (O.C.)				>45 years
Family 13					
I-2	66.67	66.67	66.67	66.67	S
II-2~	33.33	5.15	7.97	13.61	

O.C. = obligate carrier
ni = not informative
* = Newborn male at risk
~ = Male fetus at risk

and DNA data are calculated assuming the proportion of unlinked families to be 0%, 10% and 30%. Of the 17 obligate carriers, 7 are under the age of 45 years and can all be offered prenatal diagnosis, using S21 in 2, pXG12 in 4, and either probe in 1, Of the 34 females at risk of being carriers, 17 have their risks increased, 15 decreased and 2 unchanged by the RFLP results. 11 of the 17 women whose risks were increased are under 45 years and 7 of them can be offered prenatal prediction.

The impact of varying the proportion of unlinked families in predicting low risks can also be assessed. Of the 15 women whose carrier risks were decreased, 3 were already below 5% and 7 were reduced to under 5% if the proportion of unlinked families is assumed to be 10%. Of these 7, 6 would still have a risk below 5% if a figure of 30% unlinked families were used; the other would have a risk of 5.14% (Table 6).

In family 3, II-1 was shown to be at high risk of being a carrier with both S21 and pXG12. She was heterozygous for S21 and hence prenatal diagnosis was possible. She was, however, well into the last trimester of her second pregnancy before the linkage study of XLA was completed. Pregnancy proceeded to term and a male infant (III-2) was born. RFLP analysis of cord blood indicated that he ran a low risk of being affected with XLA. With the family having 3 meioses supporting linkage with S21 and the posterior probability of this family being linked as 0.95, the risk to her son is 4.25-5.62%

(assuming 10% or 30% unlinked families respectively). He was shown to have normal numbers of circulating B lymphocytes and normal immunoglobulin level and is clinically normal at the age of one year thereby confirming our prediction.

A woman (family 13) with only one son affected with XLA but no family history presented for prenatal exclusion at 9 weeks of pregnancy. She was shown to be heterozygous for S21 and the affected son inherited the 2.2kb allele. Chorionic villus sampling was performed and the fetus was shown to have 46XY karyotype and inherited the opposite 2.0kb allele to the affected boy. A residual risk of 10% of the fetus being affected with XLA was quoted to the parents. This figure takes into account the possibility that the affected boy is a new mutation and that the mother is linkage phase unknown if she were a carrier. The calculated risk was 7.97, 10.79 and 13.6% according to whether 10,20 or 30% of families are unlinked. They accepted the risk and a male infant was born at term. Analysis of both cord blood at birth and peripheral blood at two weeks of age showed a normal number of B lymphocytes. As he is still under six months of age, serum immunoglobulins are mainly of maternal origin.

4.4.3 DISCUSSION

Until recently female carriers of XLA could not be detected, but once linkage to RFLPs was demonstrated it became possible to use gene tracking in genetic prediction

(Kwan et al 1986). In our study, of 34 potential carriers, gene tracking with S21 and pXG12 gave information that could be used in genetic counselling in 32. Of a total of 19 obligate or potential carriers under the age of 45, prenatal prediction using these probes was possible in 15. However, the present sample is biased towards optimum family structure since most were selected for the original linkage study (Malcolm et al 1987). The combination of S21 (heterozygosity frequency 0.45) and pXG12 (heterozygosity frequency 0.48) can be expected to render about 70% of females in other families amenable to gene tracking. There is no evidence of linkage disequilibrium between S21 and pXG12, or between XLA and either probe. There are several potential unrelated sources of error in using linked probes. The problems of recombination, non-paternity and new mutation are well recognised (Pembrey 1986). Here the problem of non-allelic genetic heterogeneity has been addresed in particular. Until tests independent of linkage analysis can distinguish which gene locus is involved in any particular XLA family, non-allelic genetic heterogeneity must be incorporated into any genetic prediction.

It is demonstrated here how one can allow for the problem of non-allelic genetic heterogeneity in risk calculation. However, there is still a degree of uncertainty in risk prediction due to the fact that one cannot be sure about the true proportion of unlinked families, whether it is 10%, or perhaps 30%; though it is

likely to be about 10% on present evidence. This degree of uncertainty (depending on whether unlinked families are 10% or 30%) is less in a large family (family 1) with linkage data from many members than a smaller family (family 9) with little or no linkage data with the marker.

This difference in the degree of uncertainty can be illustrated by comparing II-12 in family 1 with I-2 in family 9 where the RFLP results favour a new mutation during spermatogenesis in I-1. Both are potential carriers with pedigree risks between 33% and 43% which are reduced to under 5% with the RFLP results if all XLA mutations were linked to S21/pXG12. However, if one assumes 30% of families are unlinked, the risk of I-2 in family 9 will be 20.35% while that of II-12 in family 1 is still 4.22%. The change of these two women's carrier risks with the proportion of unlinked families is shown in figure 4-3.

It is also feasible, with the linkage data, to indicate where the mutation first started in a family, e.g. in families 5 and 10, the mutation most likely arose during spermatogenesis in the grandfathers. There are both practical and biological interests in determining whether the mutation rate in males and females is equal with respect to any X-linked disease (Winter 1980). However, the immediate practical benefit in pinpointing the mutation in a family is to exclude risks in all relatives except descendants of the carrier of the new mutation.

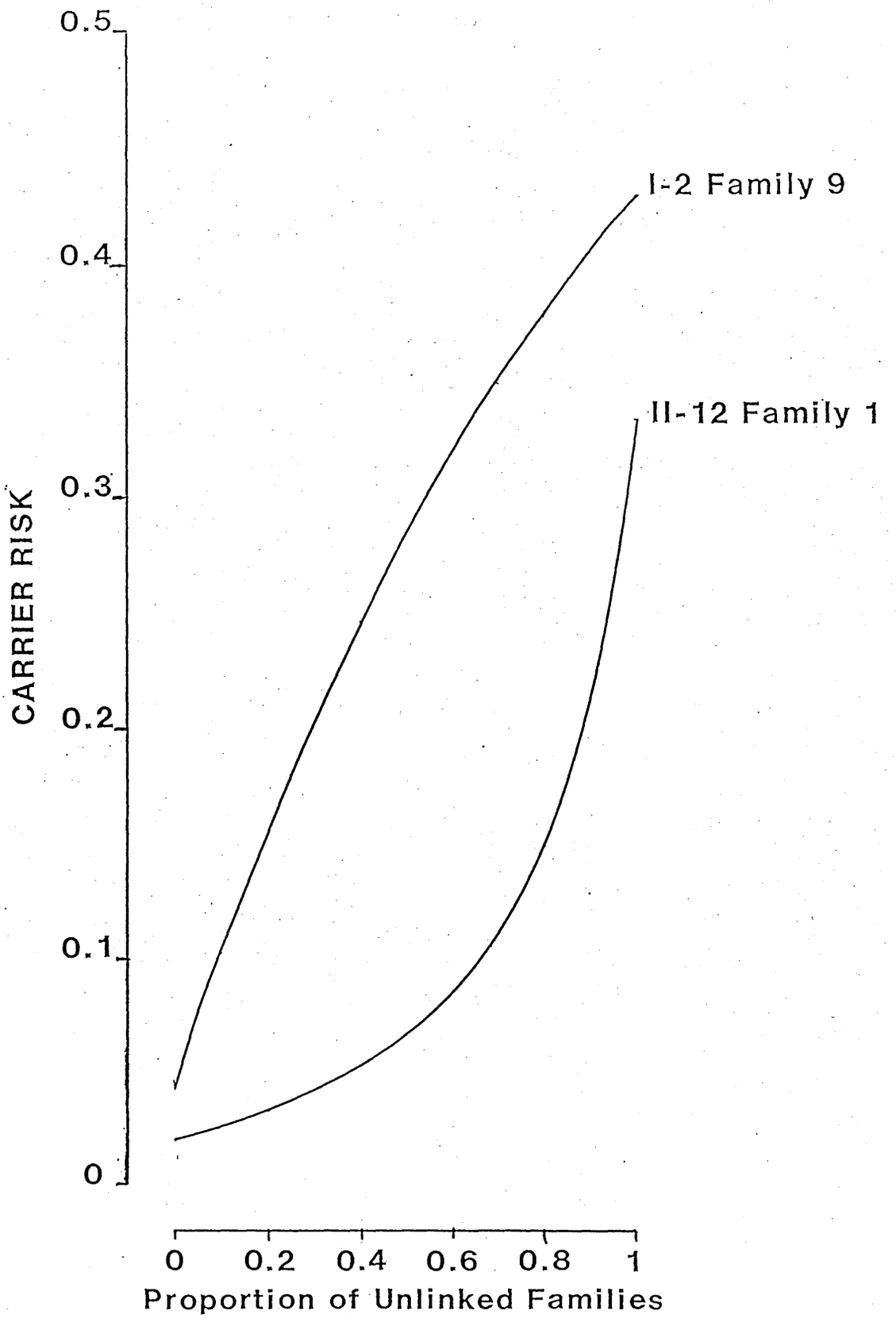


Figure 4-3 Change of carrier risks with the proportion of unlinked families rising from 0% to 100%.

Carrier risks calculated from expression (7) with LIPED and programme listed in appendix 2.

Another potential source of error in using linked probes for genetic prediction is that of non-paternity. In family 1, the prediction that the female II-13 is a carrier which is based on the pXG12 linkage data is paternity dependent; that is if her biological father were another man, this prediction could be incorrect. However, a similar prediction that she (II-13) is a carrier, based on the S21 linkage data, is paternity independent. Using pXG12, she (II-13) can also be offered prenatal diagnosis. Therefore, pXG12 and S21 used together can get round the potential error of non-paternity and provide prenatal diagnosis, hence complementing each other in such circumstances.

A more powerful and universal strategy to confirm family relationship is to use an extremely polymorphic minisatellite probe (Jeffreys et al 1985, Wong et al 1986). A locus-specific minisatellite probe, pλg3, which detects at least 77 different alleles (Wong et al 1986) has been used to confirm paternity in some of our families where predictions are paternity-dependent. The chance of two randomly-selected individuals sharing a same allele >4kb was estimated to be only 0.016 (Wong et al 1986). The family relationship of family 15 was also checked and confirmed by using pλg3. Figure 4-4 shows the Southern blot of a family using pλG3. The parents have totally different alleles as they are not related; father has alleles a and b, mother c and d. Each of their four

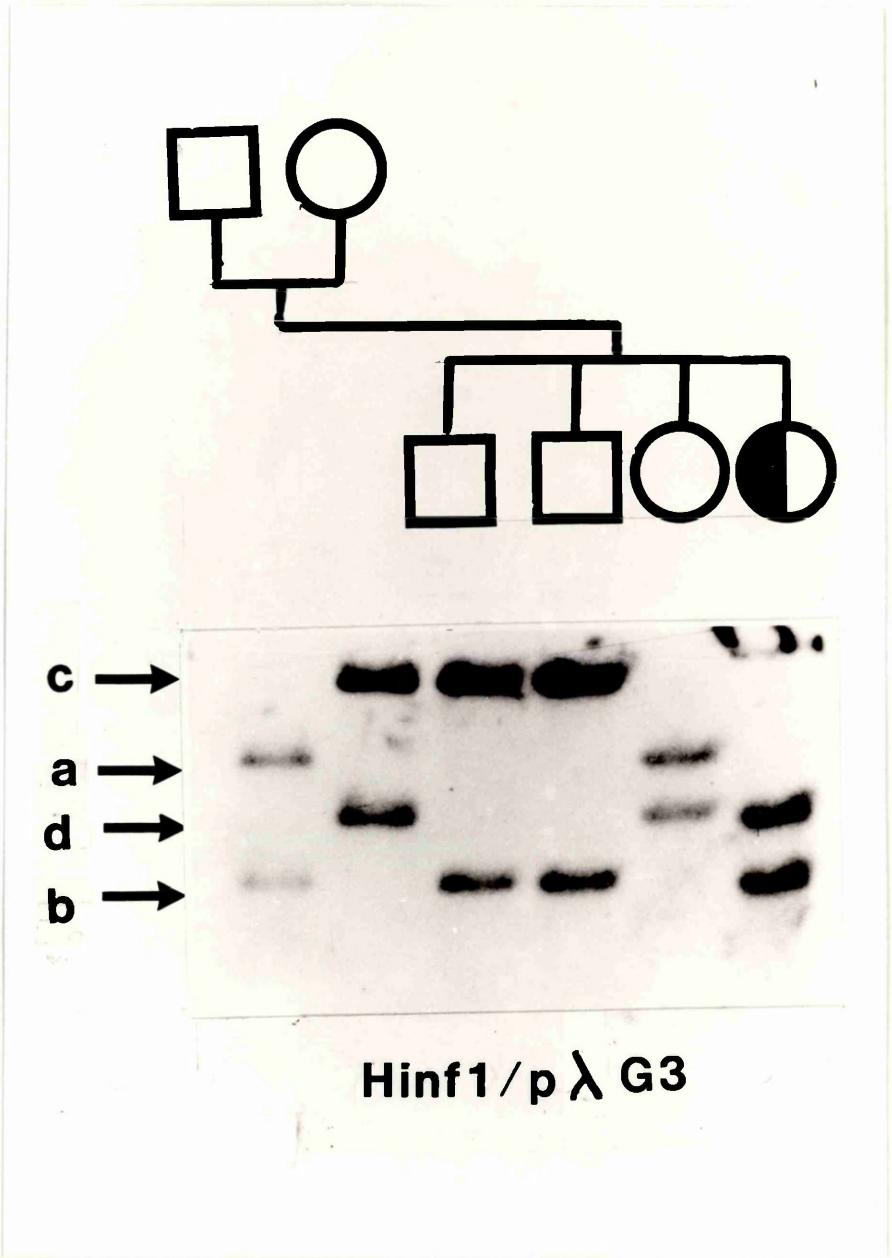


Figure 4-4 Autoradiograph showing the hypervariable DNA polymorphism detected with a locus-specfic minisatellite probe in checking family relationship.

offsprings should have either ac, ad, bc or bd.

A consensus opinion regarding screening for fetal and genetic abnormality has been reached by the King's Fund forum (1987). The purpose of carrier detection should be to assist informed decision making before pregnancy. Accurate information on possible risks will allow some couples to avoid high risk pregnancies, while other couples may decide to embark on pregnancies that they would, without this knowledge, have avoided. The purpose of prenatal diagnosis is to provide the option of not continuing with an abnormal pregnancy, but should the mother wish to continue with an affected pregnancy the prenatal diagnosis would allow the family to prepare to care for an affected child or to attempt appropriate treatment as soon as possible after birth. This is particularly relevant for severe combined immunodeficiency where both matched and mismatched bone marrow transplants are curative and the success rate is about 90% for human leucocyte antigen (HLA) matched and 50-60% for HLA haploidentical transplants (Fischer et al 1986). Therefore, the specific goal of any genetic screening programme should not be of reducing the incidence of a disease, but of maximizing options available to couples at risk for an affected child (Rowley 1984).

4.5 X-LINKED HYPERIMMUNOGLOBULINAEMIA M IS NOT AN ALLELIC GENETIC DISEASE WITH X-LINKED AGAMMAGLOBULINAEMIA

X-linked hyperimmunoglobulinaemia M (X-linked hyperIgM) shares certain similar clinical features with

that of X-linked agammaglobulinaemia (XLA) (Chapter 1, this thesis), it is therefore conceivable that X-linked hyperIgM and XLA could be two allelic forms of the same genetic disease. However this hypothesis seems very unlikely from the linkage analysis of a family with X-linked hyperIgM (family 19). The pedigree is shown in figure 4-5, with the segregation of the alleles of 19.2, pXG12 and S21. At least two cross-overs were observed between the disease locus and the 3 DNA probes linked with XLA. One cross-over with known phase was observed with probes 19.2 and pXG12 as individual III-2 carries the same haplotype as his affected brother; S21 was uninformative in this case. Another cross-over was observed as a normal brother (II-2) has inherited the same haplotype as his two carrier sisters. As I-1 is also informative for S21, the cross-over in this case does not lie between pXG12 and S21.

The results from this family clearly indicates a distinct localisation for the genetic defect in X-linked hyperIgM from that in X-linked agammaglobulinaemia. Mensink et al (1987b) has in fact mapped the gene locus for X-linked hyperIgM to Xq24-q27 with p43-15(DXS42) in one family. Unfortunately our family is not informative for the probe p43-15. Further linkage study should however be done on many other X-linked hyperIgM families before decision can be made regarding clinical application of the linked RFLP in carrier detection and prenatal diagnosis.

Family 19

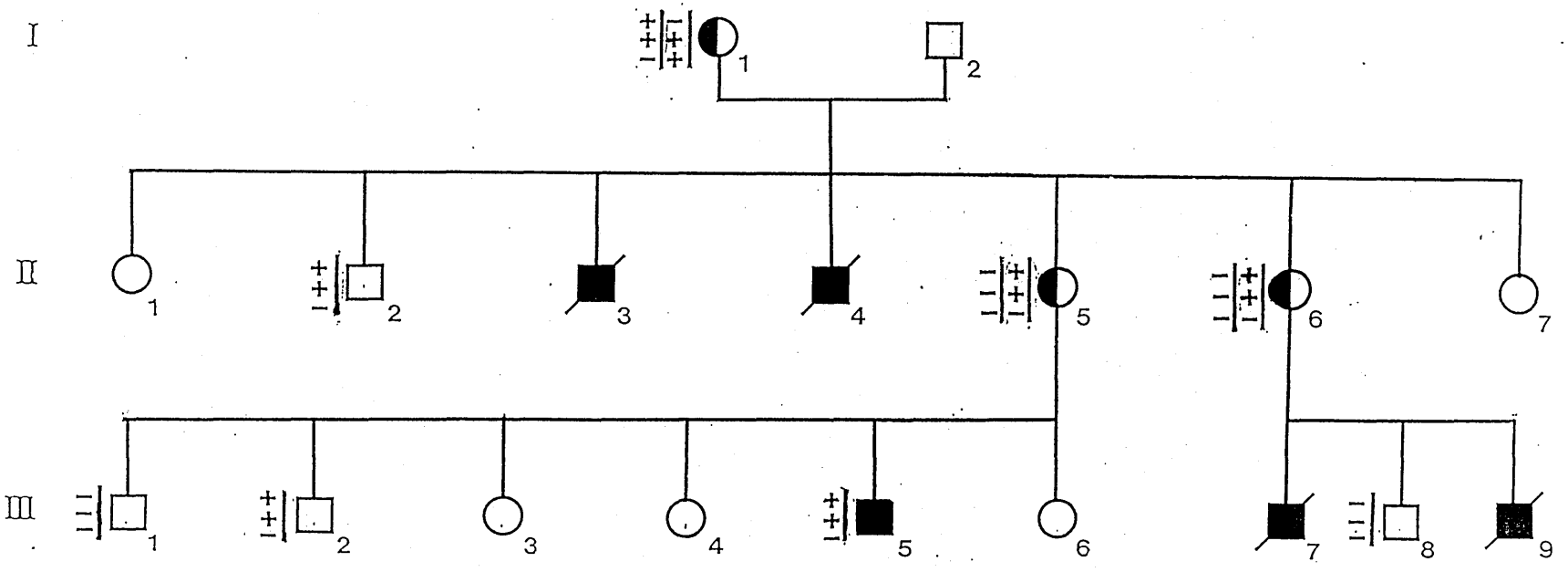


Figure 4-5 Pedigree with RFLP data of a family with X-linked hyperIgM. Results are given for 19.2(top), pXG12(centre) and S21(bottom):
+ indicates presence of restriction site,
− indicates absence of restriction site.

Chapter 5 GENETIC STUDIES OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

5.1 INTRODUCTION

The underlying defect of X-linked severe combined immunodeficency (SCID) is still unclear but the primary cellular defect seems to be a block in early T cell differentiation (Griscelli et al 1978, Gelfand and Dosch 1982, 1983). There is no immunological or biochemical method to detect carriers (Chapter 1, this thesis). Female carriers can however be now identified by the pattern of non-random X-chromosome inactivation in their mature T cells (Goodship et al 1988). Prenatal diagnosis based on enumeration of fetal T cells and their response to phytohaemagglutinin has been successful but it involves mid-trimester fetal blood sampling (Linch et al 1984). Therefore, it would be desirable to localize the gene on the X-chromosome by linkage with RFLPs; it then renders carrier detection and first trimester prenatal diagnosis feasible by gene tracking (Pembrey 1986). The linkage analysis of 3 families with X-linked SCID is presented in this Chapter, which contributes to the mapping of the gene to Xq11-q13 in a collaborative study with the French group headed by Professor C. Griscelli in Paris and Dr. J.L. Mandel in Strasbourg (de Saint Basile et al 1987).

5.2 LINKAGE ANALYSIS BETWEEN X-LINKED SEVERE COMBINED IMMUNODEFICIENCY AND DNA MARKERS FROM Xq11-q13

The three families with X-linked SCID were chosen on the following two criteria. The diagnosis of SCID was

ascertained by the demonstration of abscence of T cells, lack of mitogen-induced T cell proliferation as well as normal or increased numbers of B cells. (Rosen et al 1983) The X-linked inheritance was established unequivocally as all 3 families (families 16-18) showed segregation of the disease in 3 generations (Figure 5-1). This is essential because children with the variety of autosomal recessive SCID may have identical immunological parameters in terms of T and B cell numbers and function as X-linked SCID. It follows that a family with two or more affected sons can either be of autosomal recessive or X-linked inheritance.

The RFLP data of these three families are shown in table 7 and the pedigrees with the cpX73 and 19.2 data are shown in figure 5-1. The two-point linkage analysis between the SCID locus and the loci defined by the four DNA markers, viz cpX73 (DXS159), 19.2(DXS3), pXG12(DXS94) and S21(DXS17) were performed with the LIPED programme (Ott 1974). The input and output files are included in appendix 6. The lod scores of these three families are shown in table 8; along with the combined results obtained from six more X-linked SCID families from the French group (de Saint Basile et al 1987).

The two-point linkage data suggested that the SCID gene locus is close to cpX73 marker, which defines the locus DXS159 in Xq11-q13 (Arveiler et al 1987b). The marker cpX73 showed complete cosegregation with the

Family 16

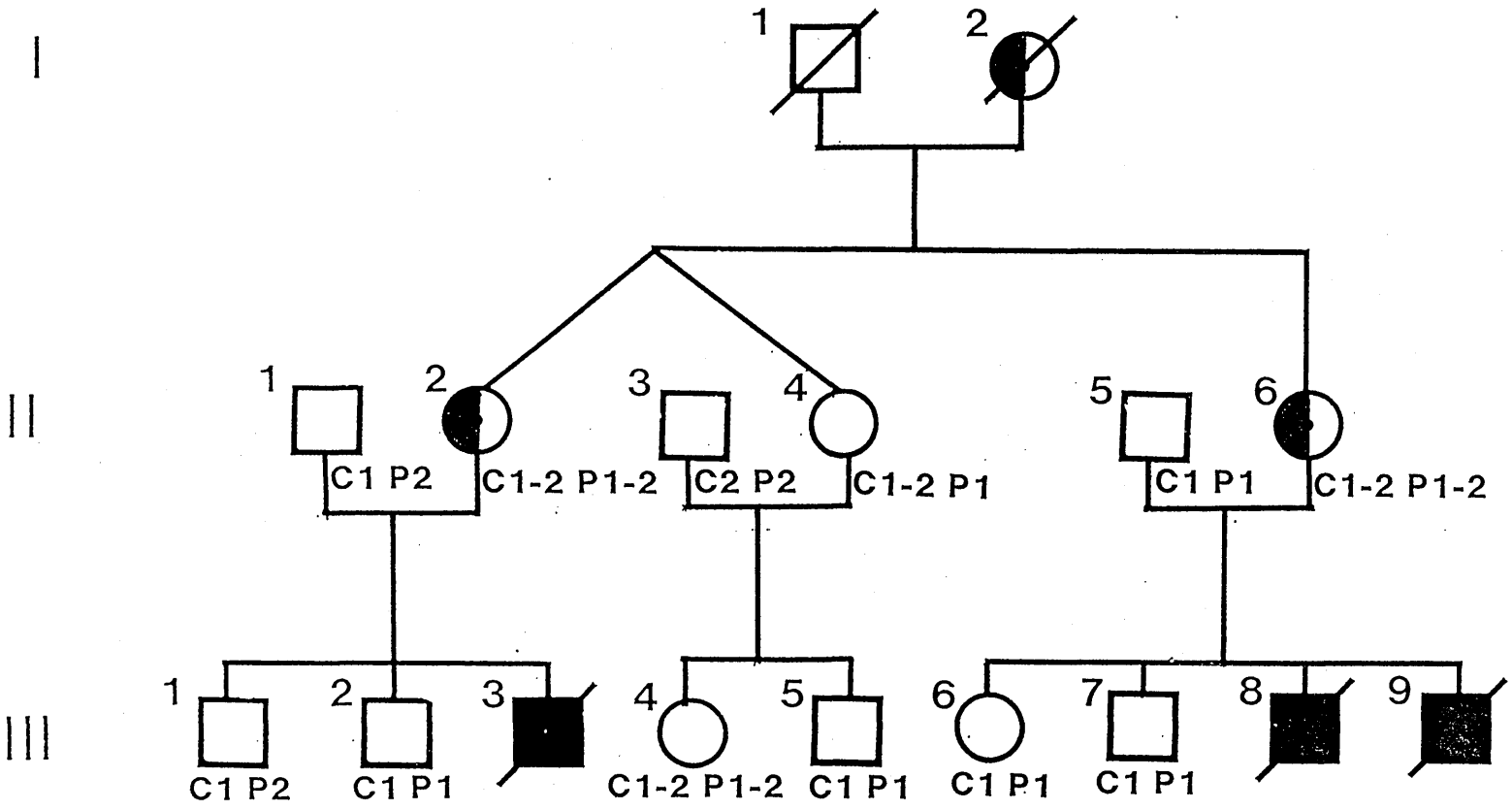


Figure 5-1 Pedigrees with RFLP data of the three families with X-linked severe combined immunodeficiency.
cpX73 Pst 1 polymorphism (C1 is 5.5kb, C2 is 1.6kb).
19.2 Taq 1 polymorphism (P1 is 5.1kb, P2 is 2.95/2.15kb).

Family 17

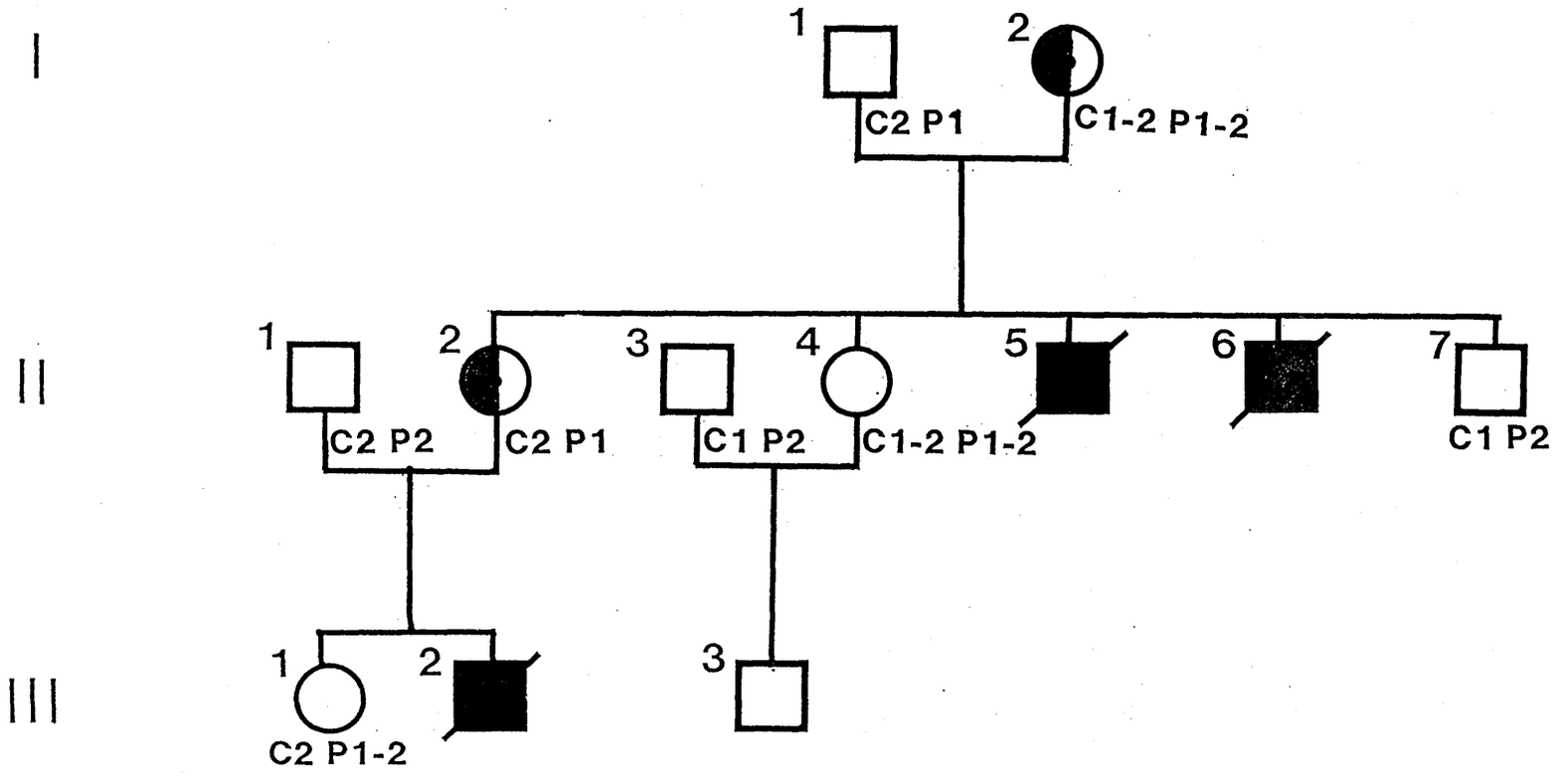


Figure 5-1 (continued)

Family 18

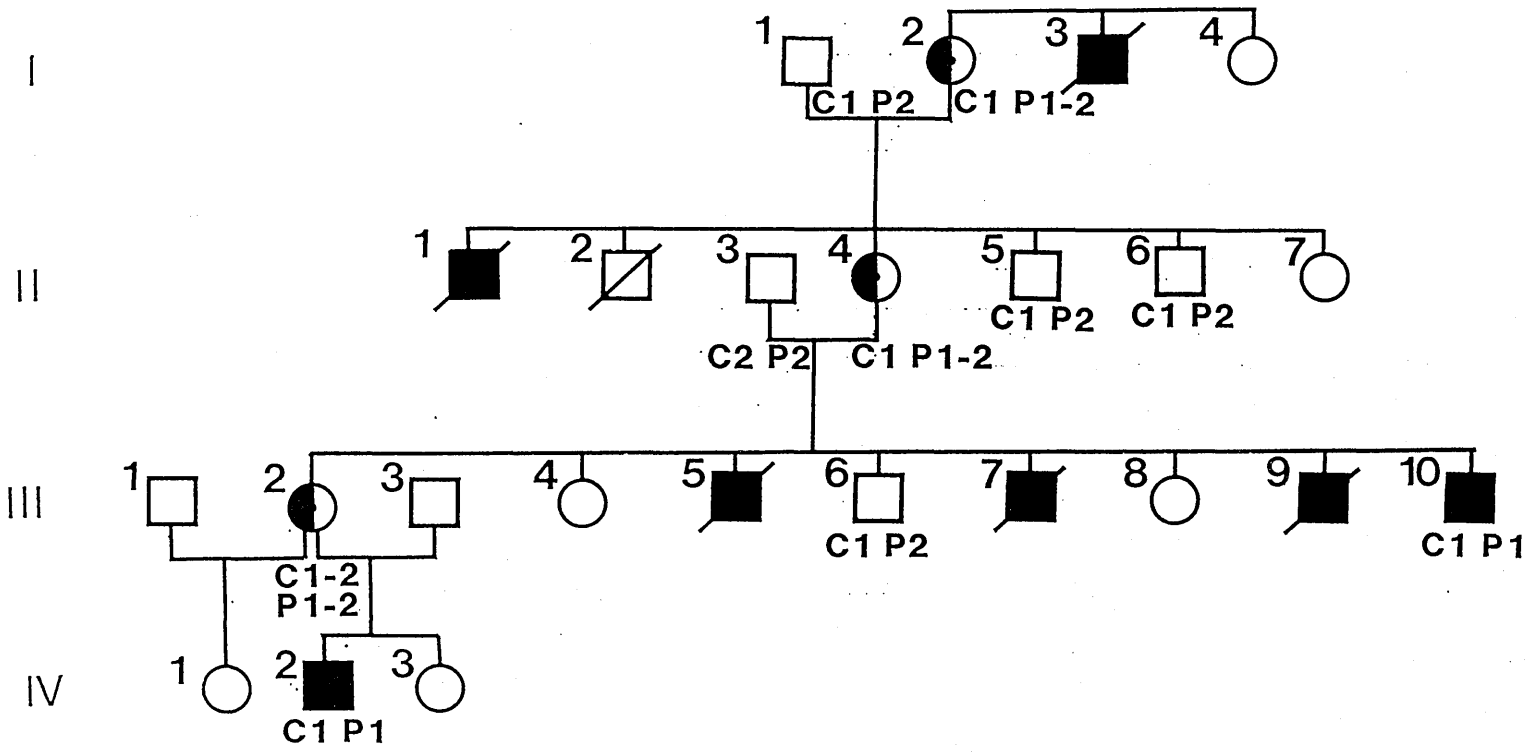


Figure 5-1 (continued)

Table 7

RFLP data of the 3 families with X-linked SCID

Family	Individual	cpX73	19.2	pXG12	S21
16	II-1	1	2	nd	1
	II-2 (O.C.)	1,2	1,2	1	1,2
	II-3	2	2	nd	1
	II-4	1,2	1	1	1,2
	II-5	1	1	nd	2
	II-6(O.C.)	1,2	1,2	1	1,2
	III-1	1	2	nd	1
	III-2	1	1	nd	1
	III-4	1,2	1,2	nd	1
	III-5	1	1	nd	2
	III-6	1	1	nd	1,2
	III-7	1	1	nd	1
17	I-1	2	1	2	nd
	I-2(O.C.)	1,2	1,2	1	1
	II-1	2	2	2	nd
	II-2(O.C.)	2	1	1,2	1
	II-3	1	2	1	nd
	II-4	1,2	1,2	1,2	1
	II-7	1	2	1	1
	III-1	2	1,2	1,2	1

(continued next page)

Table 7 (continued)

Family	Individual	cpX73	19.2	pXG12	S21
18	I-1	1	2	nd	nd
	I-2 (O.C.)	1	1,2	1,2	1
	II-3	2	2	nd	nd
	II-4 (O.C.)	1	1,2	2	1
	II-5	1	2	1	nd
	II-6	1	2	1	nd
	III-2(O.C.)	1,2	1,2	2	1,2
	III-6	1	2	nd	nd
	III-10(aff)	1	1	nd	1
	IV-2	1	1	nd	nd

O.C. = obligate carrier
aff. = affected
nd = not done
allele 1 = larger fragment
allele 2 = smaller fragment(s)

Table 8

LOD SCORES FOR FAMILIES WITH X-LINKED SCID

	LOD SCORES			
LOCUS PROBE	DXS159 cpX73	DXS3 19.2	DXS94 pXG12	DXS17 S21
θ=0.00	1.48(5.27)	-2.14(-INF)	0.62(-6.86)	0.48(-7.00)
θ=0.01	1.45(5.16)	1.50(2.22)	0.61(-1.43)	0.48(-1.87)
θ=0.05	1.30(4.70)	1.95(4.97)	0.55(0.59)	0.47(0.20)
θ=0.10	1.12(4.11)	1.93(5.51)	0.48(1.29)	0.44(0.90)
θ=0.15	0.93(3.50)	1.77(5.37)	0.40(1.51)	0.39(1.12)
θ=0.20	0.75(2.90)	1.55(4.95)	0.32(1.52)	0.33(1.10)
θ=0.30	0.39(1.71)	1.03(3.60)	0.17(1.18)	0.19(0.73)

lod scores for various recombination fractions (θ) between SCID locus and the listed loci in the three SCID families (families 16, 17, 18). The lod scores in brackets are our results combined with the results of the six French families (de Saint Basile et al 1987).

INF=infinity.

disease locus in all informative families analysed. The maximum lod score obtained with cpX73 was 5.27 at recombination fraction $\theta=0$ in the collaborative study (de Saint Basile et al 1987). The 90% confidence limit for the recombination fraction between SCID and cpX73 was estimated to be 0.09. A maximum lod score of 5.51 at $\theta=0.11$ was also obtained for the linkage between SCID and 19.2 (DXS3). The recombination fraction between cpX73 and 19.2 analysed in another study of larger set of families was estimated to be about 0.13 (Arveiler et al 1987a). This indeed suggests the SCID locus is very close to cpX73 but it is not yet decided whether the disease locus lies distal or proximal to cpX73. The relatively high recombination fraction found between SCID and pXG12 or S21 (0.18 and 0.17 respectively, see table 8) clearly shows that X-linked SCID is located in a different region from X-linked agammaglobulinaemia, which has been mapped very close to markers pXG12 and S21 (Chapter 4, this thesis).

In our three families, only one cross-over was observed in family 16; that between X-linked SCID and 19.2. Individual III-1, an unaffected male, has inherited the opposite allele of 19.2 to that of his normal brother (III-2) and cousin (III-7).

The ethnic backgrounds of our three families are quite different from one another as well as from the French families. There was however no evidence for non-allelic genetic heterogeneity since linkage with probes in

the Xq11-q21 region was observed in all nine families (de Saint Basile et al 1987).

5.3 GENETIC PREDICTION

With the locus of X-linked SCID mapped to the region of Xq11-q13 in nine families (de Saint Basile et al 1987) and the lod score of 5.27 with probe cpX73 obtained at recombination fraction $\theta=0$ (90% confidence limit is 0.09), it is then possible to use cpX73 for genetic counselling in families with X-linked SCID. There are the usual potential sources of error in using linked probes in genetic prediction, viz recombinations and non-paternity. However there was no evidence for genetic heterogeneity in these nine families for X-linked SCID which may further confound the approach of gene tracking as in X-linked agammaglobulinaemia (Chapter 4, this thesis). Using cpX73 one can make an earlier prenatal diagnosis in informative families than by using immunological methods on fetal blood (Linch et al 1984). However, because of the possibility of recombination, a control of the presence of T cells and normal mitogen-induced proliferation of T cells in fetal blood should still be performed if RFLP analysis suggests that a fetus has a low risk of being affected. As for carrier detection, we have been able to change the perceived genetic risk of being a carrier substantially in three potential female carriers in family 16 and one in family 17 using probe cpX73. It is however not possible to alter the risk of the other potential female carriers in families 17 and 18 since the key female carriers are

not informative for cpX73. Several of the obligate female carriers can be offered prenatal diagnosis using cpX73.

Using cpX73 and assuming a recombination fraction of 0.09 (the upper limit of 90% confidence intervals), the carrier risks were calculated as follows. For any individual the probability of a specific SCID genotype, given the pedigree and marker (RFLP) data, can be expressed as:

$$\Pr(\text{genotype}|\text{pedigree}) = \frac{\Pr(\text{genotype},\text{pedigree})}{\Pr(\text{pedigree})}$$

where Pr(genotype,pedigree) is the probability of the specific SCID genotype in the individual in question and the pedigree data given that the disease is linked to the marker at θ=0.09. Pr(pedigree) is the probability of the pedigree assuming that the SCID genotype of the individual at risk is unknown and given that the disease is linked to the marker at θ=0.09. These probabilities can be obtained from the computer programme LIPED (Ott 1974). The results are shown in table 9.

5.4 DISCUSSION

Of the three potential female carriers whose risks were reduced (table 9), two were reduced to 5% and one to 9%. These are not insignificant risks for a disease as X-linked SCID. Recently, we have established that DNA

Table 9

PREDICTION OF CARRIER RISKS IN X-LINKED SCID FAMILIES

Subjects	Pedigree risk (%)	Pedigree and DNA risk (%)
Family 16		
II-4	33	60
III-4	17	5
III-6	50	9
Family 17		
II-4	33	5

Carrier risks of females in SCID families as modified by RFLP results from cpX73 assuming a recombination fraction $\theta = 0.09$.

methylation analysis can be used for carrier detection in X-linked SCID (Goodship et al 1988). The principle of the method is as follows. The Lyon hypothesis states that permanent inactivation of one of the two X chromosomes occurs at random in every somatic cell in the female early in embryogenesis (Lyon 1961). The pattern of X chromosome inactivation is transmitted in stable fashion to all progeny cells. In cells requiring the mutant gene for development, cells with the normal X active will have a selective advantage, so although the initial inactivation was random, the mature cell population will show a non-random pattern of X inactivation. Goodship et al (1988) demonstrated non-random X chromosome inactivation pattern in the mature T lymphocytes of two obligate female carriers of X-linked SCID; while in normal females, the T lymphocytes showed random X chromosome inactivation pattern. This method of determining carrier status will be applicable to at least 50% of Caucasian females who are informative for the probes from the 5' end of the hypoxanthine phosphoribosyltransferase (HPRT) and phospho-glycerate kinase (PGK) genes. These probes detect both a RFLP which is used to distinguish the paternal X chromosome from the maternal X chromosome and also a difference in methylation between the active and inactive X chromosome. One endonuclease is used to demonstrate the RFLP and a methylation-sensitive endonuclease to demonstrate the methylation pattern. The methylation of the CpG-rich islands (Hpa II tiny fragments islands) situated at the 5' end of most vertebrate genes is

associated with inactivation of that gene (Bird 1986). This method of carrier detection will obviously be preferred in informative females to that of gene tracking using linked probes because it will give an absolute prediction while the latter approach only gives a probability. In fact, II-4 in family 17 has been found to have the pattern of random X chromosome inactivation in her T cells with this approach (Goodship et al 1988); hence confirming the prediction that she is not a carrier using the linked probe cpX73. Nevertheless the gene tracking approach is technically much easier and is therefore used first in informative families with clear cut X-linked pedigrees. DNA methylation analysis can also distinguish the X-linked form of SCID from recessive form in families with only two or more affected sons. This in turn can help to ascertain more X-linked families for refining the linkage data in this disease.

Chapter 6 FUNCTIONAL STUDIES OF B CELLS FROM PATIENTS WITH X-LINKED AGAMMAGLOBULINAEMIA AND WISKOTT-ALDRICH SYNDROME

6.1 INTRODUCTION

The development of B lymphocytes can be divided into two main phases, an initial antigen-independent phase (stem cell to pre-B cell to mature B lymphocyte) and an antigen dependent phase (B lymphocyte to antibody secreting plasma cell). Inherited primary immunodeficiency diseases involving the B cell may result from developmental blocks at certain points in either of the two phases. Therefore, the understanding of the normal development and differentiation of B cells is indispensable in the analysis of the possible B cell defects in X-linked agammaglobulinaemia and Wiskott-Aldrich syndrome.

6.1.1. DEVELOPMENT OF PRE-B CELL TO B LYMPHOCYTE

The earliest B cell precusors are identified in the fetal liver during the eigth or ninth week of gestation when the pluripotent haemopoeitic stem cells first migrate from the yolk sac to the fetal liver; the bone marrow is then similarly populated (Cooper 1981). Pre-B cells and their B cell progeny are subsequently generated in the bone marrow throughout life. Two biological processes are essential in the generation of a large population of B cells which are clonally diverse with respect to their antibody receptors. The first is the rearrangement of the immunoglobulin variable-region genes and the second is polyclonal proliferation (Cooper 1987). Antigens play no

part in this process since pre-B cells lack cell-surface antibodies.

The immunoglobulin heavy-chain genes are on chromosome 14, kappa-light-chain genes on chromosome 2 and lambda-light-chain genes on chromosome 22. Each gene family consists of multiple exons separated by noncoding introns. A complex but orderly rearrangement of the exons of the variable (V), diversity (D) and joining (J) genes of the heavy-chain has to be executed correctly before transcription can begin (Tonegawa 1983). When the VDJ-constant mu transcript is further processed by RNA splicing, the processed message is translated into mu-heavy-chain products (Tonegawa 1983). The cytoplasmic expression of mu chains is the first easily identifiable marker of the pre-B cell stage. The next step is the rearrangement of variable (V) and joining (J) genes of the kappa or lambda light-chain gene families in the small postmitotic cytoplasmic-mu positive pre-B cells. If the VJ rearrangement is correct, the RNA transcript can be processed to form messenger RNA for light-chain production. The light chains are assembled with the mu chains to form the complete IgM molecules which are then transported through the Golgi region to the cell surface. The expression of the surface IgM marks the end of the pre-B cell stage and the beginning of the B lymphocyte. The surface IgM positive B cells then produce a long nuclear transcript, VDJ-C*u*-C*d*, which is differentially spliced into VDJ-C*u* and VDJ-C*d* messenger RNAs; these are

subsequently translated to generate mu and delta chains (Cooper 1987). Mature B cells can thus express both IgM and IgD antibody receptors with the same specificity.

The regulatory mechanism of the complex differentiation of the pre-B cells to B lymphocytes remains largely unknown. Stromal cells in the bone marrow appear to promote pre-B cell differentiation, possibly via direct cell contact and by secretion of soluble factors (Dorshkind 1987, Collins and Dorshkind 1987). Conditioned medium from a stromal cell line, S17, has been shown to act as growth factor for murine pre-B cells (Landreth and Dorshkind 1988). Landreth et al (1985b) identified in the urine of a patient with cyclic neutropenia a lymphopoeitic activity that acts as a growth factor for human pre-B cells. Recently, a murine lymphopoeitic growth factor designated lymphopoeitin 1 has been purified to a single 25 kilodaltons species from the culture supernatant of an adherent stromal cell line (Namen et al 1988). It is capable of stimulating the proliferation and extended maintenance of precursor cells of the B cell lineage and has been shown to be distinct from IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, G-CSF, CSF-1 and IFN (Namen et al 1988).

6.1.2 ACTIVATION, GROWTH AND DIFFERENTIATION OF B LYMPHOCYTES

The generation of millions of B-cell clones to the surface IgM/IgD positive stage as described above and their migration from bone marrow to the spleen and other peripheral lymphoid tissues are all antigen-independent

events. B cells that encounter antigens complementary to their surface immunoglobulin receptors and those that receive T-cell help will undergo phases of activation, proliferation and differentiation into antibody-secreting plasma cells or long-lived memory cells. The mechanism of isotype switching involves cutting of repetitive DNA sequences in a switch (s) region upstream of the Cu gene and splicing with a complementary s region in front of the next constant-heavy gene to be expressed (Cooper 1987). The molecular basis of T-cell help has been clarified in the last few years (Gordon and Guy 1987) and it is now apparent that the various B-cell factors are not B-cell specific but can act on many other cell types; when they act on the B cells, the factors are not stage-specific, and B-cell factors can be derived from non-T cells as well (O'Garra et al 1988). Hence the control of the B-cell programme must reside in levels other than those operating through the direction of single factors.

These soluble factors were originally divided into two functional groups: B cell growth factors (BCGF), thought to be involved in B cell activation and replication, and B cell differentiation factors (BCDF), responsible for maturation of activated B cells into plasma cells (Kishimoto et al 1984). This strict classification based on function has to be reviewed as recombinant B cell factors (interleukins: IL-4, IL-5, IL-6) are now available and shown to be pleiotropic (O'Garra et al 1988). Other lymphokines originally shown to be

effective on target cells other than B cells have also been shown to affect B-cell physiology (IL-1, IL-2 and gamma-interferon). The functions of these factors on B cells are reviewed as follows:

6.1.3 INTERLEUKIN 4

Human interleukin 4 (IL-4) or B-cell stimulatory factor 1 (BSF-1) has been isolated and characterised by molecular cloning of a human cDNA based on homology with a mouse IL-4 cDNA (Yokota et al 1986). The DNA sequences of human and murine IL-4 are 70% homologous and the two factors mediate several similar activities, such as inducing IgE secretion by activated B cells, low-affinity receptors for the Fc portion of IgE (CD23) on resting B cells (DeFrance et al 1987a), the proliferation of anti-IgM stimulated B cells and the growth of helper T-cell clones (Yokota et al 1986, DeFrance et al 1987b). Murine IL-4 has also been shown to be IgG1 inducing factor and to induce the expression of class II major histocompatibility complex molecules on resting B cells (Snapper and Paul 1987, O'Garra et al 1988).

6.1.4 INTERLEUKIN 5

Human interleukin 5 (IL-5) or eosinophil differentiating factor (EDF) or T-cell replacing factor (TRF) has also been identified by molecular cloning of a cDNA from T cells, based upon its homology with murine IL-5 cDNA (Yokota et al 1987). Human IL-5 has been shown to enhance IgA production by unstimulated and Staphylococcus aureus

Cowan 1 (SAC)-activated enriched peripheral blood B cell preparations (Yokota et al 1987). In many standard human B cell growth factor assays, human IL-5 has however been shown to have no activity on human B cells at all (Clutterbuck et al 1987). The difference is probably due to the source of the B cell target population and the levels of contaminating accessory cells. The principal activity of human IL-5 is on the differentiation of eosinophils.

6.1.5 INTERLEUKIN 6

Human interleukin 6 (IL-6) or B-cell stimulatory factor 2 (BSF2) was purified and identified by its ability to induce antibody secretion by preactivated normal and Epstein-Barr virus-transformed human B cells (Hirano et al 1985). The cDNA of IL-6 was subsequently cloned by Hirano et al (1986) by probing the cDNA libraries of a human T-cell line, which constitutively produced large amounts of BSF-2/IL-6, with synthetic oligonucleoties corresponding to fragments of amino acid sequences of the purified IL-6. Human IL-6 was initially thought to be a pure differentiation factor with no growth effect on B cells (Hirano et al 1985). IL-6 is however now known to be the same as plasmacytoma/hybridoma growth factor and has been shown to be produced by human multiple myelomas as an autocrine growth factor (Kawano et al 1988). IL-6 is also identical to interferon-beta 2 (Billiau 1987). IL-6 has in addition been shown to induce acute phase proteins by hepatocytes (Gauldie et al 1987). IL-6 is therefore not

just a B cell differentiation factor as previously thought and its action on pre-B cell development remains to be elucidated (O'Garra et al 1988).

6.1.6 LOW MOLECULAR WEIGHT B CELL GROWTH FACTOR

Low molecular weight B cell growth factor (BCGF-low) has been purified from lectin-stimulated, peripheral blood mononuclear cell-conditioned medium (Mehta et al 1985) and was shown to be a heat and protease sensitive protein with a molecular weight of 12 to 14 kilodaltons. BCGF-low co-stimulates with anti-Ig to induce DNA synthesis in normal human B cells; long-term BCGF-low dependent B cell culture has also been established as an assay for BCGF-low (Maizel et al 1983). A commercially available preparation of BCGF-low (Cellular Products, Inc) has been shown to increase DNA synthesis and immunoglobulin secretion in several human B cell lines (Shields et al 1987). Sharma et al (1987) have isolated and expressed a cDNA in E. coli which appears to encode BCGF-low, but this recombinant molecule has not yet been shown to induce the effects attributed to the commercial BCGF-low. The commercial BCGF-low also contains traces of other contaminating lymphokines, such as IL-1, IL-2, interferon-gamma and tumour necrosis factor (O'Garra et al 1988), which may complicate interpretation of experiments using this commercial preparation.

6.1.7 B-CELL FACTORS FROM NON-LYMPHOID AND LYMPHOID CELL LINES

T24, a bladder carcinoma cell line, has been shown to

produce a molecule with IL-6/BSF-2 activity (Rawle et al 1986). Cardiac myxoma cells also produce high levels of a molecule with IL-6 activity which was specifically absorbed by the anti-IL-6 antibody (Hirano et al 1987). Northern blot analysis has established that the IL-6 mRNA was transcribed in both T24 cells and cardiac myxoma cells at a much higher level than activated lymphocytes (Hirano et al 1986). It is therefore feasible to use such cell lines as sources of IL-6.

KG-1a, a promyelocytic cell line, has been shown to secrete a BCGF-like factor which is not the same as IL-4 (Callard et al 1987, Shields et al unpublished), but this factor has not been characterised.

An Epstein-Barr virus (EBV)-transformed B cell line, has been shown to secrete a BCGF which appears to be distinct from IL-1, IL-2, IL-4, IL-5 and interferon-gamma (Muraguchi et al 1986). This factor induces proliferation of activated B cells that has been stimulated with Staphylococcus aureus Cowan Strain I for 3 days. Normal activated B cells also secrete this BCGF, but at a lower level. It has been postulated the constitutive production of an autostimulatory BCGF serves as part of the crucial events in the immortalisation of these cells (Gordon et al 1984). A low molecular weight BCGF (16Kilodaltons) derived from an EBV-transformed B cell line has also been purified (Buck et al 1987). A high molecular weight BCGF (50 kilodaltons) has also been generated from an IL-2-dependent helper T cell clone, this was found to be

synergistic with the BCGF-low, generated from phytohaemagglutinin-stimulated T cells, on the proliferation of anti-IgM stimulated B cells (Yoshizaki et al 1983). Another high molecular BCDF-like factor (40-60 Kilodaltons) from various human T and B cell lines has been described (Kanowith-Klein et al 1987). Its relationship to IL-6 (21 kilodaltons) has not been elucidated however.

6.1.8 INTERLEUKIN 1, INTERLEUKIN 2, INTERFERON-GAMMA

Monocyte-derived interleukin 1 (IL-1) has been shown to support the proliferation of EBV-transformed B cell lines but not resting B cells costimulated with anti-IgM (Gordon et al 1986a); Falkoff et al (1983) however demonstrated synergy between purified IL-1 and a low concentration of anti-IgM in the proliferation of B cells. Purified IL-1 alone did not induce immunoglobulin secretion by activated B cells (Falkoff et al 1984). Transformed B cells also secrete an IL-1 like factor which promotes B cell proliferation (Rimsky et al 1986) and EBV-transformed B cell lines have been shown to possess receptors for IL-1 alpha/beta (Matsushima et al 1986). The importance of interleukin 2 (IL-2) and interferon-gramma in B-cell proliferation and differentiation is still being elucidated. It is however clear that IL-2 can promote proliferation and differentiation of staphylococcus aureus Cowan Strain I activated B cells (Jelinek et al 1986). Interferon-gamma delivers an enhancing signal during initial activation that permits

subsequent growth and differentiation of activated B cells in response to IL-2 (Jelinek et al 1986). Interferon-gamma also specifically inhibits the IL-4 mediated induction of the low-affinity receptor for IgE (CD23) on B lymphocytes (DeFrance et al 1987a). In contrast, interferon-gamma was found to enhance the IL-4-induced proliferation of anti-IgM-preactivated B cells (DeFrance et al 1987b).

The activity of the various interleukins on human B cells is summarised in the table 10.

6.1.9 THE ROLE OF B-CELL SURFACE ANTIGENS IN B-CELL DIFFERENTIATION

In the third international workshop on human leucocyte differentiation antigens in Oxford, twelve cluster differentiation (CD) surface markers of B cells have been delineated (Ling et al 1987). Various functional roles have been ascribed to several of these cell surface antigens (Golay 1986, Zola 1987). Anti-CD19 antibody blocks the DNA synthesis of B cells induced by anti-IgM (Pezzutto et al 1987a) and partially inhibits the growth of long-term EBV-transformed cell lines (Golay and Crawford 1987) while CD20 antigen is involved in the activation of B cells (Golay et al 1985). Anti-CD22 antibody was costimulatory with anti-IgM in inducing small, dense tonsillar B cells to proliferate (Pezzutto et al 1987b). Monoclonal antibody to CD21 antigen, which is the

Table 10

EFFECTS OF LYMPHOKINES ON HUMAN B CELLS

FACTORS	EFFECTS ON B CELLS
Interleukin 1	induces proliferation of EBV-transformed B cell lines (Gordon et al 1986a).
Interleukin 2	induces proliferation and differentiation of staphylococcus aureus Cowan I (SAC) activated B cells (Jelinek et al 1986).
Interleukin 4	induces low-affinity receptors for the Fc portion of IgE (CD23) on resting B cells; induces proliferation and IgE secretion of activated B cells (DeFrance et al 1987 a&b).
Interleukin 5	induces IgA production by SAC activated B cells (Yokota et al 1987).
Interleukin 6	induces Ig secretion by EBV-transformed B cell lines (Hirano et al 1985); induces growth of multiple myelomas (Kawano et al 1988) and EBV-transformed B cell lines (Tosato et al 1988).
Interferon-gamma	enhances the growth and differenatiation of activated B cells in response to IL-2 (Jelinek et al 1986); inhibits the IL-4 induction of CD23 on B cells but enhances the IL-4-induced proliferation of activated B cells (DeFrance et al 1987 a&b).

receptor for both Epstein-Barr virus and complement C3d (CR2) (Frade et al 1985), is able to trigger human B cells to divide and differentiate in the presence of T cells (Nemerow et al 1985). Anti-CDw40 antibody provides a very potent co-stimulation in B cell activation as long as the signal transmitted through this CDw40 is delivered concomitantly or shortly after the initial stimulus (Clark and Ledbetter 1986). IL-4 and anti-CDW40 have been shown to synergise in promoting DNA synthesis of B cells in a restimulation assay (Gordon et al 1987).

CD23, the low-affinity receptor for IgE (45 kDa), is expressed on activated but not on resting B cells. IL-4 has been shown to induce the expression of CD23 on resting B cells (DeFrance et al 1987a). Epstein-Barr virus also induces CD23 rapidly on a large number of B cells (Wang et al 1987), the expression of which is essential for transformation to proceed (Thorley-Lawson and Mann 1985). It has been suggested CD23 acts as a central focus for growth-promoting signals delivered to the activated B cells (Gordon and Guy 1987). Anti-CD23 monoclonal antibody can promote DNA synthesis in phorbol ester activated B cells (Gordon et al 1986b) and it has been suggested that there is an association between CD23 and the receptor for BCGF-low (Gordon et al 1986c). Moreover, the binding of the anti-CD23 antibody to the 45 kDa CD23 causes the release of a 25 kDa form of the CD23 into the extracellular medium (Guy and Gordon 1987); this effect

is mimicked by the binding of BCGF-low to the activated B-cell surface. Shed CD-23 is associated with autocrine growth factor activity (Swendeman and Thorley-Lawson 1987). Furthermore, IgE has been shown to augment both BCGF-low and anti-CD23 in promoting B-cell growth, by enhancing CD23 expression (Guy and Gordon 1987). This then leads to the generation of more cleaved CD23 by BCGF-low and anti-CD23 antibody and thus provide greater autocrine activity.

The various functions mediated through the B cell surface markers are summarised in table 11.

6.1.10 FUNCTIONAL STUDIES ON EPSTEIN-BARR VIRUS TRANSFORMED B CELL LINES FROM PATIENTS WITH XLA AND WAS: RATIONALE AND HYPOTHESIS

As the normal development of the B lymphocytes is subject to such a complex network of regulatory mechanisms involving many exogenous and endogenous growth and differentiation factors which mediate their functions through binding onto their specific B-cell surface receptors, it is conceivable either a lack of factors or failure to respond to factors may lead to a developmental arrest of the B lymphocytes. In both X-linked agammaglobulinaemia (XLA) and Wiskott-Aldrich syndrome (WAS), B lymphocytes are thought to be intrinsically defective from both immunological and genetic evidence (Chapter 1). In XLA, there is a relative developmental

Table 11

EFFECTS OF MONOCLONAL ANTIBODIES
AGAINST B CELL SURFACE MARKERS ON HUMAN B CELLS

ANTIBODY TO SURFACE MARKERS ON B CELLS	EFFECTS ON B CELLS
Anti-CD19	blocks DNA synthesis of B cell induced by anti-IgM (Pezzutto et al 1987a); partially inhibits growth of EBV-transformed B cell lines (Golay and Crawford 1987).
Anti-CD20	activates B cells (Golay et al 1985).
Anti-CD21(Receptor for EBV/C3d)	induces proliferation and differentiation of B cells (Nemerow et al 1985).
Anti-CD22	costimulates with anti-IgM in inducing B cells to proliferate (Pezzutto et al 1987b).
Anti-CD23	promotes DNA synthesis in phorbol ester activated B cells (Gordon et al 1986b).
Anti-CDw40	augments the proliferation of activated B cells (Clark and Ledbetter 1986).

arrest from pre-B cells to B lymphocytes. In WAS, the B lymphocytes bear striking resemblance to the immature B cell system of the newborn, viz inability to respond to polysaccharide antigens (Blaese et al 1968, Cooper et al 1986). We therefore hypothesize that the B lymphocytes from these patients may respond abnormally to the B-cell factors, leading to the disease phenotype. A subsidary hypothesis is that these patients' B lymphocytes do not secrete autocrine growth and differentiation factors, which may be important in allowing clonal expansion.

To test the above hypothesis, we have chosen Epstein-Barr virus (EBV)-transformed B cell lines from these patients as an experimental model. Receptors for EBV occur on mature and immature B cells; pre-B cells and even cells of B lineage prior to immunoglobulin gene rearrangement can also be transformed by EBV (Fu et al 1980, Katamine et al 1984). The frequency of B-lymphocyte transformation by EBV depends on stages of cell cycle of the B cells and experimental conditions of the transformation (Chan et al 1986) and it varies from 1% to 30%. The validity of the EBV-transformed B cell lines as a model partly depends on the evidence that in a female carrier with either XLA or WAS, B cells manifest non-random X-inactivation pattern, hence B cells bearing the defective gene which is not inactivated do not mature in the female carrier (Fearon et al 1987, M. Blaese, personal communication). It is therefore most likely that all B cells in the patients with XLA or WAS will be affected by

their respective basic defects, hence transformation of a fraction of these patients' B lymphocytes by EBV should still give a valid model to study their underlying defects.

EBV-transformed B cell lines secrete and respond to various B cell growth and differentiation factors. These include B cell derived BCGF (Gordon et al 1984, Muraguchi et al 1986), monocyte derived IL-1 (Gordon et al 1986a), B cell derived IL-1 (Rimsky et al 1986), T cell derived IL-6 (Hirano et al 1985) and the soluble CD23 (Swendeman and Thorley-Lawson 1987). Such cell lines may not be representative of the normal B cell population, but their range of responses to B cell growth and differentiation factors, and availability in large numbers free of other cell types makes them suitable for studies of some aspects of B cell physiology. It is difficult to obtain enough purified peripheral blood B cells from children for measuring responses to B cell growth and differentiation factors, especially from patients with XLA. In such cases, the use of EBV-transformed B cell lines for experiments is the only viable option. The EBV-transformed B cell lines used in the following study were grown for a short period only (8-12 weeks) in bulk culture to avoid overgrowth by dominant clones.

6.2 PRELIMINARY STUDY

A series of preliminary experiments were done on several normal EBV-transformed B cell lines to determine

the optimum conditions for eliciting responses in increasing DNA synthesis and immunoglobulin secretion of these cell lines to T24-BCDF, KG1-a supernatant and BCGF-low. In later experiments, recombinant human IL-4 (rhIL-4) and IL-6 (rhIL-6) were used.

The basic methodology of these experiments are detailed in Chapter 3.

6.2.1 EFFECT OF VARYING CELL NUMBERS PER WELL

A range of cell concentrations (2.5 to 20 x 10^3 cells/well) were tested to ascertain at what range of cell concentrations these EBV-transformed B cell lines would give the optimum response to various factors added. Figures 6-1 and 6-2 show the responses of a normal EBV-transformed B cell line to BCGF-low in increasing DNA synthesis and to T24-BCDF in increasing IgG secretion at different cell concentrations. At $2.5x10^3$ cells/well, there was no response in growth or differentiation probably because EBV-transformed B cell lines need a minimum cell density to survive even in the presence of growth factors. At 5 and $10x10^3$ cells/well, there were some increases in DNA synthesis and IgG secretion in response to factors. At $20x10^3$ cells/well, the magnitude of responses increased but the control values also increased dramatically because at a high cell density, the EBV-transformed B cell lines are able to sustain its own growth via the autocrine stimulatory mechanism (Gordon et al 1984). Hence, it is critical to use a suitable range of cell concentrations in order to test whether the EBV-

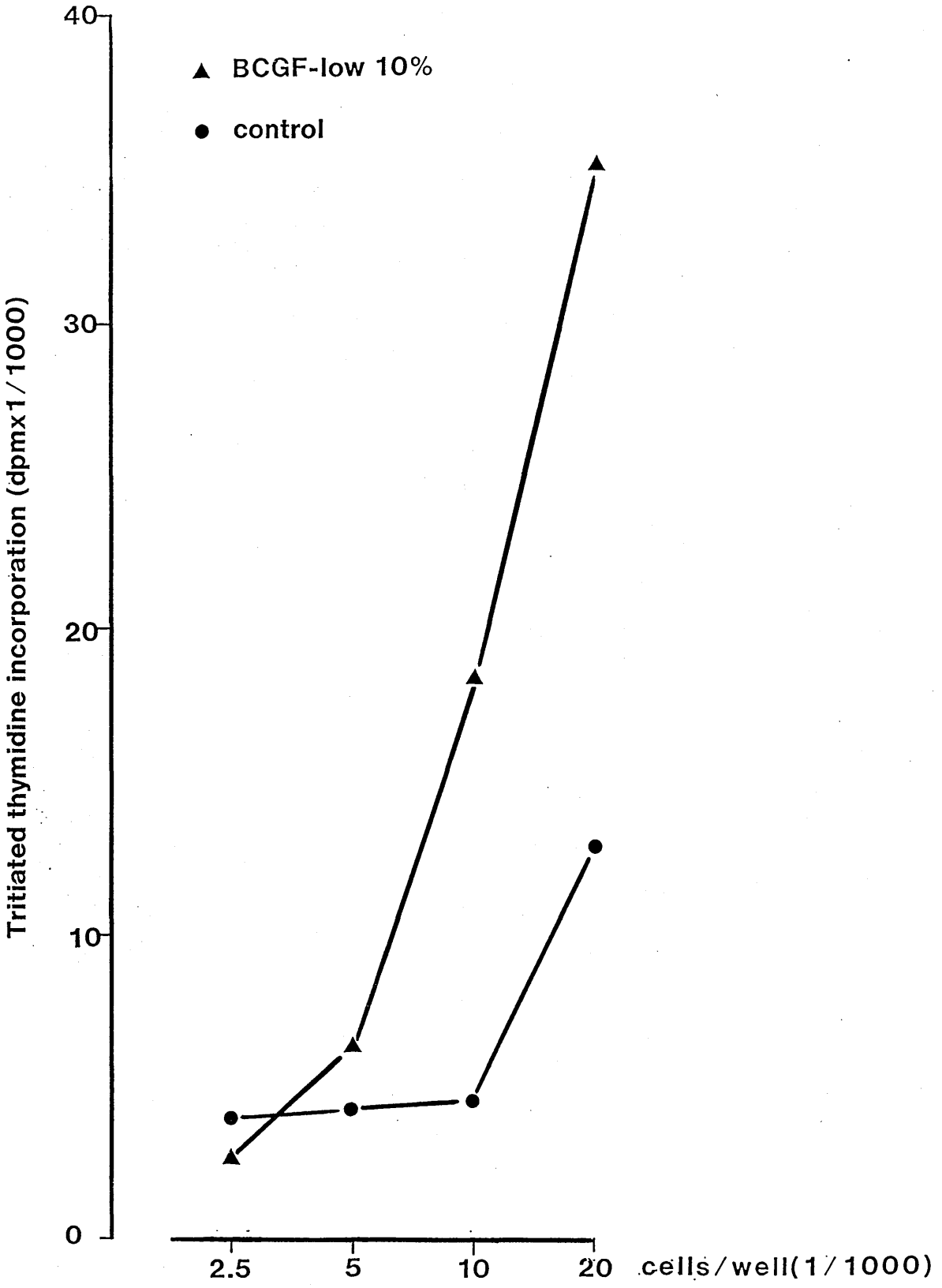


Figure 6-1 Effect of varying cell numbers per well on the proliferative response of normal EBV transformed B cell line with and without BCGF-low

Results as mean of triplicate cultures.
Standard deviations within 20% of mean.

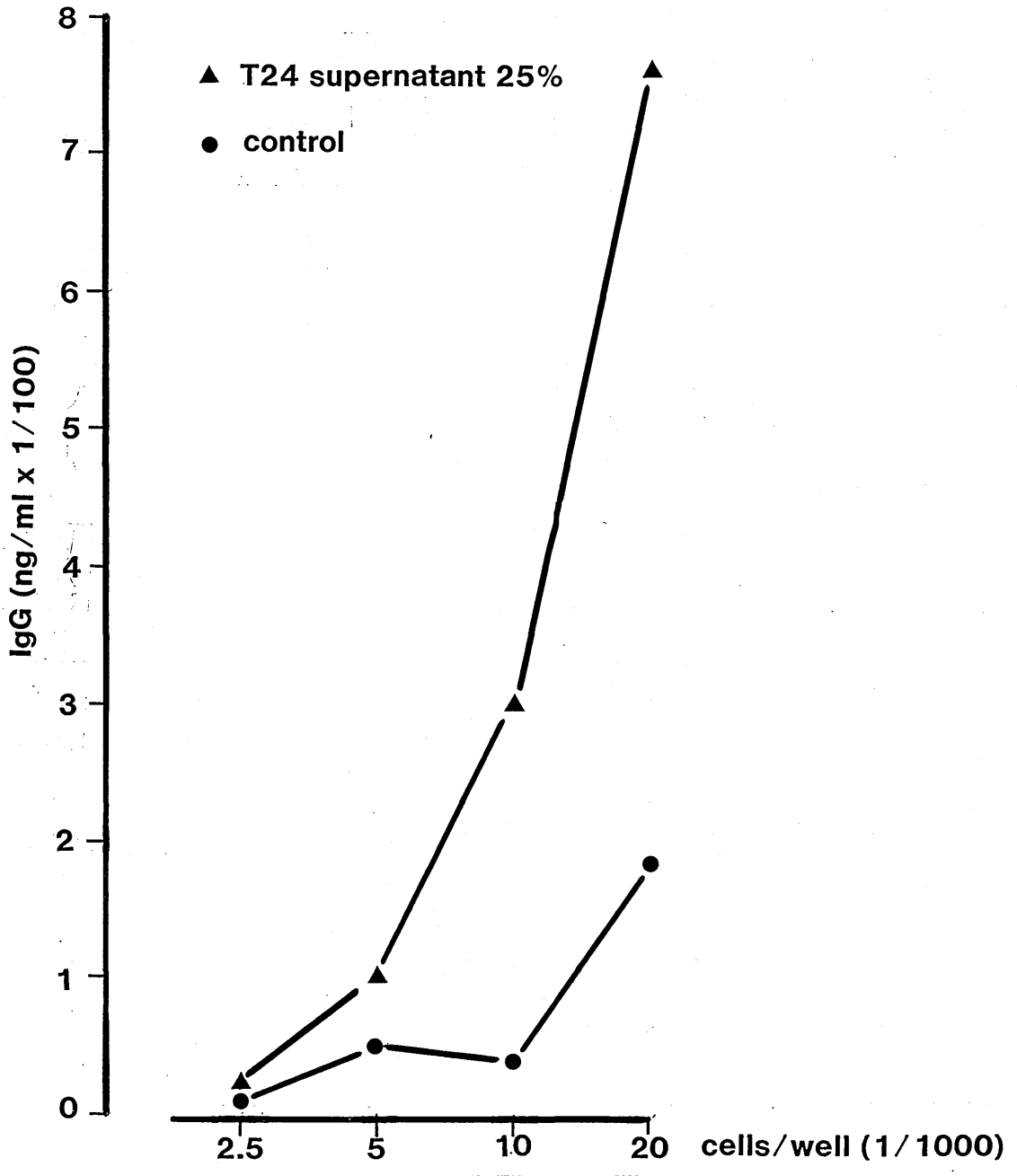


Figure 6-2 Effect of varying cell numbers per well on the IgG secretion of normal EBV transformed B cell line with and without T24-BCDF.

Results as mean of triplicate cultures.
Standard deviations within 20% of mean.

transformed B cell lines respond to factors or not. In subsequent experiments, a range of 5 to $20x10^3$ cells/well were normally used.

6.2.2 EFFECT OF VARYING NUMBER OF DAYS OF CULTURE

Several time-course experiments were done to ascertain how many days of culture would give the best response in DNA synthesis and immunoglobulin secretion of normal EBV-transformed B cell lines. DNA synthesis as measured by incorporation of tritiated thymidine is only indicative of the DNA synthesized in the last 8 hours of the cultured period and not of the total cell growth and survival at the end of the culture. Measurement of immunoglobulins in the supernatant at the end of the cultured period represents the cumulative secretion over the whole period. Figure 6-3 shows the kinetics of response of a normal EBV-transformed B cell line to BCGF-low and KG1-a supernatant in DNA synthesis from day 2 to day 6. The maximum response was seen on day 3. BCGF-low was able to sustain DNA synthesis at a much higher level and over a longer period than KG1-a supernatant. In subsequent experiments, 3 days of culture was used in assaying DNA synthesis. Figure 6-4 shows that after 5 to 7 days of culture, the accumulation of immunoglobulin secretion of a normal EBV-transformed B cell line in response to T24-BCDF had probably reached a maximum. Hence, 5 or 6 days of culture was used in assaying immunoglobulin secretion.

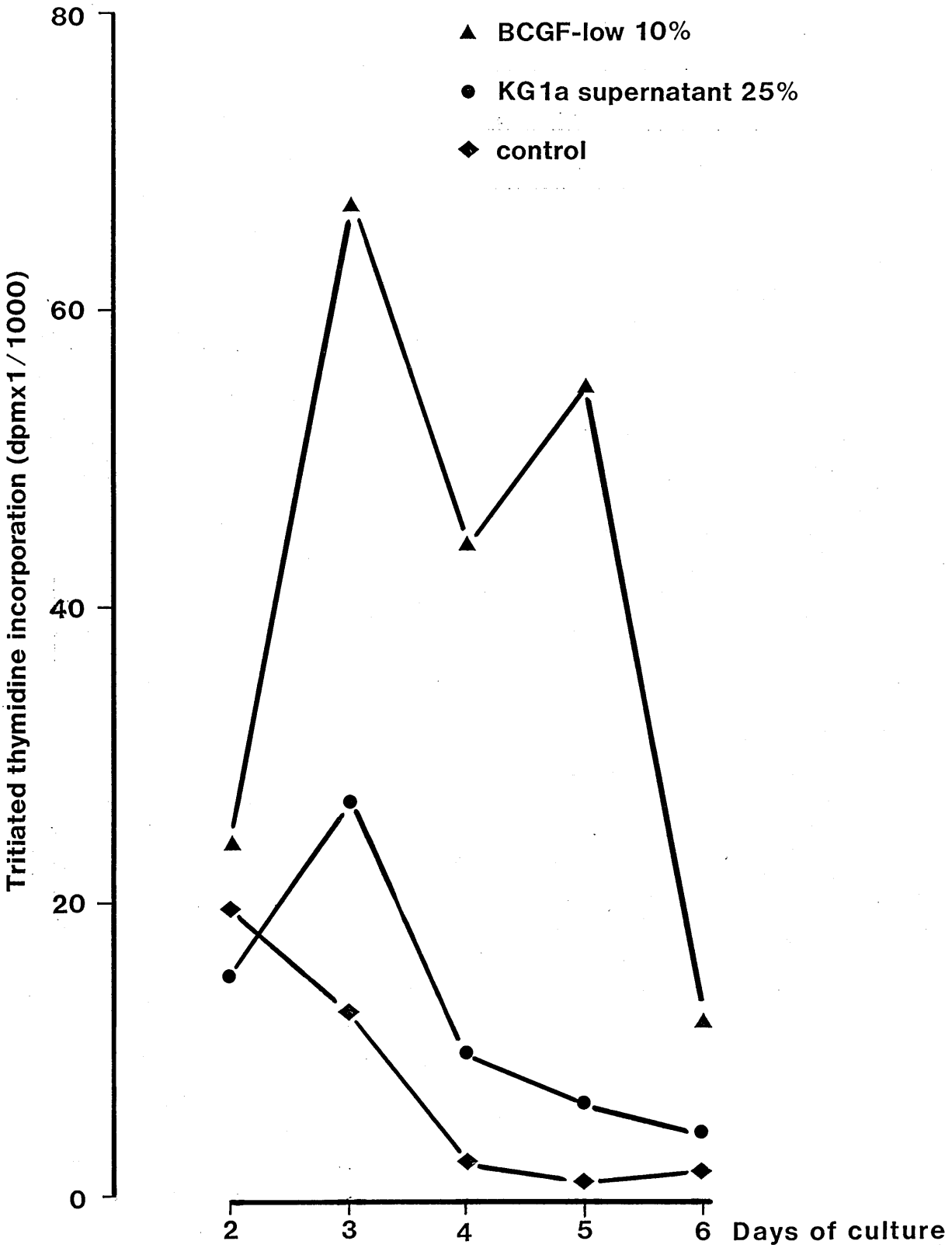
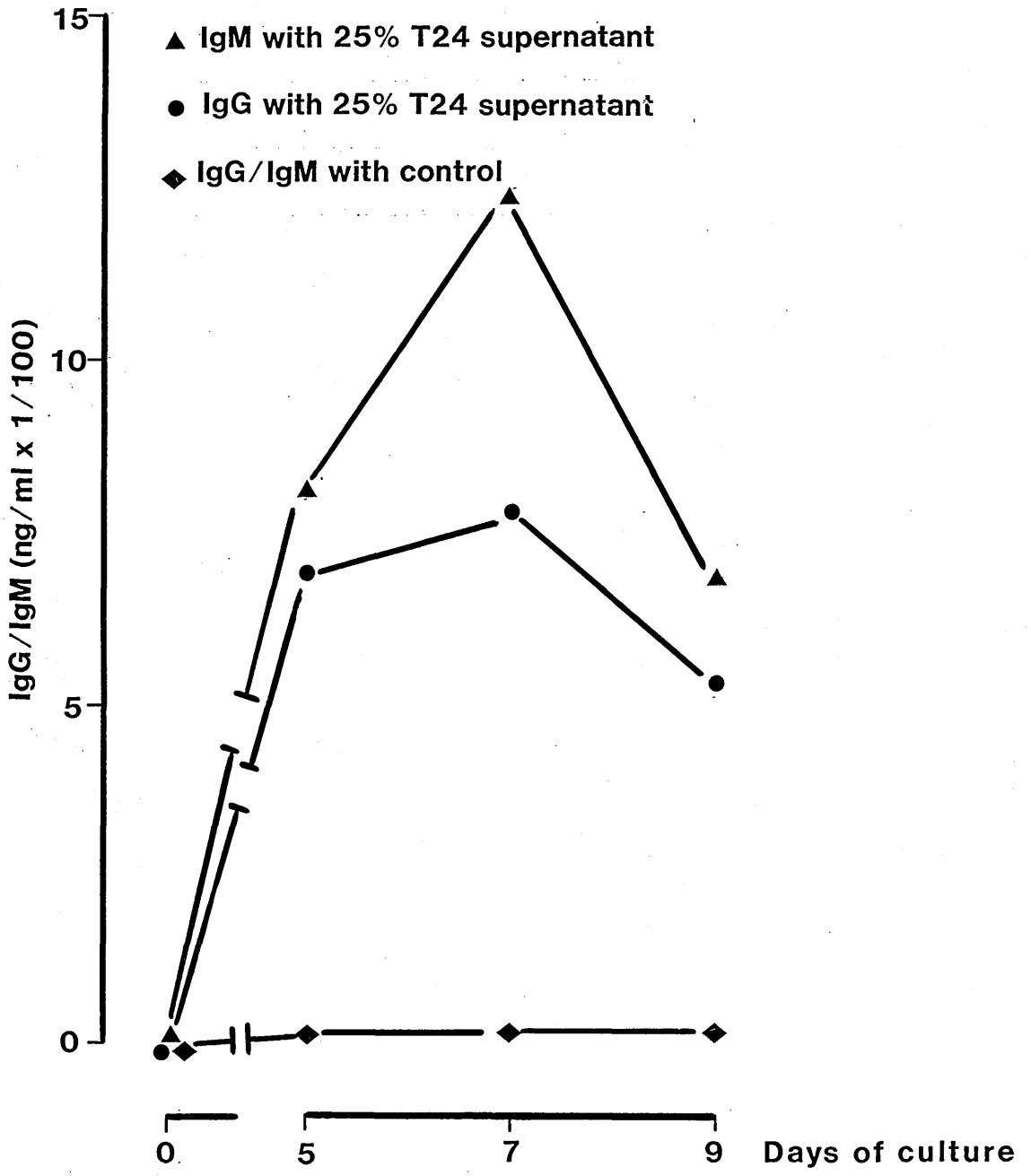


Figure 6-3 Effect of varying number of days of culture on the proliferative response of normal EBV transformed B cell line with and without BCGF-low or KG1-a supernatant.

Results as mean of triplicate cultures.
Standard deviation within 20% of mean.



jure 6-4 Effect of varying number of days of culture on the IgG/IgM cretion of normal EBV transformed B cell line with and without T24-BCDF.

sults as mean of triplicate cultures.
andard deviations within 20% of mean.

6.2.3 EFFECT OF VARYING CONCENTRATION OF FACTORS

Since T24-BCDF and KG1-a supernatant were generated from cell lines in our laboratory, it is essential to test each batch of supernatants to ensure the presumed activities are present. Figure 6-5 shows the dose-response curves of IgG secretion of a normal EBV-transformed B cell line to T24-BCDF and KG1-a supernatant. 25% of either factor elicited appreciable response and this concentration was used throughout all subsequent experiments. The same batches of T24-BCDF and KG1-a supernatants were used in all experiments.

A commercial source of BCGF-low (Cellular Products, Inc) was used. Figure 6-6 shows a dose-response curve of IgM secretion of a normal EBV-transformed B cell line to BCGF-low. 5% of BCGF-low elicited appreciable response and was used in all experiments for differentiation subsequently, while 10% of BCGF-low was used for growth assay.

Recombinant human interleukins, rhIL-4 and rhIL-6, were used at various concentrations as indicated in separate experiments.

6.3 PROLIFERATIVE RESPONSES TO KG1-A SUPERNATANT, BCGF-LOW AND rhIL-4

EBV-transformed B cell lines from 7 normal subjects, 3 XLA and 6 WAS patients were tested for their proliferative responses to KG1-a supernatant, BCFG-low and rhIL-4. rhIL-4 was in the form of supernatant from COS-7 cells transfected with plasmids bearing the human IL-4

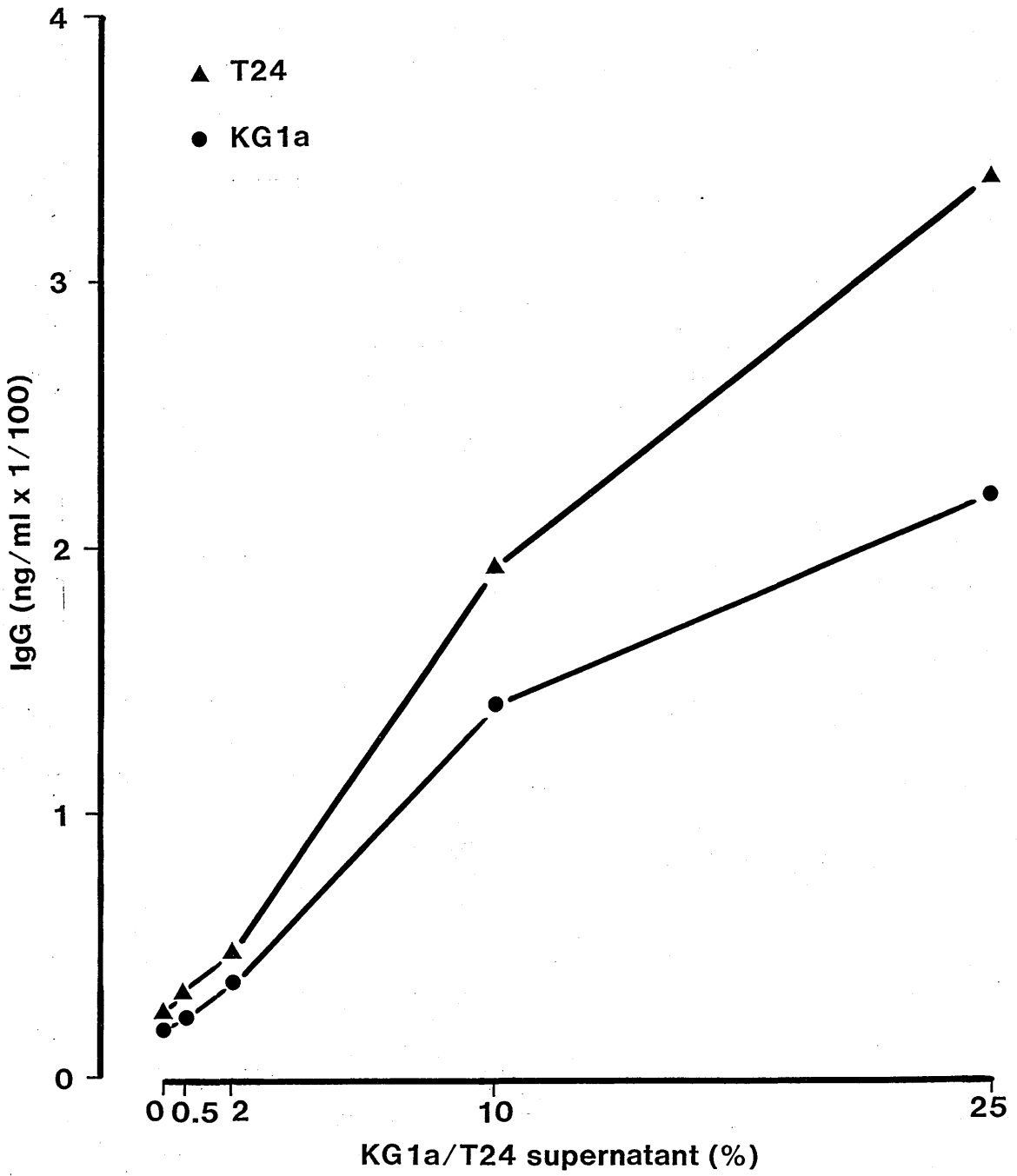


Figure 6-5 Dose-response curve of IgG secretion of normal EBV transformed B cell line to T24-BCDF and KG1-a supernatant.

Results as mean of triplicate cultures.
Standard deviations within 20% of mean.

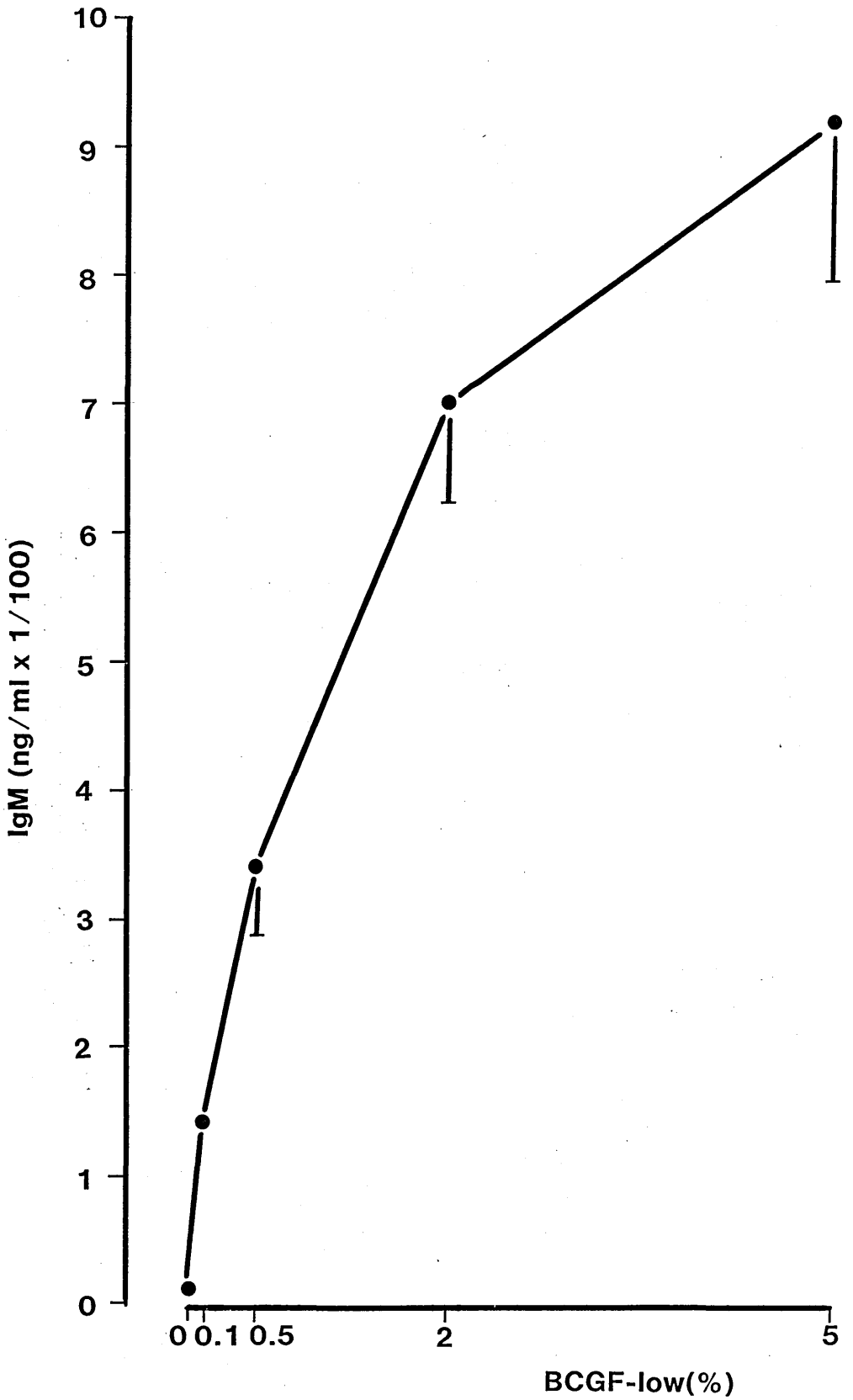


Figure 6-6 Dose-response curve of IgM secretion of normal EBV transformed B cell line to BCGF-low.

Results as mean of triplicate cultures with one standard deviation bar.

gene and was used at a concentration of 1%. All cell lines were tested in at least two separate experiments at a range of cell concentrations (5 to $20x10^3$ cells/well). The results observed with the optimal cell concentration for each cell line are presented in Table 12. All of the normal, XLA and WAS B cell lines responded significantly to BCGF-low. rhIL-4 induced a small but significant increase in proliferation in some normal (EG and JR) and WAS (GB and RF) B cell lines. None of the 3 XLA B cell lines proliferated in response to rhIL-4. KG1-a supernatant induced significant proliferative response in all normal and WAS B cell lines, but neither of the 2 XLA B cell lines tested has shown any significant response to KG1-a supernatant.

6.4 RESPONSES OF IMMUNOGLOBULIN SECRETION TO KG1-A SUPERNATANT, BCGF-LOW, T24-BCDF AND rhIL-4

The same EBV-transformed B cell lines shown in Table 12 were tested for their responses in increasing IgG and IgM secretion to KG1-a supernatant, BCGF-low, T24-BCDF and rhIL-4. The results from the optimal cell concentration in at least two separate experiments for each cell line are resented in tables 13 and 14. KG1-a supernatant, BCGF-low and T24-BCDF induced significant increase in IgM secretion from all normal, XLA and WAS B cell lines. Five out of six normal and four out of six

Table 12

PROLIFERATION OF EBV-TRANSFORMED B CELL LINES IN THE ABSENCE AND PRESENCE OF KG1-A, BCGF-LOW AND IL-4

		TRITIATED THYMIDINE INCORPORATION (10^{-3}dpm/WELL)			
	CELL NO. (10^{-3}/WELL)	MEDIUM	KG1-A	BCGF-LOW	IL4
Normal					
SH	10	23.5±2.6*	70.4±1.4	109.3±9.4	21.1±0.8
VR	10	5.5±0.5	15.9±1.9	28.9±9.8	6.1±1.0
JS	10	5.2±0.9	15.2±0.6	18.9±2.6	6.6±0.7
EG	10	3.1±0.2	28.8±1.7	60.6±5.6	16.3±3.2
BJ	20	19.1±2.6	46.3±1.2	134.5±7.4	30.3±3.2
JR	10	19.9±0.9	46.3±4.2	68.8±9.8	45.8±4.3
DB	20	30.1±7.8	79.2±7.1	123.7±31.9	24.5±1.1
XLA					
BW	10	9.8±3.1	13.1±3.9	28.0±11.9	7.6±1.6
RH	10	6.1±0.5	nd	249.3±1.9	9.4±1.9
MH	10	12.9±2.2	17.8±0.9	75.8±6.0	2.4±0.4
WAS					
GB	20	13.7±1.1	49.4±4.5	208.1±61.7	29.3±1.8
DR	20	44.7±6.1	76.1±1.5	92.5±7.7	34.8±1.8
PM	10	38.3±0.7	158.9±6.3	236.9±4.8	34.2±2.7
RF	10	15.9±1.4	46.9±0.5	208.6±10.7	31.3±2.8
SS	20	7.5±1.3	15.3±0.9	25.3±10.7	17.7±5.0
MC	20	22.3±3.4	68.8±5.5	97.7±5.5	32.8±7.2

* = mean of triplicate cultures ±1S.D.
nd = not done

Table 13

IgM SECRETION OF EBV-TRANSFORMED B CELL LINES IN THE ABSENCE AND PRESENCE OF KG1-A SUPERNATANT, BCGF-LOW, T24-BCDF AND IL4

		IgM (microgram/ml)				
	Cell No. ($x10^{-3}$)/well	**Medium**	**KG1-a**	**BCGF-low**	**T24-BCDF**	**IL4**
Normal						
SH	5	<0.06	0.4±0.1*	1.7±0.1	0.8±0.2	nd
VR	5	0.9±0.2	7.6±0.7	8.1±0.2	11.3±0.0	0.5±0.4
JS	10	0.8±0.0	5.4±0.5	24.0±4.5	8.1+0.8	2.0+0.3
EG	5	0.5±0.2	3.6±0.4	11.8±1.4	9.5±1.4	0.6±0.2
BJ	20	1.3±0.2	4.1±0.9	9.9±0.8	5.8±1.4	3.1±0.5
JR	5	1.2±0.5	5.6±1.4	16.8±8.9	6.7±3.9	8.1±3.3
DB	5	0.5±0.1	3.2±0.4	18.8±2.7	9.8±1.5	0.5±0.1
XLA						
BW	5	0.5±0.2	1.4±0.5	4.8±1.2	1.8±0.4	0.3±0.0
RH	5	1.3±0.2	nd	36.5±3.0	16.8±1.4	0.5±0.0
MH	10	0.7±0.2	3.4±0.9	4.2±0.8	4.0±0.4	1.2±0.1
WAS						
GB	20	17.4±4.4	31.8±2.8	94.5±26.8	62.7±10.4	35.3±7.7
DR	20	8.0±0.7	14.0±2.8	21.0±6.1	16.8±2.7	8.1±1.1
PM	20	0.4±0.2	1.3±0.1	3.5±0.3	4.1±0.6	0.9±0.6
RF	10	0.3±0.0	3.3±1.5	24.3±0.6	9.9±3.8	0.5±0.2
SS	5	0.5±0.4	2.3±0.5	3.2±1.2	8.9±4.2	2.9±1.1
MC	5	6.8±0.7	15.6±1.1	28.5±9.6	49.7±4.1	72.7±47.3

* = mean of triplicate cultures ±1S.D.
nd= not done

Table 14

IgG SECRETION OF EBV-TRANSFORMED B CELL LINES IN THE ABSENCE AND PRESENCE OF KG1-A SUPERNATANT, BCGF-LOW, T24-BCDF AND IL4

		IgG (ng/ml)				
	Cell No. ($x10^{-3}$/well)	**Medium**	**KG1-a**	**BCGF-low**	**T24-BCDF**	**IL4**
Normal						
SH	5	441±45	1059±381	1430±420	2031±516	678±180
VR	5	249±33	1089±120	1439±399	1539±420	183± 12
JS	5	6± 3	81± 36	513± 72	69± 12	15± 3
EG	10	24± 3	138± 6	581±174	273± 57	69± 48
BJ	20	9± 3	27± 12	69± 15	57± 21	30± 6
JR	20	57±12	252± 57	459±108	477± 18	153± 39
DB	10	<3	<3	<3	<3	<3
XLA						
BW	20	<3	<3	<3	<3	<3
RH	10	<3	nd	<3	<3	<3
MH	10	<3	<3	<3	<3	<3
WAS						
GB	20	81± 3	291± 45	1215±567	366±123	396± 96
DR	20	<3	<3	<3	<3	<3
PM	20	87±30	843±267	>3000	>3000	102± 9
RF	10	27± 9	246± 54	408±230	426± 45	54± 39
SS	5	912±174	1197±231	1635±591	2337±606	984±141
MC	5	<9	<9	<9	<9	<9

* = mean of triplicate cultures ±1S.D.
nd= not done

WAS B cell lines also increased IgG secretion in response to KG1-a supernatant, BCGF-low and T24-BCDF. None of the 3 XLA B cell lines secreted IgG constitutively or in response to any of the factors.

rhIL-4 unexpectedly induced some increase in IgM and IgG secretion in several normal B cell lines (BJ, JS and JR) and one WAS B cell line (GB). Two other WAS B cell lines (SS and MC) also increased the IgM secretion in response to rhIL-4. None of the 3 XLA B cell lines responded to rhIL-4.

6.5 RESPONSES OF IMMUNOGLOBULIN SECRETION TO COMBINATION OF FACTORS

Several cell lines which were examined above were also tested for their responses in increasing immunoglobulin secretion to a combination of two factors. The non-IgG secreting cell lines were included to see whether a combination of rhIL-4 & BCGF-low, rhIL-4 & T24-BCDF or BCFG-low & T24-BCDF would switch these cell lines into IgG secretion. The results from the optimal cell concentration for each cell line tested are presented in tables 15 and 16.

The various combination of factors did not switch on IgG secretion in the non-IgG secreting cell lines, whether

Table 15

IgM SECRETION OF EBV-TRANSFORMED B CELL LINES
IN THE ABSENCE AND PRESENCE OF COMBINATIONS OF FACTORS

	IgM (microgram/ml)				
	Normal		XLA	WAS	
Cell lines	JR	DB	BW	DR	RF
Cell No. ($x10^{-3}$/well)	5	5	20	5	5
Medium	0.9±0.2*	0.6±0.1	3.8±0.8	2.5±0.9	0.6±0.2
IL4	1.7±0.1	0.9±0.2	4.3±0.4	10.9±4.5	1.2±0.2
BCGF-low	2.7±0.1	1.9±0.2	19.4±6.4	4.9±0.6	1.9±0.2
T24	2.9±0.6	2.3±0.4	13.3±4.4	19.3±7.0	4.1±1.1
IL4+BCGF-low	6.2±0.3	2.9±0.4	27.6±3.4	12.9±5.0	2.6±0.7
IL4+T24	5.9±1.0	2.9±0.2	13.9±5.6	48.8±21.9	3.8±0.7
BCGF-low+T24	5.6±0.5	3.7±0.2	26.2±4.6	16.4±3.8	8.3±3.0

* = mean of triplicate cultures ±1S.D.

Table 16

IgG SECRETION OF EBV-TRANSFORMED B CELL LINES IN THE ABSENCE AND PRESENCE OF COMBINATIONS OF FACTORS

IgG (ng/ml)					
	Normal		**XLA**	**WAS**	
Cell lines	JR	DB	BW	DR	RF
Cell No. ($x10^{-3}$/well)	5	5	20	5	5
Medium	27± 3*	<3	<3	<3	27± 9
IL4	45±12	<3	<3	<3	54±39
BCGF-low	141± 6	<3	<3	<3	408±23
T24	132±15	<3	<3	<3	426±45
IL4+BCGF-low	198±15	<3	<3	<3	nd
IL4+T24	156±30	<3	<3	<3	nd
BCGF-low+T24	237±15	<3	<3	<3	nd

* = mean of triplicate cultures ±1S.D.
nd= not done

they are normal, XLA or WAS B cell lines. However, the combination of factors did have some additive effects on increasing either IgM or IgG secretion in cell lines already secreting IgM/IgG constitutively. No significant difference was observed in this response among the 2 normal, 1 XLA and 2 WAS B cell lines tested.

6.6 B CELL LINES FROM NORMAL AND XLA PATIENTS SECRETE B CELL GROWTH AND DIFFERENTIATION FACTORS

A hypogammaglobulinaemic patient has been reported to be deficient in the production of B cell differentiation factor by his peripheral blood mononuclear cells (Matheson and Green 1987). Supernatants from normal and XLA EBV-transformed B cell lines were hence assayed for B cell growth and differentiation factors using B cell indicator cell lines L4, HFB-1 and CESS (Shields et al 1987, Hunter et al 1982, Olsson et al 1983, Muraguchi et al 1981). These supernatants were used at a final concentration of 25%. The results from the optimal cell concentration for each cell line are presented in tables 17 and 18.

Both normal and XLA B cell lines secreted B cell growth factor(s) which increased the proliferation of two indicator B cell lines, L4 and HFB-1 (Table 17). Similarly, they also secreted factor(s) which increased IgG secretion by L4 and CESS (Table 18).

Table 17

BCGF ACTIVITY IN THE SUPERNATANTS OF NORMAL AND XLA EBV-TRANSFORMED B CELL LINES

	TRITIATED THYMIDINE INCORPORATION (10^{-3}dpm/well)	
	L4 ($10x10^3$cells/well)	**HFB1** ($5X10^3$cells/well)
Medium	4.5± 0.2	6.8± 2.0
BCGF-low	148.7±10.0	74.3±16.1
Normal supernatant	56.0± 3.7	150.6± 4.2
XLA supernatant	199.4± 7.4	179.9± 8.6

Table 18

BCDF ACTIVITY IN THE SUPERNATANTS OF NORMAL AND XLA EBV-TRANSFORMED B CELL LINES

	IgG (ng/ml)	
	L4 ($20x10^3$cells/well)	**CESS** ($2.5X10^3$cells/well)
Medium	<9	39± 18
T24	180±21*	966±129
Normal supernatant	118±21	507±141
XLA supernatant	258± 6	216± 45

Using L4, HFB1 and CESS as indicator B cell lines to assay BCGF and BCDF activities in the supernatants of normal and XLA EBV-transformed B cell lines.

* = mean of triplicate cultures ±1S.D.

6.7 CELL SURFACE PHENOTYPES OF NORMAL AND XLA B CELL LINES

Since the EBV-transformed B cell lines from patients with XLA differed in certain aspects in their responses to the factors investigated above from the normal control B cell lines, it is conceivable that the XLA B cell lines may have differences in their cell surface phenotypes from the normal. EBV-transformed B cell lines from two patients with XLA and two normal control were therefore typed with a panel of B cell surface markers (Table 19). Monoclonal antibodies used for surface phenotype analysis were 8EBI (CD19), B1(CD20), BL13/10B1(CD21), MHM6(CD23), and G28-10 (CD39) as described in the third international workshop on human leucocyte differentiation antigens (Ling et al 1987). No significant differences were seen between the cell surface phenotypes of XLA and normal B cell lines.

6.8 PROLIFERATIVE RESPONSES OF NORMAL AND XLA B CELL LINES TO rhIL-6

Recombinant human IL-6 became available towards the end of the tenure of my research fellowship and with the realisation that it may also be a growth promoting factor for B cells (Kawano et al 1988) rather than just a pure differentiation factor as previously thought (Hirano et al 1985), EBV-transformed B cell lines from the three XLA patients and three normal control were tested for their proliferative responses to rhIL-6. Two sources of rhIL-6, from Immunex Corporation, Seattle, USA and Dr T.

Table 19

PHENOTYPES OF EBV-TRANSFORMED B CELL LINES FROM PATIENTS WITH XLA AND NORMAL CONTROL

	NORMAL		XLA	
	(JS)	(BJ)	(MH)	(RH)
CD19	+	++	++	+
CD20	+	++	++	++
CD21	+	+	+	+
CD23	++	++	+++	++
CD39	++	+++	+++	nd

nd = not done

Kishimoto, Japan, were used at two final concentrations of 0.1 unit/ml and 1 unit/ml. All three XLA cell lines were tested at least in two separate experiments at a range of cell concentrations (2.5 to $20x10^3$ cells/well). BCGF-low at 5% final concentrations was used as a positive control in these experiments. No significant difference was observed between the responses to the two different sources of rhIL-6 or to the two different concentrations. Therefore, only the results with rhIL-6 from Immunex at 1 unit/ml are presented. The results observed with the optimal cell concentration for each cell line are presented in Table 20. There were no consistent and significant proliferative responses to rhIL-6 in either normal or XLA B cell lines as they did to BCGF-low. However, there were minor proliferative responses in one normal (SS) and two XLA (MH, RH) EBV-transformed B cell lines.

Table 20

PROLIFERATIVE RESPONSES OF EBV-TRANSFORMED B CELL LINES TO rhIL-6 AND BCGF-LOW

		TRITIATED THYMIDINE INCORPORATION (dpm/well)		
	CELL NUMBER ($x10^{-3}$/well)	**MEDIUM**	**BCGF-LOW**	**rhIL-6**
NORMAL				
S.S.	10	10269±1047*	277942± 6217	22240±2833
M.B.	20	13729±2020	69206± 7008	18125±1235
V.R.	20	12104±4494	262983±11676	19283±3697
XLA				
B.W.	20	14079±4301	41866± 1988	12003±1809
M.H.	20	28396±6851	155859±28048	49263± 676
R.H.	10	6125± 472	249266± 1942	54078±3830

* = Mean of triplicate cultures ±1S.D.

6.9 DISCUSSION

EBV-transformed B cell lines from both XLA and WAS patients proliferated in response to BCGF-low (Table 12). In each case, the response was not appreciably different from that of normal EBV-transformed B cell lines. However there were differences in proliferative responses to both KG1-a supernatant and rhIL-4. EBV-transformed B cell lines from both normal control and WAS patients showed significant proliferative responses to KG1-a supernatant but none of the XLA B cell lines showed any significant response (Table 12). KG1-a is a promyelocytic cell line derived from a human acute myelogenous leukaemia cell line (Koeffler et al 1980); it has been shown to produce B cell growth and differentiation factors, which are not IL-1, IL-2, IL-4, or interferon-gamma (Shields et al unpublished observation). The nature of the B cell growth factor in KG1-a supernatant is not clear but its activity is similar to that of BCGF-low in that it can promote DNA synthesis in indicator B cell lines L4 and HFB1, as well as TPA (12-O-tetradecanoylphorbol 13-acetate) activated tonsillar E negative cells (non sheep red blood cells-rosette forming cells) (Shields et al 1987 and unpublished observations). However, it is likely that the BCGF activity in KG1-a supernatant is not the same as BCGF-low since the EBV-transformed B cell lines from XLA patients proliferated in response to BCGF-low but not to KG1-a supernatant (Table 12). It will be important to characterise the BCGF activity in the KG1-a supernatant in

order to investigate further the difference in proliferative response between the XLA and normal EBV-transformed B cell lines.

Unexpectedly, rhIL-4 promoted DNA synthesis in some normal and WAS EBV-transformed B cell lines, but had no effect on the XLA EBV-transformed B cell lines (Table 12). rhIL-4 is a BCGF in costimulation assay with anti-IgM for normal B cells (DeFrance et al 1987b) but does not usually induce proliferation of other B cell lines (Shields et al 1987). It is not clear why some normal and WAS EBV-transformed B cell lines proliferate in response to rhIL-4. It may be a property of B cell subpopulations transformed by EBV, or of B cells at a particular stage of differentiation represented by these lines, or EBV cell lines transformed relatively recently.

In the differentiation assay, both normal and WAS EBV-transformed B cell lines increased secretion of IgM and IgG in response to BCGF-low, T24-BCDF and KG1-a supernatant (Tables 13 and 14). Both BCGF-low and KG1-a supernatant have been shown to increase immunoglobulin secretion by B cell lines (Shields et al 1987 and unpublished obervation); T24, a bladder carcinoma cell line, is known to secrete IL-6 (Rawle et al 1986, Hirano et al 1986) which is a BCDF for EBV-transformed B cell line such as CESS (Hirano et al 1985). Unexpectedly, some normal and WAS EBV-transformed B cell lines also increased secretion of IgM and IgG in response to rhIL-4. (Tables 13 and 14). Murine IL-4 has been shown to induce IgG1

secretion (Snapper and Paul 1987) and it will be of interest to investigate further whether the IgG response of these EBV-transformed B cell lines, which showed increase in total IgG secretion in response to rhIL-4, are of any particular IgG subclass.

In contrast to the EBV-transformed B cell lines from normal control and WAS patients, those obtained from XLA patients responded quite differently in the differentiation assays. Although the XLA lines did increase IgM secretion in response to BCGF-low, T24-BCDF and KG1-a supernatant, they did not secrete IgG constitutively or in response to any of the factors (Tables 13 and 14). This is consistent with earlier findings that XLA EBV-transformed B cell lines can secrete IgM without being stimulated (Levitt et al 1984). None of the XLA EBV-transformed B cell lines responded to rhIL-4. (Tables 13 and 14).

Patients with XLA do have low levels of serum IgG. The lack of IgG secretion by the XLA EBV-transformed B cell lines may, therefore, be due to an inability to transform pre-committed IgG precursors with EBV (Stein et al 1983), or a failure to switch from IgM to IgG secretion. Various combinations of factors (IL-4 + BCGF-low, IL-4 + T24-BCDF, and BCGF-low + T24-BCDF) were tried in an attempt to induce switching of the IgM producing EBV-transformed B cell lines from the XLA patients and the one normal and two WAS EBV-transformed B cell lines which

did not secrete detectable IgG, but without success (Table 16).

These results suggest that the non-IgG secreting XLA EBV-transformed B cell lines probably result from the much lower frequency of pre-committed IgG precursors which are transformable with EBV in patients with XLA as compared with normal control and patients with WAS. The question of IgG, IgM, IgA precursor frequencies in normal control and various disease states can be investigated by limiting dilution analysis, but these experiments have not been performed. The combination of two factors however did have at least an additive effect in most cases in increasing IgM and/or IgG secretion by B cell lines which were secreting IgM and/or IgG constitutively (Tables 15 and 16).

B cells are known to secrete and respond to growth and differentiation factors (Gordon et al 1984, Muraguchi et al 1986, Rimsky et al 1986, Buck et al 1987). EBV-transformed B cell lines depend on an autocrine growth factor for continuous proliferation (Gordon et al 1984) and it is conceivable that autostimulation is also sustained briefly for rapid expansion of normal B cells in areas of high B-cell density (Gordon and Guy 1987). It was, therefore, of interest to determine B cells derived from our patients were able to produce autocrine factors. Using the indicator B cell lines L4, HFB1 and CESS, for assaying B cell growth and differentiation factors, supernatants from XLA EBV-transformed B cell lines were

shown to contain BCGF and BCDF (Tables 17 and 18). Several B cell derived factors were reported to be involved in autocrine proliferation (Buck et al 1987, Swendeman and Thorley-Lawson 1987, Muraguchi et al 1986). The nature of the factors secreted by the XLA B cell lines has however not been investigated further.

There are immunodeficiency syndromes resulting from defective expression of cell surface antigens which have vital immunological functions, viz leucocyte adherence defect and bare lymphocyte syndrome (Lisowska-Grospierre et al 1985, 1986). It was, therefore, of interest to determine whether the surface phenotypes of XLA EBV-transformed B cell lines differed from that of normal. Using a panel of B cell monoclonal antibodies, no significant difference was found (Table 19).

Although rhIL-6 has been shown to be a growth promoting factor for a range of B cells, such as hybridoma and multiple myeloma (Billiau 1987, Kawano et al 1988), it was previously thought to be purely a B cell differentiation factor (Hirano et al 1985). It has, in addition, been shown recently that rhIL-6 can promote the proliferation of EBV-transformed B cell lines (Tosato et al 1988). Two XLA and one normal EBV-transformed B cell lines showed some proliferative response to rhIL-6 (Table 20). The reason why not all cell lines proliferated in response to rhIL-6 could be that the concentration of rhIL-6 used was suboptimal since Tosato et al (1988) used

100 units/ml of rhIL-6 while only 1 unit/ml was used here.

In conclusion, the EBV transformed B cells from the patients with WAS did not differ from the normal control in any of the functional assays I have tested. However differences were found in the B cells from the patients with XLA. XLA EBV-transformed B cell lines did not proliferate in response to KG1-a supernatant; they did not produce IgG in the presence or absence of various B cell growth and differentiation factors.

Chapter 7 CONCLUSION

7.1 EXPERIMENTS OF NATURE

The incidence of primary immunodeficiency diseases is comparatively low (Chapter 1) but their importance as "experiments of nature" extends far beyond the immediate problems faced by these relatively low number of patients and the physicians caring for them. Ever since Bruton (1952) described the first case of agammaglobulinaemia and the subsequent description of many other forms of human immunodeficiency, a deeper understanding of how the various arms of immune system cooperate and function has been achieved by the detailed investigation and interpretation of these nature's experiments, coupled with the development and study of experimental models (Good 1976).

The present study is mainly concerned with the lymphoid system which, through nature's experiments, has deviated from normality and resulted in diseases such as X-linked agammaglobulinaemia (XLA), X-linked severe combined immunodeficiency (X-linked SCID) and Wiskott-Aldrich syndrome (WAS). The elucidation of the underlying defects of these diseases will no doubt throw a ray of light into the understanding of the normal physiology of the lymphoid system, and may perhaps lead to better management of these patients and their families. Two approaches of investigation are feasible in any genetic diseases of Mendelian inheritance. One is to identify the mutant gene and the other is to pinpoint the underlying biochemical abnormality. The former approach depends

firstly on genetic mapping to give the genomic position, and secondly on molecular techniques to identify coding sequences corresponding to the locus. This strategy has been coined reverse genetics (Orkin 1986a) and it has been successfully applied in the isolation and cloning of the gene of X-linked chronic granulomatous disease (X-linked CGD) (Baehner et al 1986, Royer-Pokora et al 1986). The latter approach depends firstly on identifying which cell types are intrinsically defective in function, and secondly on biochemical and biophysical techniques to characterise the biochemical defect which leads to the dysfunction of the cell types involved. This strategy has also been successful in elucidating the biochemical defect in the neutrophils of X-linked CGD (Segal et al 1983). The two approaches finally converged in establishing the basic defect in X-linked CGD as that in the beta-chain of cytochrome b complex (Teahan et al 1987, Dinauer et al 1987).

7.2 THE APPROACH OF MOLECULAR BIOLOGY - CLONING THE GENE

This present study has been able to achieve the first step in the approach of isolating the mutant genes responsible for XLA and X-linked SCID (Chapters 4 and 5), viz mapping their gene loci to Xq21.3-q22 and Xq11-q13 respectively. For XLA, we have confirmed the linkage with S21 (DXS17) and 19.2 (DXS3) (Kwan et al 1986); and extended the linkage with another probe, pXG12 (DXS94) (Malcolm et al 1987). Evidence for non-allelic genetic heterogeneity in XLA soon emerged and has complicated the

approach of gene tracking in carrier detection and prenatal diagnosis (Mensink et al 1986a, 1987a, Ott et al 1986). Further evidence of non-allelic genetic heterogeneity in XLA was presented in this thesis and the percentage of XLA families unlinked to Xq21.3-q22 was estimated (Chapter 4). A method of risk calculation to allow for non-allelic genetic heterogeneity was developed and applied in the genetic counselling of thirteen XLA families (Lau et al 1988b). The genetic locus of X-linked hyperIgM was also shown to be distinct from that of XLA (Chapter 4). The next step should be finer mapping of this region of the X chromosome using more probes in larger number of families (Arveiler et al 1987a) or in isolated patients with chromosomal duplication or deletion (Cremers et al 1987, Schwartz 1988); and establishing closely linked probes which flank the disease locus. This will not only improve the certainty of genetic prediction but also the chance of cloning the gene. Future progress in isolating the gene however depends on recent advances in techniques for cloning long segments of DNA, such as the chromosome hopping procedure (Collins et al 1987); as well as the breakthrough in the ability to display and isolate large DNA fragments up to several megabase pairs by pulsed-field gel electrophoresis (Schwartz and Cantor 1984, Weissman 1987). In the past, the successful cloning of disease genes without the benefit of these novel techniques has always depended on exploitation of isolated patients with chromosomal deletions, such as that found in

association with X-linked chronic granulomatous disease (Baehner et al 1986, Royer-Pokora et al 1986). For both XLA and X-linked SCID, no patients have been described with the corresponding chromosomal deletion or translocation to allow for a strategy of rapid cloning of the defective genes. One has to rely on the recently established molecular genetic techniques of mapping the human genome (Weissman 1987).

Pulsed-field gel electrophoresis subjects DNA after cleavage by rare-cutting endonucleases to non-uniform (or uniform) field pulses applied at an angle greater than 90^{o} to each other; the DNA size range separable by this technique extends from 0.005 Mbp (megabase pairs) to more than 9.0 Mbp (Barlow and Lehrach 1987). This technique has enabled one to generate physical long-range restriction maps covering several million basepairs by single and double enzyme digests, as well as deliberate partial digests showing the physical distances separating genetically linked markers (Burmeister and Lehrach 1986). If the two linked markers are known from linkage analysis to flank the disease gene and the physical distance between them is within the capacity of cloning technique available, the DNA fragment separated by pulsed-field gel electrophoresis which is recognised by the two linked markers can be excised from preparative low melting point agarose gels, and after a second digestion used to generate fragments that can be cloned into lambda vectors (Barlow and Lehrach 1987). The library hence generated

will provide enriched sources of DNA from around the disease locus. A further step of purification is possible by cleavage of the DNA contained in the excised agarose strip with a second rare-cutting endonuclease that changes the fragment length and re-electrophoresis on a pulsed-field gel, before cloning into vectors (Barlow and Lehrach 1987).

The construction of long-range restriction maps also allows the genetic distances deduced from linkage analysis to be compared with the physical length in kilobase pairs; thereby identifying regions with high and low recombinations and may lead to understanding the regulation of recombination frequency. (Barlow and Lehrach 1987).

All the rare-cutting endonucleases used for pulsed-field gel electrophoresis (except Sfi I) contain one or more CpG dinucleotides in their recognition site (Weissman 1987), and because their ability to cleave genomic DNA is inhibited by methylation of the cytosine residue, a cleaved rare-cutter site identifies a methylation-free CpG-containing region. Clusters of unmethylated CpG dinucleotides (Hpa II tiny fragments or HTF islands) were thought to be associated with the 5' end of most vertebrate genes (Bird 1986, Burmeister and Lehrach 1986). HTF islands can be readily identified by pulsed-field gel electrophoresis as a cluster of several different rare-cutter sites (Brown and Bird 1986, Burmeister and Lehrach 1986) in the long-range restriction map and may lead in

turn to the associated gene.

The novel techniques of molecular biology will certainly accelerate the pace of identifying the genetic defects of XLA and X-linked SCID. The eventual identification of the gene may not necessarily lead to better treatment of the disease but at least open up the futuristic option of gene therapy, which is however more feasible in genetic diseases due to enzymatic defects such as adenosine deaminase deficiency (Orkin 1986b).

7.3 THE APPROACH OF CELL BIOLOGY - DEFINING THE CELLULAR AND BIOCHEMICAL DEFECT

The present study has also followed the second approach in investigating XLA and Wiskott-Aldrich syndrome (WAS), viz attempting to elucidate the functional defects of the B lymphocytes which are known from both clinical and genetic evidence to be affected in both XLA and WAS (Chapter 6). Epstein-Barr virus (EBV)-transformed B cell lines were established and characterised as an experimental system for testing B cell function in vitro for normal subjects and then extended to patients with XLA and WAS. The differences detected in the XLA EBV-transformed B cell lines were that they did not proliferate in response to KG1-a supernatant and did not secrete IgG in the absence or presence of the various B cell growth and differentiation factors. No abnormalities were detected in the EBV-transformed B cell lines from patients with WAS in terms of their responses to various B cell growth and differentiation factors. XLA EBV-

transformed B cell lines were also shown to secrete B cell growth and differentiation factors and have similar surface phenotypes as normal B cell lines.

With the availability of recombinant lymphokines (rhIL-4, rhIL-6) recently, it was discovered that factors originally thought to act only on B cells are, in fact, pleiotropic, i.e. acting on many other cell types (O'Garra et al 1988). In addition, each factor mediates multiple effects on a single target cell population. Therefore, it seems unlikely that the defect in response to and/or production of these factors will account for the underlying defect in XLA, which seems to affect the B cells only. For WAS, it is still conceivable that a defect in response to such a pleiotropic factor could lead to functional impairment in B cells, T cells and platelets. It is easier to interpret an experiment if recombinant lymphokines are used rather than factors-containing supernatants from cell lines or activated T cells; and such recombinant lymphokines were only available near the end of my research fellowship. However, it is interesting to note that one consistent functional difference identified in this study is the failure of XLA EBV-transformed B cell lines to proliferate in response to KG1-a supernatant, which no doubt contains a mixture of active factors. Since XLA EBV-transformed B cell lines increased IgM secretion in response to KG1-a supernatant as the normal B cell lines, the growth-promoting factor in KG1-a supernatant would seem to be

different from that which increased immunoglobulin secretion. Moreover, the growth-promoting factor in KG1-a supernatant is not BCGF-low as XLA EBV-transformed B cell lines proliferated in response to BCGF-low but not to KG1-a supernatant. It is important to confirm this finding in more XLA B cell lines before attempting to isolate and purify this growth-promoting factor in KG1-a supernatant which has so far failed to stimulate two XLA EBV-transformed B cell lines to proliferate but stimulated all normal and WAS B cell lines to proliferate. This factor may be important in the further elucidation of the the underlying defect of XLA.

Another experimental model which may throw some light in the early events of pre-B cell development, which is defective in XLA, is long-term culture of bone marrow pre-B cells, which can then be investigated for their requirements of growth and differentiation. Some advances in this direction have been made in the mouse system recently (Landreth and Dorshkind 1988, Namen et al 1988) and a purified murine pre-B cell growth factor of 25 kilodaltons has been reported (Namen et al 1988). It is important to set up parallel human system to identify human pre-B cell growth and differentiation factor(s) and investigate their possible role in the pathogenesis of XLA. This may be more fruitful as the intrinsic defect of XLA seems to be somewhere along the developmental pathway between pre-B cell and mature B cell; and the understanding of regulation of this phase of B cell

development lags much behind that from mature B cells to plasma cells.

For WAS, the present study has not identified any functional abnormality in the EBV-transformed B cell lines from six patients. It could be the measurement of total IgG and IgM was too crude to detect the abnormalities in WAS. It will be interesting therefore to see whether the ability and inability to mount antibody response to polysaccharide antigens in normal subjects and patients with WAS respectively (Blaese et al 1968) are also reflected in their EBV-transformed B cell lines; if so, it will be important to test whether any B cell growth and differentiation factor may influence the magnitude and/or ability to mount antibody response to polysaccharide antigens in the normal and WAS EBV-transformed B cell lines. It will also be interesting to see the IgG subclass profile in the antibody responses since IgG antibodies to polysaccharides are predominantly restricted to IgG2 subclass in man, although it has been shown patients with WAS have normal IgG2 levels (Nahm et al 1986).

7.4 THE INTEGRATION

The complementary nature of the two approaches above has been instrumental in the success of determining the underlying defect of X-linked CGD (Teahan et al 1987, Dinauer et al 1987) and will no doubt be successful for the other X-linked immunodeficiencies in the near future. Similarly, the integration of clinical medicine and

vigorous scientific experimentation has been the hallmark of many successes.

I would like to end this dissertation with a quote from Robert A. Good in his presidential address to the American Association of Immunologists on April 13, 1976:

"the very great strengths of immunology, from its very beginning....., is that this discipline originated and has been maintained by the interaction between the clinic and effective scientific investigation."

APPENDIX 1 MATERIALS

A. CULTURE MEDIUM AND ADDITIVES

Culture medium used for in vitro experiments with human B cells is RPMI 1640 supplemented with 25mM Hepes, 2mM glutamine and fetal calf serum (Gibco, Paisley, Scotland). Gentamicin at 50 microgram/ml has been used to inhibit bacterial growth in long term culture.

B. SALT SOLUTIONS

Stock solutions were prepared as follows:

1. 1M Tris

Dissolve 121.1 g Tris base in 800 ml of water. Adjust the pH to the desired value by adding concentrated hydrochloric acid. Then make up the volume of the solution to 1 litre.

2. 0.5M Ethylenediamine tetraacetate (EDTA)

Add 186.1 g of disodium ethylenediamine tetra-acetate.$2H_2O$ to 800 ml of water. Stir vigorously on a magnetic stirrer. Adjust pH to 8.0 with sodium hydroxide pellets.

3. 5M Sodium Chloride (NaCl)

Dissolve 292.2 g of NaCl in 800ml of water. Adjust volume to 1 litre.

4. 3M Sodium Acetate (ph5.2)

Dissolve 408.1 g of sodium acetate.$3H_2O$ in 800 ml of water. Adjust pH to 5.2 with glacial acetic acid. Adjust volume to 1 litre.

5. 10% Sodium Dodecyl Sulphate (SDS)

Dissolve 100 g of electrophoresis-grade SDS in 900 ml of water. Heat to 68°C to assist dissolution. Adjust pH to 7.2 by concentrated hydrochloric acid. Adjust volume to 1 litre.

6. 20x Sodium Chloride and Sodium Citrate (SSC)

Dissolve 175.3 g of sodium chloride and 88.2 g of sodium citrate in 800 ml of water. Adjust pH to 7.0 with 10N solution of sodium hydroxide. Adjust volume to 1 litre.

7. Ethidium Bromide (10mg/ml)

Add 1 g of ethidium bromide to 100 ml of water. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil and store at 4° C.

8. 1M Magnesium Chloride ($MgCl_2$)

Dissolve 203.3 g of $MgCl_2.6H_2O$ in 800 ml of water. Adjust volume to 1 litre.

9. TE solution

A solution of 10mM Tris and 1mM EDTA.

10. 10% Bovine Serum Albumin (BSA)

Dissolve 1 g of BSA in 10 ml of water.

11. 50x Denhardt's Solution

5 g of ficoll, 5 g of polyvinylpyrrolidone and 5 g of bovine serum albumin (BSA) to 500 ml of

water. Filter and dispense into 25ml aliquots and store at -20°C.

12. Beta-Mercaptoethanol (BME)

Usually obtained as a 14.4M solution. Store in a dark bottle at 4°C.

13. TM Solution

A solution of 250mM Tris (pH8), 25mM $MgCl_2$ and 50mM BME.

14. DTM Solution

A solution of 100 microM of dATP, dGTP and TTP in TM solution.

15. OL Solution

To 250 microlitres of 90 units/ml of oligo-nucleotide (17-base),add 1 microlitre of 0.25M EDTA and 1 microlitre of 0.25M Tris (pH7.5).

16. LS Solution

A solution of 25 parts of 1M Hepes (pH 6.6), 25 parts of DTM and 7 parts of OL solution.

17. Prehybridization Solution

A solution of 10x Denhardt's, 4xSSC, 0.1% SDS, 10 micrograms/ml polyA and 50 micrograms/ml herring sperum. Herring sperm should be boiled for 5 minutes and the SDS to be added last.

18. 10x Tris E Buffer

Dissolve 193.6 g of Tris, 65.6 g sodium acetate and 29.6 g EDTA in 3 litres of water. Adjust pH to 7.7 with glacial acetic acid. Make final volume to 4 litres.

19. Denaturing Solution

A solution of 0.5M sodium hydroxide and 0.5M sodium chloride.

20. Neutralising Solution

Dissolve 242 g of Tris, 700 g of sodium chloride and 353 g of sodium citrate in 3 litres of water. Adjust pH to 5.5 with concentrated hydrochloric acid. Make final volume to 4 litres.

21. Bicarbonate Buffer (pH9.6)

Dissolve 1.59 g of sodium carbonate, 2.93 g of sodium hydrogen carbonate and 0.20 g of sodium azide in 1 litre of distilled water.

22. Phosphate Citrate Buffer (pH5.0)

28.4 g of sodium hydrogen phosphate in 1 litre of distilled buffer (pH5.0) water. 21.0 g of citric acid in 1 litre of distilled water. Mix equal volumes of each just prior to use.

23. Saline/Tween 20

Dissolve 45 g of NaCl in 5 litres of distilled water and add 2.5 ml of Tween 20.

C. B CELL GROWTH AND DIFFERENTIATION FACTORS

Partially purified low molecular weight BCGF (BCGF-low) prepared by Cellular Products was purchased from Sera Lab, Crawley Down, UK (Mehta et al 1985). Recombinant 1L4 was kindly provided by Dr.J. Banchereau, UNICET, France as supernatants from COS 7 cells transfected with cDNA encoding human 1L-4 (Yokota et al 1986). Supernatants from the bladder carcinoma cell line, T24 and promyelocytic cell line, KG1-a were prepared by seeding $2.5x10^5$ cells/ml in medium supplemented with 5% FCS, and incubated at 37^oC for 5 days. Cells were removed by centrifugation and the supernatants then filtered through an 0.22 micron filter, aliquoted and stored at -70^oC (Rawle et al 1986, Callard et al 1986) Recombinant 1L-6 was kindly provided by both Immunex and Dr. Kishimoto, Japan.

D. CELL LINES

Indicator B cell lines for assaying B cell growth and differentiation factors are HFB1, L4 and CESS (Shields et al 1987, Hunter et al 1982, Olsson et al 1983, Muraguchi et al 1981). L4 and HFB1 are mycoplasma free as shown by repeated testing with orcein acetate staining and culture on PPLO agar. DNA dot blots with a mycoplasma specfic probe have also shown these lines to be mycoplasma free. CESS, however, does contain mycoplasma. An EBV producing marmoset B cell line, B95-8, was used to generate EBV containing supernatants for human B cell transformation (Miller et al 1972). All these B cell lines were

maintained in cultures in Hepes buffered RPMI 1640 supplemented with 2mM glutamine and 10% FCS.

E. RESTRICTION ENDONUCLEASES, POLYMERASE AND DNA PROBES

Restriction endonucleases, TaqI, PstI, Hind III, AluI, MspI and HinfI, were purchased from either Pharmacia or Northumbria Biologicals Ltd, Cramlington, England. DNA polymerase 1 Klenow fragment was purchased from Pharmacia. Various DNA probes used with their polymorphisms and allele frequency are listed in table 21.

F. OTHER MATERIALS

Reagents used in the ELISA assay for IgG and IgM were purchased from Sigma, viz horseradish peroxidase (HRP) conjugated affinity goat anti-human IgG/IgM, o-phenylene-diamine (OPD) and affinity purified goat anti-human IgG/IgM.

Other materials used in the Southern blotting include nitrocellulose or nylon filters and [^{32}P] dCTP purchased from Du Point or Amersham, fast film (XAR-5) from Kodak, and electrophoresis-grade agarose powder from Betheseda Research Lab.

Table 21

DNA probes used in the linkage studies

CHROMOSOME REGION	PROBE(LOCUS)	ENZYME	CONSTANT BAND	ALLELE LENGTH(kb)	FREQUENCY
Xq11-q13	cpX73(DXS159)	Pst 1	-	5.5	.67
				1.6	.33
Xq21.3-q22	19.2(DXS3)	Taq 1	-	5.1	.38
				2.95,2.15	.62
Xq21.3-q22	pXG12(DXS94)	Pst 1	-	7.2	.40
				6.5	.60
Xq21.3-q22	S21(DXS17)	Taq 1	4.0,1.0	2.2	.65
				2.0	.35

cpX73 (Arveiler et al 1987);
19.2 (Aldridge et al 1984, Menlove et al 1985);
pXG12 (Davatelis et al 1985);
S21 (Drayna and White 1985).

Appendix 2 COMPUTER PROGRAMME FOR CALCULATING THE VARIATION OF THE CARRIER RISK OF AN INDIVIDUAL WITH VARYING PROPORTION OF UNLINKED FAMILIES

```
program xla(input, output);
{
    XLA CALCULATION
    ===============

    Function     :
             This program calculates the carrier risk of an
             individual depending on the proportion of the
             unlinked families.

             The data can be either entered from a data file or
             keyed-in interactively. The data required are as
             follows :-
                1) Family name
                2) Patient name
                3) Aa  (probability of being a carrier when the
                        probe is linked with the disease locus)
                4) Aa' (probability of being a carrier when the
                        probe is unlinked with the disease locus)
                5) aa  (probability of being a normal genotype
                        when the probe is linked with the disease
                        locus)
                6) aa' (probability of being a normal genotype
                        when the probe is unlinked with the
                        disease locus)
                7) AA  (probability of being affected when the
                        probe is linked with the disease locus)
                8) AA' (probability of being affected when the
                        probe is unlinked with the disease locus)

                (If data file is used, each parameter must be in
                 a new line)

             The result of the XLA calculation is outputed to a
             file named 'XLA.OUT'.

    Date          : July 1987
    Version       : 1
    Language      : Turbo Pascal
    To be run on : IBM PC and compatible using MSDOS operating

}
var
  family : string[50];
  patient : string[50];
  in_file_name : string[30];
  in_file_var : text;
  out_file_name : string[30];
  out_file_var : text;
  d_in, d_out : text;
  from_data_file, error : boolean;
```

```
  a1, a2, a3, a4, a5, a6 : real;
  b1, b2, b3, b4, b5, b6 : real;


  procedure GetInFile;
  var
    io_ok : boolean;
  begin
    error := false;
    from_data_file := false;
    in_file_name := '                          ';
    write(' Enter data filename  (ret = from keyboard) : ');
    readln(input, in_file_name);
    if (in_file_name <> '') then begin
      assign(in_file_var, in_file_name);
      {$I-} reset(in_file_var); {$I+}
      io_ok := (IOresult = 0);
      if not io_ok then begin
        writeln('Input data file not found');
        error := true;
      end else begin
        from_data_file := true;
        readln(in_file_var, family);
        readln(in_file_var, patient);
        readln(in_file_var, a1);
        readln(in_file_var, a2);
        readln(in_file_var, a3);
        readln(in_file_var, a4);
        readln(in_file_var, a5);
        readln(in_file_var, a6);
        close(in_file_var);
      end;
    end;
  end;


  procedure GetName(var d_in, d_out : text);
  begin
    write(d_out,' Enter family : ');
    readln(d_in, family);
    write(d_out, ' Enter Patient : ');
    readln(d_in, patient);
  end;


  procedure PrName(var d_out : text);
  begin
    writeln(d_out);
    writeln(d_out, '     FAMILY  : ', family);
    writeln(d_out, '     PATIENT : ', patient);
  end;
```

```
function power(y : real) : real;
begin
  if (y <= -38.0) then
    power := 0.0
  else
    power := exp(y * ln(10));
end;


procedure anti_log(var a1, a2, a3, a4, a5, a6 : real);
begin
  a1 := power(a1);
  a2 := power(a2);
  a3 := power(a3);
  a4 := power(a4);
  a5 := power(a5);
  a6 := power(a6);
end;


procedure GetPar(var d_in, d_out : text;
                 var a1, a2, a3, a4, a5, a6 : real);
begin
  write(d_out, 'Enter Aa  : ');
  readln(d_in, a1);
  write(d_out, 'Enter Aa" : ');
  readln(d_in, a2);
  write(d_out, 'Enter aa  : ');
  readln(d_in, a3);
  write(d_out, 'Enter aa" : ');
  readln(d_in, a4);
  write(d_out, 'Enter AA  : ');
  readln(d_in, a5);
  write(d_out, 'Enter AA" : ');
  readln(d_in, a6);
end;


procedure PrProbs(var d_out : text;
                  a1, a2, a3, a4, a5, a6 : real);
begin
  writeln(d_out);
  writeln(d_out, ' Aa  = ', b1:10:6, ', prob(Aa ) = ', a1:25:20);
  writeln(d_out, ' Aa" = ', b2:10:6, ', prob(Aa") = ', a2:25:20);
  writeln(d_out, ' aa  = ', b3:10:6, ', prob(aa ) = ', a3:25:20);
  writeln(d_out, ' aa" = ', b4:10:6, ', prob(aa") = ', a4:25:20);
  writeln(d_out, ' AA  = ', b5:10:6, ', prob(AA ) = ', a5:25:20);
  writeln(d_out, ' AA" = ', b6:10:6, ', prob(AA") = ', a6:25:20);
  writeln(d_out);
end;
```

```
function CalcXLA(a1, a2, a3, a4, a5, a6, alpha : real) : real;
var
  x1, x2, x3, x4 : real;
begin
  x1 := (1 - alpha) * a1;
  x2 := alpha * a2;
  x3 := (1 - alpha) * (a1 + a3 + a5);
  x4 := alpha * (a2 + a4 + a6);
  CalcXLA := (x1 + x2) / (x3 + x4);
end;


procedure LoopXLA(var d_out : text;
                  a1, a2, a3, a4, a5, a6 : real);
var
  i : integer;

  alpha, step, result : real;
begin
  alpha := 0.0;
  step  := 0.1;

  for i := 1 to 11 do begin
    result := CalcXLA(a1, a2, a3, a4, a5, a6, alpha) * 100.0;
    writeln(d_out, '     Unlinked family = ', alpha:4:1,
                   ',    Risk = ', result:7:2);
    alpha := alpha + step;
  end;
end;


procedure OpenOutFile;
begin
  out_file_name := 'xla.out';
  assign(out_file_var, out_file_name);
  rewrite(out_file_var);
end;


procedure CloseOutFile;
begin
  close(out_file_var);
end;


procedure save_input;
begin
  b1 := a1;
  b2 := a2;
  b3 := a3;
  b4 := a4;
  b5 := a5;
  b6 := a6;
end;
```

```
begin   { MAIN }
  writeln;
  writeln('XLA Calculation');
  writeln('===============');
  writeln;
  GetInFile;
  if not error then begin
    if from_data_file = false then begin
      GetName(input, output);
      GetPar(input, output, a1, a2, a3, a4, a5, a6);
    end;
    save_input;
    anti_log(a1, a2, a3, a4, a5, a6);

    OpenOutFile;

    PrName(output);
    PrProbs(output, a1, a2, a3, a4, a5, a6);
    LoopXLA(output, a1, a2, a3, a4, a5, a6);

    PrName(out_file_var);
    PrProbs(out_file_var, a1, a2, a3, a4, a5, a6);
    LoopXLA(out_file_var, a1, a2, a3, a4, a5, a6);

    CloseOutFile;
    writeln;
    writeln('Output in XLA.OUT.');
  end;
end.    { MAIN }
```

Appendix 3 **INPUT AND OUTPUT FILES OF LIPED FOR THE ANALYSIS OF S21, pXG21 AND 19.2 DATA IN THE FAMILIES WITH XLA**

```
 1 0010 0.00001      XLA Linkage Study  (S21)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0200 0200
 0300 0300
 0400 0400
 0500 0500
 0600 0600
 0700 0700
 0800 0800
 0900 0900
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
XLA     A   a aff n.a
    0001     9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
S21     U   L  UU  UL  LL
    6500     3500
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
   5   0Fox/Berryman(family 11)
 1.1            m n.a
 1.2            f n.a  UU
 2.1            m n.a
 2.2 1.1 1.2    f n.a  UU
 3.1 2.1 2.2    m aff  UU
7000
   5   0Parry(family 10)
 1.1            m n.a
 1.2            f n.a  UU
 2.1 1.1 1.2    m aff
```

```
 2.2 1.1 1.2   m aff
 2.3 1.1 1.2   m aff
7000
   8   ORushin/Graham/Golder(family 3)
 1.1           m n.a  UU
 1.2           f n.a  UL
 2.1 1.1 1.2   m n.a  UU
 2.2 1.1 1.2   m aff  LL
 2.3 1.1 1.2   m n.a  UU
 2.4 1.1 1.2   f n.a  UL
 2.5           m n.a
 3.1 2.5 2.4   m n.a  UU
7000
   5   OBarton(family 5)
 1.1           m n.a
 1.2           f n.a  UU
 2.1 1.1 1.2   m aff
 2.2 1.1 1.2   m n.a
 2.3 1.1 1.2   m aff
7000
   4   OWilliams(family 6)
 1.1           m n.a
 1.2           f n.a  UU
 2.1 1.1 1.2   m aff
 2.2 1.1 1.2   m aff
7000
  11   OPatterson/Williamson/Carse(family 7)
 1.1           m n.a
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UU
 2.3           m n.a
 2.4 1.1 1.2   f n.a  UU
 2.5 1.1 1.2   m n.a  LL
 3.1 2.1 2.2   m aff  UU
 3.2 2.1 2.2   m n.a  UU
 3.3 2.3 2.4   m aff  UU
 3.4 2.3 2.4   m aff  UU
7000
  12   OKing/Pomfrett(family 1)
 1.1           m n.a
 1.2           f n.a
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3 1.1 1.2   m n.a  UU
 2.4           m n.a
 2.5 1.1 1.2   f n.a  UL
 2.6 1.1 1.2   f n.a  UL
 2.7 1.1 1.2   f n.a  UL
 2.8 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m aff  UU
 3.2 2.4 2.5   m aff  UU
7000
   4   OKirsopp(family 4)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
```

```
 2.2 1.1 1.2   m aff  LL
   4   0Cox
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  LL
7000
   9   0Lucken/Gach(family 2)
 1.1           m n.a
 1.2           f n.a
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m n.a  LL
 3.2 2.1 2.2   m aff  UU
 3.3 2.3 2.4   m aff  UU
7000
   6   0Gerrard(family 12)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m n.a  UU
 2.2 1.1 1.2   m aff  LL
 2.3 1.1 1.2   m aff  LL
 2.4 1.1 1.2   m aff  LL
7000
   9   0Chaperon(French 1)
 1.1           m n.a  LL
 1.2           f n.a  UU
 2.1 1.1 1.2   m n.a  UU
 2.2           m n.a
 2.3 1.1 1.2   f n.a  UL
 2.4 1.1 1.2   m aff  UU
 2.5 1.1 1.2   m n.a  UU
 2.6 1.1 1.2   m aff  UU
 3.1 2.2 2.3   m aff  UU
7000
   7   0Gatte(French 2)
 1.1           m n.a
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  LL
 2.3 1.1 1.2   m aff  LL
 3.1 2.1 2.2   m aff  LL
 3.2 2.1 2.2   m n.a  LL
7000
   9   0Joliton(French 3)
 1.1           m n.a  LL
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  LL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  LL
 3.1 2.1 2.2   m n.a  LL
 3.2 2.1 2.2   m aff  LL
 3.3 2.3 2.4   m aff  LL
9000
```

```
Program LIPED  Version for IBM PC   March 1987    J. Ott
---------------------------------------------------------

 PROBLEM  1    XLA Linkage Study
 **********

 PEDIGREE  1   Fox/Berryman
 -----------
                   5 PERSONS

 LOCUS  0   XLA       VS.      LOCUS  1   S21
 ------------------------------------------
 Mutation rate at current main locus =   .000010000000

 GENE FREQUENCIES FOR  0   XLA     .0001   .9999
 GENE FREQUENCIES FOR  1   S21     .6500   .3500

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000        -4.71629       .00000
   .0000   .0000        -4.71629       .00000
   .0010   .0010        -4.71629       .00000
   .0100   .0100        -4.71629       .00000
   .0200   .0200        -4.71629       .00000
   .0300   .0300        -4.71629       .00000
   .0400   .0400        -4.71629       .00000
   .0500   .0500        -4.71629       .00000
   .0600   .0600        -4.71629       .00000
   .0700   .0700        -4.71629       .00000
   .0800   .0800        -4.71629       .00000
   .0900   .0900        -4.71629       .00000
   .1000   .1000        -4.71629       .00000
   .1500   .1500        -4.71629       .00000
   .2000   .2000        -4.71629       .00000
   .2500   .2500        -4.71629       .00000
   .3000   .3000        -4.71629       .00000
   .3500   .3500        -4.71629       .00000
   .4000   .4000        -4.71629       .00000
   .4500   .4500        -4.71629       .00000


 PEDIGREE  2   Parry
 -----------
                   5 PERSONS

 LOCUS  0   XLA       VS.      LOCUS  1   S21
 ------------------------------------------
 Mutation rate at current main locus =   .000010000000

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000        -4.97631       .00000
   .0000   .0000        -4.97631       .00000
```

.0010	.0010	-4.97631	.00000
.0100	.0100	-4.97631	.00000
.0200	.0200	-4.97631	.00000
.0300	.0300	-4.97631	.00000
.0400	.0400	-4.97631	.00000
.0500	.0500	-4.97631	.00000
.0600	.0600	-4.97631	.00000
.0700	.0700	-4.97631	.00000
.0800	.0800	-4.97631	.00000
.0900	.0900	-4.97631	.00000
.1000	.1000	-4.97631	.00000
.1500	.1500	-4.97631	.00000
.2000	.2000	-4.97631	.00000
.2500	.2500	-4.97631	.00000
.3000	.3000	-4.97631	.00000
.3500	.3500	-4.97631	.00000
.4000	.4000	-4.97631	.00000
.4500	.4500	-4.97631	.00000

PEDIGREE 3 Rushin/Graham/Golder

8 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.57575	.00000
.0000	.0000	-5.99298	.58276
.0010	.0010	-5.99456	.58118
.0100	.0100	-6.00879	.56696
.0200	.0200	-6.02461	.55114
.0300	.0300	-6.04044	.53531
.0400	.0400	-6.05628	.51947
.0500	.0500	-6.07211	.50363
.0600	.0600	-6.08795	.48780
.0700	.0700	-6.10377	.47198
.0800	.0800	-6.11957	.45618
.0900	.0900	-6.13534	.44041
.1000	.1000	-6.15107	.42468
.1500	.1500	-6.22879	.34695
.2000	.2000	-6.30379	.27196
.2500	.2500	-6.37406	.20168
.3000	.3000	-6.43719	.13856
.3500	.3500	-6.49060	.08515
.4000	.4000	-6.53207	.04368
.4500	.4500	-6.56036	.01539

PEDIGREE 4 Barton

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-4.97631	.00000
.0000	.0000	-4.97631	.00000
.0010	.0010	-4.97631	.00000
.0100	.0100	-4.97631	.00000
.0200	.0200	-4.97631	.00000
.0300	.0300	-4.97631	.00000
.0400	.0400	-4.97631	.00000
.0500	.0500	-4.97631	.00000
.0600	.0600	-4.97631	.00000
.0700	.0700	-4.97631	.00000
.0800	.0800	-4.97631	.00000
.0900	.0900	-4.97631	.00000
.1000	.1000	-4.97631	.00000
.1500	.1500	-4.97631	.00000
.2000	.2000	-4.97631	.00000
.2500	.2500	-4.97631	.00000
.3000	.3000	-4.97631	.00000
.3500	.3500	-4.97631	.00000
.4000	.4000	-4.97631	.00000
.4500	.4500	-4.97631	.00000

PEDIGREE 5 Williams

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-4.67528	.00000
.0000	.0000	-4.67528	.00000
.0010	.0010	-4.67528	.00000
.0100	.0100	-4.67528	.00000
.0200	.0200	-4.67528	.00000
.0300	.0300	-4.67528	.00000
.0400	.0400	-4.67528	.00000
.0500	.0500	-4.67528	.00000
.0600	.0600	-4.67528	.00000
.0700	.0700	-4.67528	.00000
.0800	.0800	-4.67528	.00000
.0900	.0900	-4.67528	.00000
.1000	.1000	-4.67528	.00000
.1500	.1500	-4.67528	.00000

.2000	.2000	-4.67528	.00000
.2500	.2500	-4.67528	.00000
.3000	.3000	-4.67528	.00000
.3500	.3500	-4.67528	.00000
.4000	.4000	-4.67528	.00000
.4500	.4500	-4.67528	.00000

PEDIGREE 6 Patterson/Williamson/Carse

11 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-7.23847	.00000
.0000	.0000	-6.63646	.60201
.0010	.0010	-6.63776	.60071
.0100	.0100	-6.64955	.58892
.0200	.0200	-6.66278	.57569
.0300	.0300	-6.67613	.56234
.0400	.0400	-6.68961	.54886
.0500	.0500	-6.70322	.53525
.0600	.0600	-6.71696	.52151
.0700	.0700	-6.73082	.50765
.0800	.0800	-6.74480	.49366
.0900	.0900	-6.75891	.47956
.1000	.1000	-6.77313	.46534
.1500	.1500	-6.84581	.39266
.2000	.2000	-6.92044	.31803
.2500	.2500	-6.99546	.24301
.3000	.3000	-7.06823	.17024
.3500	.3500	-7.13468	.10379
.4000	.4000	-7.18926	.04921
.4500	.4500	-7.22563	.01284

PEDIGREE 7 King/Pomfrett

12 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.61601	.00000
.0000	.0000	-6.03439	.58162
.0010	.0010	-6.03523	.58078
.0100	.0100	-6.04283	.57319
.0200	.0200	-6.05135	.56467
.0300	.0300	-6.05995	.55606
.0400	.0400	-6.06865	.54737

.0500	.0500	-6.07743	.53858
.0600	.0600	-6.08631	.52971
.0700	.0700	-6.09528	.52073
.0800	.0800	-6.10435	.51166
.0900	.0900	-6.11352	.50249
.1000	.1000	-6.12280	.49322
.1500	.1500	-6.17080	.44521
.2000	.2000	-6.22181	.39421
.2500	.2500	-6.27621	.33980
.3000	.3000	-6.33446	.28155
.3500	.3500	-6.39705	.21897
.4000	.4000	-6.46450	.15151
.4500	.4500	-6.53735	.07867

PEDIGREE 8 Kirsopp

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.24516	.00000
.0000	.0000	-4.94414	.30102
.0010	.0010	-4.94500	.30015
.0100	.0100	-4.95282	.29234
.0200	.0200	-4.96150	.28365
.0300	.0300	-4.97018	.27498
.0400	.0400	-4.97884	.26632
.0500	.0500	-4.98749	.25767
.0600	.0600	-4.99611	.24904
.0700	.0700	-5.00471	.24044
.0800	.0800	-5.01329	.23187
.0900	.0900	-5.02182	.22333
.1000	.1000	-5.03032	.21484
.1500	.1500	-5.07198	.17318
.2000	.2000	-5.11162	.13353
.2500	.2500	-5.14825	.09691
.3000	.3000	-5.18070	.06446
.3500	.3500	-5.20773	.03743
.4000	.4000	-5.22812	.01703
.4500	.4500	-5.24083	.00432

PEDIGREE 9 Cox

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE

.5000	.5000	-5.24516	.00000
.0000	.0000	-4.94414	.30102
.0010	.0010	-4.94500	.30015
.0100	.0100	-4.95282	.29234
.0200	.0200	-4.96150	.28365
.0300	.0300	-4.97018	.27498
.0400	.0400	-4.97884	.26632
.0500	.0500	-4.98749	.25767
.0600	.0600	-4.99611	.24904
.0700	.0700	-5.00471	.24044
.0800	.0800	-5.01329	.23187
.0900	.0900	-5.02182	.22333
.1000	.1000	-5.03032	.21484
.1500	.1500	-5.07198	.17318
.2000	.2000	-5.11162	.13353
.2500	.2500	-5.14825	.09691
.3000	.3000	-5.18070	.06446
.3500	.3500	-5.20773	.03743
.4000	.4000	-5.22812	.01703
.4500	.4500	-5.24083	.00432

PEDIGREE 10 Lucken/Gach

---

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.57425	.00000
.0000	.0000	-5.84731	.72694
.0010	.0010	-5.84892	.72533
.0100	.0100	-5.86342	.71083
.0200	.0200	-5.87963	.69463
.0300	.0300	-5.89592	.67833
.0400	.0400	-5.91230	.66196
.0500	.0500	-5.92876	.64549
.0600	.0600	-5.94531	.62894
.0700	.0700	-5.96193	.61232
.0800	.0800	-5.97864	.59561
.0900	.0900	-5.99542	.57883
.1000	.1000	-6.01227	.56198
.1500	.1500	-6.09746	.47679
.2000	.2000	-6.18360	.39065
.2500	.2500	-6.26943	.30482
.3000	.3000	-6.35283	.22142
.3500	.3500	-6.43049	.14376
.4000	.4000	-6.49751	.07674
.4500	.4500	-6.54763	.02662

PEDIGREE 11 Gerrard
---

6 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.44928	.00000
.0000	.0000	-5.54620	.90308
.0010	.0010	-5.54794	.90134
.0100	.0100	-5.56366	.88562
.0200	.0200	-5.58130	.86798
.0300	.0300	-5.59911	.85016
.0400	.0400	-5.61711	.83216
.0500	.0500	-5.63530	.81398
.0600	.0600	-5.65368	.79560
.0700	.0700	-5.67225	.77702
.0800	.0800	-5.69102	.75825
.0900	.0900	-5.70999	.73929
.1000	.1000	-5.72916	.72011
.1500	.1500	-5.82810	.62118
.2000	.2000	-5.93214	.51713
.2500	.2500	-6.04062	.40866
.3000	.3000	-6.15139	.29789
.3500	.3500	-6.25948	.18979
.4000	.4000	-6.35530	.09398
.4500	.4500	-6.42393	.02535

PEDIGREE 12 Chaperon
---

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.63640	.00000
.0000	.0000	-6.33538	.30102
.0010	.0010	-6.33582	.30058
.0100	.0100	-6.33975	.29665
.0200	.0200	-6.34415	.29224
.0300	.0300	-6.34861	.28779
.0400	.0400	-6.35311	.28329
.0500	.0500	-6.35766	.27874
.0600	.0600	-6.36225	.27415
.0700	.0700	-6.36690	.26950
.0800	.0800	-6.37159	.26481
.0900	.0900	-6.37634	.26006
.1000	.1000	-6.38114	.25526
.1500	.1500	-6.40596	.23044

.2000	.2000	-6.43229	.20411
.2500	.2500	-6.46032	.17608
.3000	.3000	-6.49028	.14612
.3500	.3500	-6.52246	.11394
.4000	.4000	-6.55722	.07918
.4500	.4500	-6.59501	.04139

PEDIGREE 13 Gatte

7 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.30317	.00000
.0000	.0000	-6.00217	.30100
.0010	.0010	-6.00304	.30013
.0100	.0100	-6.01086	.29231
.0200	.0200	-6.01954	.28363
.0300	.0300	-6.02821	.27496
.0400	.0400	-6.03687	.26630
.0500	.0500	-6.04552	.25765
.0600	.0600	-6.05415	.24902
.0700	.0700	-6.06275	.24042
.0800	.0800	-6.07132	.23185
.0900	.0900	-6.07986	.22331
.1000	.1000	-6.08835	.21482
.1500	.1500	-6.13000	.17316
.2000	.2000	-6.16965	.13352
.2500	.2500	-6.20627	.09690
.3000	.3000	-6.23872	.06445
.3500	.3500	-6.26575	.03742
.4000	.4000	-6.28614	.01703
.4500	.4500	-6.29885	.00432

PEDIGREE 14 Joliton

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.60421	.00000
.0000	.0000	-6.30324	.30098
.0010	.0010	-6.30411	.30011
.0100	.0100	-6.31192	.29229
.0200	.0200	-6.32060	.28361
.0300	.0300	-6.32928	.27494
.0400	.0400	-6.33794	.26628

.0500	.0500	-6.34658	.25763
.0600	.0600	-6.35521	.24900
.0700	.0700	-6.36381	.24040
.0800	.0800	-6.37238	.23183
.0900	.0900	-6.38092	.22330
.1000	.1000	-6.38941	.21480
.1500	.1500	-6.43106	.17315
.2000	.2000	-6.47070	.13351
.2500	.2500	-6.50733	.09689
.3000	.3000	-6.53977	.06444
.3500	.3500	-6.56680	.03742
.4000	.4000	-6.58719	.01703
.4500	.4500	-6.59989	.00432

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	4.90144
.0010	.0010	4.89047
.0100	.0100	4.79144
.0200	.0200	4.68090
.0300	.0300	4.56986
.0400	.0400	4.45831
.0500	.0500	4.34629
.0600	.0600	4.23382
.0700	.0700	4.12091
.0800	.0800	4.00760
.0900	.0900	3.89391
.1000	.1000	3.77988
.1500	.1500	3.20590
.2000	.2000	2.63019
.2500	.2500	2.06166
.3000	.3000	1.51357
.3500	.3500	1.00509
.4000	.4000	.56242
.4500	.4500	.21753

```
 1 0010 0.00001      XLA Linkage Study  (pXG12)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0200 0200
 0300 0300
 0400 0400
 0500 0500
 0600 0600
 0700 0700
 0800 0800
 0900 0900
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
XLA    A   a aff n.a
    0001    9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
XG12   U   L  UU  UL  LL
    4000    6000
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
   5   0Fox/Berryman(family 11)
 1.1           m n.a  UU
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m aff  LL
7000
   5   0Parry(family 10)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  UU
 2.2 1.1 1.2   m aff  UU
 2.3 1.1 1.2   m aff  UU
7000
   5   0Rushin/Graham/Golder(family 3)
 1.1           m n.a  UU
 1.2           f n.a  UL
```

```
 2.1 1.1 1.2   m n.a  LL
 2.2 1.1 1.2   m aff  UU
 2.3 1.1 1.2   m n.a  LL
7000
   5   OBarton(family 5)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m n.a  UU
 2.3 1.1 1.2   m aff  LL
7000
   4   OWilliams(family 6)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  LL
7000
  11   OPatterson/Williamson/Carse(family 7)
 1.1           m n.a
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  LL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  LL
 2.5 1.1 1.2   m n.a  UU
 3.1 2.1 2.2   m aff  LL
 3.2 2.1 2.2   m n.a  LL
 3.3 2.3 2.4   m aff  LL
 3.4 2.3 2.4   m aff  LL
7000
  12   OKing/Pomfrett(family 1)
 1.1           m n.a  UU
 1.2           f n.a  LL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3 1.1 1.2   m n.a  LL
 2.4           m n.a
 2.5 1.1 1.2   f n.a  UL
 2.6 1.1 1.2   f n.a  UL
 2.7 1.1 1.2   f n.a  UL
 2.8 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m aff  LL
 3.2 2.4 2.5   m aff  LL
7000
   4   OKirsopp(family 4)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  UU
 2.2 1.1 1.2   m aff  UU
   4   OCox(family 8)
 1.1           m n.a
 1.2           f n.a  LL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  LL
7000
   9   OChaperon(French 1)
 1.1           m n.a  UU
```

```
 1.2                f n.a  UL
 2.1 1.1 1.2        m n.a  UU
 2.2                m n.a
 2.3 1.1 1.2        f n.a  UL
 2.4 1.1 1.2        m aff  LL
 2.5 1.1 1.2        m n.a  UU
 2.6 1.1 1.2        m aff  LL
 3.1 2.2 2.3        m aff  LL
7000
   7   0Gatte(French 2)
 1.1                m n.a  UU
 1.2                f n.a  LL
 2.1                m n.a
 2.2 1.1 1.2        f n.a  UL
 2.3 1.1 1.2        m aff  LL
 3.1 2.1 2.2        m aff  LL
 3.2 2.1 2.2        m n.a  UU
7000
   9   0Joliton(French 3)
 1.1                m n.a  UU
 1.2                f n.a  LL
 2.1                m n.a
 2.2 1.1 1.2        f n.a  UL
 2.3                m n.a
 2.4 1.1 1.2        f n.a  UL
 3.1 2.1 2.2        m n.a  UU
 3.2 2.1 2.2        m aff  LL
 3.3 2.3 2.4        m aff  LL
9000
```

```
Program LIPED  Version for IBM PC   March 1987    J. Ott
-------------------------------------------------------------

 PROBLEM  1    XLA Linkage Study
 **********

 PEDIGREE  1   Fox/Berryman
 -----------
                   5 PERSONS

 LOCUS  0   XLA       VS.      LOCUS  1   XG12
 ------------------------------------------
 Mutation rate at current main locus =    .000010000000

 GENE FREQUENCIES FOR  0   XLA     .0001    .9999
 GENE FREQUENCIES FOR  1   XG12    .4000    .6000

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000        -5.47379       .00000
   .0000   .0000        -5.23971       .23408
   .0010   .0010        -5.24007       .23372
   .0100   .0100        -5.24335       .23045
   .0200   .0200        -5.24701       .22678
   .0300   .0300        -5.25071       .22308
   .0400   .0400        -5.25444       .21936
   .0500   .0500        -5.25820       .21560
   .0600   .0600        -5.26199       .21180
   .0700   .0700        -5.26581       .20798
   .0800   .0800        -5.26967       .20412
   .0900   .0900        -5.27357       .20022
   .1000   .1000        -5.27750       .19629
   .1500   .1500        -5.29770       .17609
   .2000   .2000        -5.31889       .15490
   .2500   .2500        -5.34117       .13262
   .3000   .3000        -5.36465       .10914
   .3500   .3500        -5.38947       .08432
   .4000   .4000        -5.41580       .05799
   .4500   .4500        -5.44383       .02996

 PEDIGREE  2   Parry
 -----------
                   5 PERSONS

 LOCUS  0   XLA       VS.      LOCUS  1   XG12
 ------------------------------------------
 Mutation rate at current main locus =    .000010000000

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000        -5.82398       .00000
   .0000   .0000        -5.22194       .60205
```

.0010	.0010	-5.22324	.60074
.0100	.0100	-5.23503	.58895
.0200	.0200	-5.24825	.57573
.0300	.0300	-5.26161	.56238
.0400	.0400	-5.27509	.54889
.0500	.0500	-5.28870	.53528
.0600	.0600	-5.30244	.52154
.0700	.0700	-5.31630	.50768
.0800	.0800	-5.33029	.49370
.0900	.0900	-5.34439	.47959
.1000	.1000	-5.35861	.46537
.1500	.1500	-5.43130	.39269
.2000	.2000	-5.50593	.31805
.2500	.2500	-5.58095	.24303
.3000	.3000	-5.65373	.17026
.3500	.3500	-5.72018	.10380
.4000	.4000	-5.77477	.04922
.4500	.4500	-5.81115	.01284

PEDIGREE 3 Rushin/Graham/Golder

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.07583	.00000
.0000	.0000	-5.57850	.49733
.0010	.0010	-5.57968	.49615
.0100	.0100	-5.59038	.48544
.0200	.0200	-5.60235	.47347
.0300	.0300	-5.61441	.46142
.0400	.0400	-5.62654	.44929
.0500	.0500	-5.63875	.43708
.0600	.0600	-5.65103	.42479
.0700	.0700	-5.66338	.41244
.0800	.0800	-5.67580	.40002
.0900	.0900	-5.68828	.38755
.1000	.1000	-5.70081	.37501
.1500	.1500	-5.76407	.31176
.2000	.2000	-5.82750	.24833
.2500	.2500	-5.88951	.18631
.3000	.3000	-5.94779	.12803
.3500	.3500	-5.99924	.07659
.4000	.4000	-6.04011	.03572
.4500	.4500	-6.06662	.00921

PEDIGREE 4 Barton

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.82399	.00000
.0000	.0000	-5.22194	.60205
.0010	.0010	-5.22324	.60075
.0100	.0100	-5.23503	.58896
.0200	.0200	-5.24826	.57573
.0300	.0300	-5.26161	.56238
.0400	.0400	-5.27509	.54890
.0500	.0500	-5.28870	.53528
.0600	.0600	-5.30244	.52155
.0700	.0700	-5.31630	.50768
.0800	.0800	-5.33029	.49370
.0900	.0900	-5.34439	.47960
.1000	.1000	-5.35862	.46537
.1500	.1500	-5.43130	.39269
.2000	.2000	-5.50593	.31806
.2500	.2500	-5.58096	.24303
.3000	.3000	-5.65373	.17026
.3500	.3500	-5.72019	.10380
.4000	.4000	-5.77477	.04922
.4500	.4500	-5.81115	.01284

PEDIGREE 5 Williams

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.22193	.00000
.0000	.0000	-4.92091	.30102
.0010	.0010	-4.92177	.30015
.0100	.0100	-4.92959	.29234
.0200	.0200	-4.93827	.28365
.0300	.0300	-4.94695	.27498
.0400	.0400	-4.95561	.26632
.0500	.0500	-4.96426	.25767
.0600	.0600	-4.97288	.24904
.0700	.0700	-4.98148	.24044
.0800	.0800	-4.99006	.23187
.0900	.0900	-4.99859	.22333
.1000	.1000	-5.00709	.21484
.1500	.1500	-5.04875	.17318

.2000	.2000	-5.08839	.13353
.2500	.2500	-5.12502	.09691
.3000	.3000	-5.15747	.06446
.3500	.3500	-5.18450	.03743
.4000	.4000	-5.20489	.01703
.4500	.4500	-5.21760	.00432

PEDIGREE 6 Patterson/Williamson/Carse

11 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-7.25000	.00000
.0000	.0000	-6.64799	.60201
.0010	.0010	-6.64929	.60071
.0100	.0100	-6.66108	.58892
.0200	.0200	-6.67431	.57569
.0300	.0300	-6.68766	.56234
.0400	.0400	-6.70114	.54886
.0500	.0500	-6.71475	.53525
.0600	.0600	-6.72849	.52151
.0700	.0700	-6.74235	.50765
.0800	.0800	-6.75634	.49366
.0900	.0900	-6.77044	.47956
.1000	.1000	-6.78466	.46534
.1500	.1500	-6.85734	.39266
.2000	.2000	-6.93197	.31803
.2500	.2500	-7.00699	.24301
.3000	.3000	-7.07976	.17024
.3500	.3500	-7.14621	.10379
.4000	.4000	-7.20079	.04921
.4500	.4500	-7.23716	.01284

PEDIGREE 7 King/Pomfrett

12 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.64794	.00000
.0000	.0000	-6.04593	.60201
.0010	.0010	-6.04680	.60114
.0100	.0100	-6.05466	.59328
.0200	.0200	-6.06347	.58447
.0300	.0300	-6.07238	.57556
.0400	.0400	-6.08138	.56656

.0500	.0500	-6.09048	.55746
.0600	.0600	-6.09967	.54827
.0700	.0700	-6.10896	.53898
.0800	.0800	-6.11835	.52959
.0900	.0900	-6.12784	.52010
.1000	.1000	-6.13744	.51050
.1500	.1500	-6.18708	.46086
.2000	.2000	-6.23974	.40820
.2500	.2500	-6.29579	.35215
.3000	.3000	-6.35571	.29223
.3500	.3500	-6.42008	.22786
.4000	.4000	-6.48959	.15835
.4500	.4500	-6.56516	.08278

PEDIGREE 8 Kirsopp

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.22193	.00000
.0000	.0000	-4.92091	.30102
.0010	.0010	-4.92177	.30015
.0100	.0100	-4.92959	.29234
.0200	.0200	-4.93827	.28365
.0300	.0300	-4.94695	.27498
.0400	.0400	-4.95561	.26632
.0500	.0500	-4.96426	.25767
.0600	.0600	-4.97288	.24904
.0700	.0700	-4.98148	.24044
.0800	.0800	-4.99006	.23187
.0900	.0900	-4.99859	.22333
.1000	.1000	-5.00709	.21484
.1500	.1500	-5.04875	.17318
.2000	.2000	-5.08839	.13353
.2500	.2500	-5.12502	.09691
.3000	.3000	-5.15747	.06446
.3500	.3500	-5.18450	.03743
.4000	.4000	-5.20489	.01703
.4500	.4500	-5.21760	.00432

PEDIGREE 9 Cox

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE

.5000	.5000	-4.74480	.00000
.0000	.0000	-4.74480	.00000
.0010	.0010	-4.74480	.00000
.0100	.0100	-4.74480	.00000
.0200	.0200	-4.74480	.00000
.0300	.0300	-4.74480	.00000
.0400	.0400	-4.74480	.00000
.0500	.0500	-4.74480	.00000
.0600	.0600	-4.74480	.00000
.0700	.0700	-4.74480	.00000
.0800	.0800	-4.74480	.00000
.0900	.0900	-4.74480	.00000
.1000	.1000	-4.74480	.00000
.1500	.1500	-4.74480	.00000
.2000	.2000	-4.74480	.00000
.2500	.2500	-4.74480	.00000
.3000	.3000	-4.74480	.00000
.3500	.3500	-4.74480	.00000
.4000	.4000	-4.74480	.00000
.4500	.4500	-4.74480	.00000

PEDIGREE 10 Chaperon

---

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-8.02814	.00000
.0000	.0000	-6.52302	1.50512
.0010	.0010	-6.52563	1.50251
.0100	.0100	-6.54921	1.47893
.0200	.0200	-6.57566	1.45248
.0300	.0300	-6.60239	1.42575
.0400	.0400	-6.62939	1.39875
.0500	.0500	-6.65668	1.37146
.0600	.0600	-6.68425	1.34389
.0700	.0700	-6.71212	1.31602
.0800	.0800	-6.74029	1.28785
.0900	.0900	-6.76877	1.25937
.1000	.1000	-6.79756	1.23059
.1500	.1500	-6.94643	1.08171
.2000	.2000	-7.10405	.92409
.2500	.2500	-7.27086	.75728
.3000	.3000	-7.44619	.58195
.3500	.3500	-7.62630	.40184
.4000	.4000	-7.80037	.22777
.4500	.4500	-7.94516	.08298

PEDIGREE 11 Gatte

7 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.34689	.00000
.0000	.0000	-5.74485	.60204
.0010	.0010	-5.74572	.60117
.0100	.0100	-5.75358	.59331
.0200	.0200	-5.76240	.58449
.0300	.0300	-5.77131	.57558
.0400	.0400	-5.78031	.56658
.0500	.0500	-5.78940	.55748
.0600	.0600	-5.79859	.54829
.0700	.0700	-5.80788	.53900
.0800	.0800	-5.81727	.52961
.0900	.0900	-5.82677	.52012
.1000	.1000	-5.83636	.51052
.1500	.1500	-5.88601	.46088
.2000	.2000	-5.93867	.40822
.2500	.2500	-5.99472	.35216
.3000	.3000	-6.05465	.29224
.3500	.3500	-6.11901	.22787
.4000	.4000	-6.18853	.15835
.4500	.4500	-6.26411	.08278

PEDIGREE 12 Joliton

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.94896	.00000
.0000	.0000	-6.04592	.90304
.0010	.0010	-6.04723	.90173
.0100	.0100	-6.05902	.88994
.0200	.0200	-6.07224	.87672
.0300	.0300	-6.08561	.86335
.0400	.0400	-6.09911	.84985
.0500	.0500	-6.11275	.83621
.0600	.0600	-6.12654	.82242
.0700	.0700	-6.14047	.80849
.0800	.0800	-6.15456	.79440
.0900	.0900	-6.16880	.78017
.1000	.1000	-6.18319	.76577
.1500	.1500	-6.25766	.69130

.2000	.2000	-6.33664	.61232
.2500	.2500	-6.42073	.52823
.3000	.3000	-6.51061	.43835
.3500	.3500	-6.60716	.34180
.4000	.4000	-6.71144	.23752
.4500	.4500	-6.82480	.12417

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	6.75176
.0010	.0010	6.73892
.0100	.0100	6.62285
.0200	.0200	6.49286
.0300	.0300	6.36180
.0400	.0400	6.22966
.0500	.0500	6.09645
.0600	.0600	5.96216
.0700	.0700	5.82681
.0800	.0800	5.69040
.0900	.0900	5.55294
.1000	.1000	5.41444
.1500	.1500	4.70699
.2000	.2000	3.97727
.2500	.2500	3.23165
.3000	.3000	2.48161
.3500	.3500	1.74653
.4000	.4000	1.05741
.4500	.4500	.45902

```
 1 0010 0.00001     XLA Linkage Study  (19.2)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0200 0200
 0300 0300
 0400 0400
 0500 0500
 0600 0600
 1000 1000
 1400 1400
 1500 1500
 1600 1600
 1900 1900
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
XLA    A   a aff n.a
    0001    9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
19-2   U   L  UU  UL  LL
    3800    6200
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
   5   0Fox/Berryman(family 11)
 1.1           m n.a
 1.2           f n.a  UU
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UU
 3.1 2.1 2.2   m aff  UU
7000
   5   0Parry(family 10)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  LL
 2.3 1.1 1.2   m aff  LL
7000
   9   0Rushin/Graham/Golder(family 3)
 1.1           m n.a
 1.2           f n.a  LL
```

```
 2.1 1.1 1.2   m n.a  LL
 2.2 1.1 1.2   m aff  LL
 2.3 1.1 1.2   m n.a  LL
 2.4 1.1 1.2   f n.a  UL
 2.5           m n.a
 3.1 2.4 2.5   m aff
 3.2 2.4 2.5   m aff
7000
   5   OBarton(family 5)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m n.a  UU
 2.3 1.1 1.2   m aff  LL
7000
   4   OWilliams(family 6)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  UU
 2.2 1.1 1.2   m aff  UU
7000
  11   OPatterson/Williamson/Carse(family 7)
 1.1           m n.a
 1.2           f n.a  LL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  LL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  LL
 2.5 1.1 1.2   m n.a  LL
 3.1 2.1 2.2   m aff
 3.2 2.1 2.2   m n.a
 3.3 2.3 2.4   m aff
 3.4 2.3 2.4   m aff
7000
  12   OKing/Pomfrett(family 1)
 1.1           m n.a
 1.2           f n.a
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3 1.1 1.2   m n.a  UU
 2.4           m n.a
 2.5 1.1 1.2   f n.a  UL
 2.6 1.1 1.2   f n.a  UL
 2.7 1.1 1.2   f n.a  UL
 2.8 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m aff  UU
 3.2 2.4 2.5   m aff  UU
7000
   4   OKirsopp(family 4)
 1.1           m n.a
 1.2           f n.a  LL
 2.1 1.1 1.2   m aff
 2.2 1.1 1.2   m aff
7000
   4   OCox(family 8)
 1.1           m n.a
 1.2           f n.a  LL
```

```
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  LL
7000
   9   OLucken/Gach(family 2)
 1.1           m n.a
 1.2           f n.a
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m n.a  LL
 3.2 2.1 2.2   m aff  UU
 3.3 2.3 2.4   m aff  UU
7000
   6   OGerrard(family 12)
 1.1           m n.a
 1.2           f n.a  LL
 2.1 1.1 1.2   m n.a
 2.2 1.1 1.2   m aff
 2.3 1.1 1.2   m aff
 2.4 1.1 1.2   m aff
7000
   5   OMann(family 14)
 1.1           m n.a
 1.2           f n.a  UL
 2.1 1.1 1.2   m aff  LL
 2.2 1.1 1.2   m aff  UU
 2.3 1.1 1.2   m aff  UU
7000
   9   OChaperon(French 1)
 1.1           m n.a  UU
 1.2           f n.a
 2.1 1.1 1.2   m n.a  LL
 2.2           m n.a
 2.3 1.1 1.2   f n.a  UL
 2.4 1.1 1.2   m aff  LL
 2.5 1.1 1.2   m n.a  LL
 2.6 1.1 1.2   m aff  LL
 3.1 2.2 2.3   m aff  LL
7000
   7   OGatte(French 2)
 1.1           m n.a  LL
 1.2           f n.a  UU
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3 1.1 1.2   m aff  UU
 3.1 2.1 2.2   m aff  UU
 3.2 2.1 2.2   m n.a  LL
7000
   9   OJoliton(French 3)
 1.1           m n.a  LL
 1.2           f n.a  UL
 2.1           m n.a
 2.2 1.1 1.2   f n.a  UL
 2.3           m n.a
 2.4 1.1 1.2   f n.a  UL
 3.1 2.1 2.2   m n.a  LL
```

```
 3.2 2.1 2.2    m aff  UU
 3.3 2.3 2.4    m aff  UU
9000
```

Program LIPED Version for IBM PC March 1987 J. Ott

PROBLEM 1 XLA Linkage Study
****

PEDIGREE 1 Fox/Berryman
---

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

GENE FREQUENCIES FOR 0 XLA .0001 .9999
GENE FREQUENCIES FOR 1 19-2 .3800 .6200

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.41568	.00000
.0000	.0000	-5.41568	.00000
.0010	.0010	-5.41568	.00000
.0100	.0100	-5.41568	.00000
.0200	.0200	-5.41568	.00000
.0300	.0300	-5.41568	.00000
.0400	.0400	-5.41568	.00000
.0500	.0500	-5.41568	.00000
.0600	.0600	-5.41568	.00000
.1000	.1000	-5.41568	.00000
.1400	.1400	-5.41568	.00000
.1500	.1500	-5.41568	.00000
.1600	.1600	-5.41568	.00000
.1900	.1900	-5.41568	.00000
.2000	.2000	-5.41568	.00000
.2500	.2500	-5.41568	.00000
.3000	.3000	-5.41568	.00000
.3500	.3500	-5.41568	.00000
.4000	.4000	-5.41568	.00000
.4500	.4500	-5.41568	.00000

PEDIGREE 2 Parry
---

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.83202	.00000
.0000	.0000	-5.22997	.60205

.0010	.0010	-5.23128	.60074
.0100	.0100	-5.24307	.58895
.0200	.0200	-5.25629	.57573
.0300	.0300	-5.26964	.56238
.0400	.0400	-5.28313	.54889
.0500	.0500	-5.29674	.53528
.0600	.0600	-5.31047	.52154
.1000	.1000	-5.36665	.46537
.1400	.1400	-5.42460	.40741
.1500	.1500	-5.43933	.39269
.1600	.1600	-5.45414	.37788
.1900	.1900	-5.49894	.33308
.2000	.2000	-5.51396	.31805
.2500	.2500	-5.58899	.24303
.3000	.3000	-5.66176	.17026
.3500	.3500	-5.72822	.10380
.4000	.4000	-5.78280	.04922
.4500	.4500	-5.81918	.01284

PEDIGREE 3 Rushin/Graham/Golder

---

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.34070	.00000
.0000	.0000	-6.34070	.00000
.0010	.0010	-6.34070	.00000
.0100	.0100	-6.34070	.00000
.0200	.0200	-6.34070	.00000
.0300	.0300	-6.34070	.00000
.0400	.0400	-6.34070	.00000
.0500	.0500	-6.34070	.00000
.0600	.0600	-6.34070	.00000
.1000	.1000	-6.34070	.00000
.1400	.1400	-6.34070	.00000
.1500	.1500	-6.34070	.00000
.1600	.1600	-6.34070	.00000
.1900	.1900	-6.34070	.00000
.2000	.2000	-6.34070	.00000
.2500	.2500	-6.34070	.00000
.3000	.3000	-6.34070	.00000
.3500	.3500	-6.34070	.00000
.4000	.4000	-6.34070	.00000
.4500	.4500	-6.34070	.00000

PEDIGREE 4 Barton

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.83203	.00000
.0000	.0000	-5.22998	.60205
.0010	.0010	-5.23128	.60075
.0100	.0100	-5.24307	.58896
.0200	.0200	-5.25629	.57573
.0300	.0300	-5.26965	.56238
.0400	.0400	-5.28313	.54890
.0500	.0500	-5.29674	.53528
.0600	.0600	-5.31048	.52155
.1000	.1000	-5.36665	.46537
.1400	.1400	-5.42461	.40742
.1500	.1500	-5.43934	.39269
.1600	.1600	-5.45414	.37788
.1900	.1900	-5.49895	.33308
.2000	.2000	-5.51397	.31806
.2500	.2500	-5.58899	.24303
.3000	.3000	-5.66177	.17026
.3500	.3500	-5.72822	.10380
.4000	.4000	-5.78281	.04922
.4500	.4500	-5.81919	.01284

PEDIGREE 5 Williams

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.22996	.00000
.0000	.0000	-4.92894	.30102
.0010	.0010	-4.92981	.30015
.0100	.0100	-4.93763	.29234
.0200	.0200	-4.94631	.28365
.0300	.0300	-4.95498	.27498
.0400	.0400	-4.96364	.26632
.0500	.0500	-4.97229	.25767
.0600	.0600	-4.98092	.24904
.1000	.1000	-5.01513	.21484
.1400	.1400	-5.04858	.18138
.1500	.1500	-5.05678	.17318
.1600	.1600	-5.06490	.16506
.1900	.1900	-5.08871	.14126

.2000	.2000	-5.09643	.13353
.2500	.2500	-5.13306	.09691
.3000	.3000	-5.16551	.06446
.3500	.3500	-5.19254	.03743
.4000	.4000	-5.21293	.01703
.4500	.4500	-5.22564	.00432

PEDIGREE 6 Patterson/Williamson/Carse

11 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.42913	.00000
.0000	.0000	-6.42913	.00000
.0010	.0010	-6.42913	.00000
.0100	.0100	-6.42913	.00000
.0200	.0200	-6.42913	.00000
.0300	.0300	-6.42913	.00000
.0400	.0400	-6.42913	.00000
.0500	.0500	-6.42913	.00000
.0600	.0600	-6.42913	.00000
.1000	.1000	-6.42913	.00000
.1400	.1400	-6.42913	.00000
.1500	.1500	-6.42913	.00000
.1600	.1600	-6.42913	.00000
.1900	.1900	-6.42913	.00000
.2000	.2000	-6.42913	.00000
.2500	.2500	-6.42913	.00000
.3000	.3000	-6.42913	.00000
.3500	.3500	-6.42913	.00000
.4000	.4000	-6.42913	.00000
.4500	.4500	-6.42913	.00000

PEDIGREE 7 King/Pomfrett

12 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.82002	.00000
.0000	.0000	-6.25233	.56769
.0010	.0010	-6.25311	.56691
.0100	.0100	-6.26018	.55984
.0200	.0200	-6.26812	.55190
.0300	.0300	-6.27617	.54385
.0400	.0400	-6.28432	.53571

.0500	.0500	-6.29257	.52745
.0600	.0600	-6.30092	.51910
.1000	.1000	-6.33546	.48456
.1400	.1400	-6.37189	.44813
.1500	.1500	-6.38132	.43870
.1600	.1600	-6.39088	.42914
.1900	.1900	-6.42037	.39965
.2000	.2000	-6.43049	.38953
.2500	.2500	-6.48336	.33666
.3000	.3000	-6.54038	.27964
.3500	.3500	-6.60203	.21799
.4000	.4000	-6.66885	.15117
.4500	.4500	-6.74136	.07866

PEDIGREE 8 Kirsopp

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-4.71632	.00000
.0000	.0000	-4.71632	.00000
.0010	.0010	-4.71632	.00000
.0100	.0100	-4.71632	.00000
.0200	.0200	-4.71632	.00000
.0300	.0300	-4.71632	.00000
.0400	.0400	-4.71632	.00000
.0500	.0500	-4.71632	.00000
.0600	.0600	-4.71632	.00000
.1000	.1000	-4.71632	.00000
.1400	.1400	-4.71632	.00000
.1500	.1500	-4.71632	.00000
.1600	.1600	-4.71632	.00000
.1900	.1900	-4.71632	.00000
.2000	.2000	-4.71632	.00000
.2500	.2500	-4.71632	.00000
.3000	.3000	-4.71632	.00000
.3500	.3500	-4.71632	.00000
.4000	.4000	-4.71632	.00000
.4500	.4500	-4.71632	.00000

PEDIGREE 9 Cox

4 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-4.71632	.00000
.0000	.0000	-4.71632	.00000
.0010	.0010	-4.71632	.00000
.0100	.0100	-4.71632	.00000
.0200	.0200	-4.71632	.00000
.0300	.0300	-4.71632	.00000
.0400	.0400	-4.71632	.00000
.0500	.0500	-4.71632	.00000
.0600	.0600	-4.71632	.00000
.1000	.1000	-4.71632	.00000
.1400	.1400	-4.71632	.00000
.1500	.1500	-4.71632	.00000
.1600	.1600	-4.71632	.00000
.1900	.1900	-4.71632	.00000
.2000	.2000	-4.71632	.00000
.2500	.2500	-4.71632	.00000
.3000	.3000	-4.71632	.00000
.3500	.3500	-4.71632	.00000
.4000	.4000	-4.71632	.00000
.4500	.4500	-4.71632	.00000

PEDIGREE 10 Lucken/Gach

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.55906	.00000
.0000	.0000	-5.83212	.72694
.0010	.0010	-5.83396	.72510
.0100	.0100	-5.85058	.70848
.0200	.0200	-5.86912	.68994
.0300	.0300	-5.88775	.67131
.0400	.0400	-5.90646	.65260
.0500	.0500	-5.92524	.63381
.0600	.0600	-5.94410	.61495
.1000	.1000	-6.02015	.53891
.1400	.1400	-6.09684	.46222
.1500	.1500	-6.11604	.44302
.1600	.1600	-6.13522	.42384
.1900	.1900	-6.19253	.36652

.2000	.2000	-6.21150	.34756
.2500	.2500	-6.30422	.25484
.3000	.3000	-6.39059	.16847
.3500	.3500	-6.46544	.09362
.4000	.4000	-6.52234	.03672
.4500	.4500	-6.55491	.00415

PEDIGREE 11 Gerrard

6 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.31838	.00000
.0000	.0000	-5.31838	.00000
.0010	.0010	-5.31838	.00000
.0100	.0100	-5.31838	.00000
.0200	.0200	-5.31838	.00000
.0300	.0300	-5.31838	.00000
.0400	.0400	-5.31838	.00000
.0500	.0500	-5.31838	.00000
.0600	.0600	-5.31838	.00000
.1000	.1000	-5.31838	.00000
.1400	.1400	-5.31838	.00000
.1500	.1500	-5.31838	.00000
.1600	.1600	-5.31838	.00000
.1900	.1900	-5.31838	.00000
.2000	.2000	-5.31838	.00000
.2500	.2500	-5.31838	.00000
.3000	.3000	-5.31838	.00000
.3500	.3500	-5.31838	.00000
.4000	.4000	-5.31838	.00000
.4500	.4500	-5.31838	.00000

PEDIGREE 12 Mann

5 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-5.83202	.00000
.0000	.0000	-10.22997	-4.39795
.0010	.0010	-8.22608	-2.39407
.0100	.0100	-7.23390	-1.40188
.0200	.0200	-6.93750	-1.10548
.0300	.0300	-6.76593	-.93392
.0400	.0400	-6.64553	-.81351

.0500	.0500	-6.55319	-.72117
.0600	.0600	-6.47862	-.64660
.1000	.1000	-6.27569	-.44367
.1400	.1400	-6.14931	-.31730
.1500	.1500	-6.12443	-.29241
.1600	.1600	-6.10154	-.26953
.1900	.1900	-6.04271	-.21069
.2000	.2000	-6.02583	-.19381
.2500	.2500	-5.95695	-.12493
.3000	.3000	-5.90774	-.07572
.3500	.3500	-5.87298	-.04096
.4000	.4000	-5.84975	-.01773
.4500	.4500	-5.83638	-.00436

PEDIGREE 13 Chaperon

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.62540	.00000
.0000	.0000	-6.34071	.28469
.0010	.0010	-6.34114	.28426
.0100	.0100	-6.34505	.28035
.0200	.0200	-6.34938	.27602
.0300	.0300	-6.35371	.27169
.0400	.0400	-6.35805	.26736
.0500	.0500	-6.36238	.26302
.0600	.0600	-6.36673	.25867
.1000	.1000	-6.38431	.24109
.1400	.1400	-6.40237	.22303
.1500	.1500	-6.40698	.21842
.1600	.1600	-6.41165	.21376
.1900	.1900	-6.42596	.19944
.2000	.2000	-6.43085	.19455
.2500	.2500	-6.45638	.16902
.3000	.3000	-6.48402	.14138
.3500	.3500	-6.51423	.11117
.4000	.4000	-6.54748	.07792
.4500	.4500	-6.58433	.04107

PEDIGREE 14 Gatte

7 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.55329	.00000
.0000	.0000	-5.95126	.60203
.0010	.0010	-5.95212	.60117
.0100	.0100	-5.95998	.59331
.0200	.0200	-5.96880	.58449
.0300	.0300	-5.97771	.57558
.0400	.0400	-5.98671	.56658
.0500	.0500	-5.99581	.55748
.0600	.0600	-6.00500	.54829
.1000	.1000	-6.04277	.51052
.1400	.1400	-6.08226	.47103
.1500	.1500	-6.09241	.46088
.1600	.1600	-6.10269	.45060
.1900	.1900	-6.13428	.41901
.2000	.2000	-6.14507	.40822
.2500	.2500	-6.20113	.35216
.3000	.3000	-6.26105	.29224
.3500	.3500	-6.32542	.22787
.4000	.4000	-6.39494	.15835
.4500	.4500	-6.47051	.08278

PEDIGREE 15 Joliton

9 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-7.24379	.00000
.0000	.0000	-6.03974	1.20405
.0010	.0010	-6.04191	1.20188
.0100	.0100	-6.06152	1.18227
.0200	.0200	-6.08343	1.16036
.0300	.0300	-6.10546	1.13832
.0400	.0400	-6.12763	1.11616
.0500	.0500	-6.14992	1.09387
.0600	.0600	-6.17233	1.07146
.1000	.1000	-6.26319	.98060
.1400	.1400	-6.35588	.88791
.1500	.1500	-6.37931	.86447
.1600	.1600	-6.40285	.84093
.1900	.1900	-6.47404	.76975

.2000	.2000	-6.49794	.74584
.2500	.2500	-6.61865	.62513
.3000	.3000	-6.74099	.50280
.3500	.3500	-6.86457	.37922
.4000	.4000	-6.98923	.25455
.4500	.4500	-7.11530	.12849

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	.49257
.0010	.0010	2.48688
.0100	.0100	3.39261
.0200	.0200	3.59234
.0300	.0300	3.66657
.0400	.0400	3.68899
.0500	.0500	3.68270
.0600	.0600	3.65800
.1000	.1000	3.45758
.1400	.1400	3.17124
.1500	.1500	3.09163
.1600	.1600	3.00956
.1900	.1900	2.75109
.2000	.2000	2.66153
.2500	.2500	2.19585
.3000	.3000	1.71378
.3500	.3500	1.23395
.4000	.4000	.77645
.4500	.4500	.36077

Appendix 4 INPUT AND OUTPUT FILES OF LIPED FOR THE ANALYSIS OF S21 AND 19.2 DATA IN FAMILY 15

```
 1 0010 0.00001      XLA Linkage Study  (S21)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0200 0200
 0300 0300
 0400 0400
 0500 0500
 0600 0600
 0700 0700
 0800 0800
 0900 0900
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
XLA    A   a aff n.a
    0001    9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
S21    U   L  UU  UL  LL
    6500    3500
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
  14   015
 1.1            m n.a  UU
 1.2            f n.a  UL
 2.1            m n.a
 2.2 1.1 1.2    f n.a  UL
 2.3 1.1 1.2    f n.a  UU
 2.4            m n.a
 2.5 1.1 1.2    m aff
 2.6 1.1 1.2    m aff
 2.7 1.1 1.2    m aff
 3.1 2.1 2.2    m n.a  LL
 3.2 2.1 2.2    m aff
 3.3 2.1 2.2    m aff
 3.4 2.3 2.4    m aff
 3.5 2.3 2.4    m aff
9000
```

```
Program LIPED  Version for IBM PC   March 1987    J. Ott
-------------------------------------------------------

 PROBLEM  1    XLA Linkage Study
 **********

 PEDIGREE  1    15
 -----------
                  14 PERSONS

 LOCUS  0   XLA       VS.      LOCUS  1   S21
 ------------------------------------------
 Mutation rate at current main locus =    .000010000000

 GENE FREQUENCIES FOR  0   XLA     .0001    .9999
 GENE FREQUENCIES FOR  1   S21     .6500    .3500

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000        -8.14157       .00000
   .0000   .0000       -12.53951     -4.39794
   .0010   .0010       -12.46041     -4.31884
   .0100   .0100       -11.22147     -3.07990
   .0200   .0200       -10.63972     -2.49814
   .0300   .0300       -10.29501     -2.15344
   .0400   .0400       -10.05070     -1.90913
   .0500   .0500        -9.86193     -1.72036
   .0600   .0600        -9.70843     -1.56686
   .0700   .0700        -9.57935     -1.43778
   .0800   .0800        -9.46817     -1.32660
   .0900   .0900        -9.37068     -1.22911
   .1000   .1000        -9.28402     -1.14245
   .1500   .1500        -8.95679      -.81522
   .2000   .2000        -8.73329      -.59171
   .2500   .2500        -8.56752      -.42594
   .3000   .3000        -8.43913      -.29755
   .3500   .3500        -8.33743      -.19585
   .4000   .4000        -8.25621      -.11464
   .4500   .4500        -8.19170      -.05012


 SUMMARY
 -------

  R MALE   R FEM.    SUM OF LODS
   .0000   .0000        -4.39794
   .0010   .0010        -4.31884
   .0100   .0100        -3.07990
   .0200   .0200        -2.49814
   .0300   .0300        -2.15344
   .0400   .0400        -1.90913
   .0500   .0500        -1.72036
   .0600   .0600        -1.56686
```

.0700	.0700	-1.43778
.0800	.0800	-1.32660
.0900	.0900	-1.22911
.1000	.1000	-1.14245
.1500	.1500	-.81522
.2000	.2000	-.59171
.2500	.2500	-.42594
.3000	.3000	-.29755
.3500	.3500	-.19585
.4000	.4000	-.11464
.4500	.4500	-.05012

```
 1 0010 0.00001      XLA Linkage Study  (19.2)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0200 0200
 0300 0300
 0400 0400
 0500 0500
 0600 0600
 0700 0700
 0800 0800
 0900 0900
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
XLA     A    a aff n.a
     0001     9999
    A    A 1 0
    A    a 0 1
    a    a 0 1
19.2    U    L  UU  UL  LL
     6500     3500
    U    U 1 0 0
    U    L 0 1 0
    L    L 0 0 1
   14    015
 1.1              m n.a  UU
 1.2              f n.a  LL
 2.1              m n.a
 2.2 1.1 1.2      f n.a  UL
 2.3 1.1 1.2      f n.a  UL
 2.4              m n.a
 2.5 1.1 1.2      m aff
 2.6 1.1 1.2      m aff
 2.7 1.1 1.2      m aff
 3.1 2.1 2.2      m n.a  LL
 3.2 2.1 2.2      m aff
 3.3 2.1 2.2      m aff
 3.4 2.3 2.4      m aff
 3.5 2.3 2.4      m aff  LL
9000
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Program LIPED Version for IBM PC March 1987 J. Ott

PROBLEM 1 XLA Linkage Study
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PEDIGREE 1 15
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14 PERSONS

LOCUS 0 XLA VS. LOCUS 1 19.2

Mutation rate at current main locus = .000010000000

GENE FREQUENCIES FOR 0 XLA .0001 .9999
GENE FREQUENCIES FOR 1 19.2 .6500 .3500

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-8.41042	.00000
.0000	.0000	-12.80836	-4.39794
.0010	.0010	-10.80449	-2.39407
.0100	.0100	-9.81231	-1.40189
.0200	.0200	-9.51590	-1.10548
.0300	.0300	-9.34434	-.93392
.0400	.0400	-9.22394	-.81352
.0500	.0500	-9.13160	-.72118
.0600	.0600	-9.05702	-.64661
.0700	.0700	-8.99473	-.58431
.0800	.0800	-8.94144	-.53102
.0900	.0900	-8.89504	-.48462
.1000	.1000	-8.85409	-.44367
.1500	.1500	-8.70284	-.29242
.2000	.2000	-8.60423	-.19381
.2500	.2500	-8.53536	-.12494
.3000	.3000	-8.48614	-.07572
.3500	.3500	-8.45138	-.04096
.4000	.4000	-8.42815	-.01773
.4500	.4500	-8.41479	-.00437

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	-4.39794
.0010	.0010	-2.39407
.0100	.0100	-1.40189
.0200	.0200	-1.10548
.0300	.0300	-.93392
.0400	.0400	-.81352
.0500	.0500	-.72118
.0600	.0600	-.64661

.0700	.0700	-.58431
.0800	.0800	-.53102
.0900	.0900	-.48462
.1000	.1000	-.44367
.1500	.1500	-.29242
.2000	.2000	-.19381
.2500	.2500	-.12494
.3000	.3000	-.07572
.3500	.3500	-.04096
.4000	.4000	-.01773
.4500	.4500	-.00437

APPENDIX 5 OUTPUT FILES OF HOMOG FOR THE ANALYSIS OF 19.2 AND S21 DATA IN THE FAMILIES WITH X-LINKED AGAMMAGLOBULINAEMIA

Program HOMOG J. Ott

Heterogeneity: two family types, one linked, the other unlinked

XLAHOMO-S21

Family	Lod scores						
1	0.5400	0.5300	0.5200	0.5150	0.5050	0.5000	0.4500
	0.3900	0.3500	0.2800	0.2300	0.1700	0.1200	0.0900
2	0.5400	0.5300	0.5200	0.5150	0.5050	0.5000	0.4500
	0.3900	0.3500	0.2800	0.2300	0.1700	0.1200	0.0900
3	0.5400	0.5300	0.5200	0.5150	0.5050	0.5000	0.4500
	0.3900	0.3500	0.2800	0.2300	0.1700	0.1200	0.0900
4	0.5400	0.5300	0.5200	0.5150	0.5050	0.5000	0.4500
	0.3900	0.3500	0.2800	0.2300	0.1700	0.1200	0.0900
5	3.9100	3.6000	3.2000	2.9000	2.7500	2.6800	2.0800
	1.5800	1.2000	0.9000	0.6800	0.4600	0.2800	0.1300
6	-3.8000	-2.8000	-2.2100	-1.8700	-1.6300	-1.4400	-0.8900
	-0.5800	-0.3900	-0.2500	-0.1500	-0.0800	-0.0400	-0.0100
7	-4.4000	-3.0800	-2.5000	-2.1500	-1.9100	-1.7200	-1.1400
	-0.8200	-0.5900	-0.4300	-0.3000	-0.2000	-0.1200	-0.0500
8	0.5820	0.5670	0.5510	0.5350	0.5194	0.5000	0.4300
	0.3500	0.2700	0.2000	0.1400	0.0900	0.0400	0.0200
9	0.6020	0.5889	0.5757	0.5623	0.5489	0.5353	0.4700
	0.3900	0.3200	0.2400	0.1700	0.1000	0.0500	0.0138
10	0.5820	0.5732	0.5647	0.5561	0.5474	0.5400	0.4900
	0.4400	0.3900	0.3400	0.2800	0.2200	0.1500	0.0800
11	0.3010	0.2920	0.2836	0.2749	0.2663	0.2600	0.2100
	0.1700	0.1300	0.0900	0.0600	0.0400	0.0200	0.0043
12	0.3010	0.2920	0.2836	0.2749	0.2663	0.2600	0.2100
	0.1700	0.1300	0.0900	0.0600	0.0400	0.0200	0.0043
13	0.7269	0.7108	0.6946	0.6783	0.6620	0.6500	0.5600
	0.4700	0.3900	0.3000	0.2200	0.1400	0.0700	0.0300
14	0.9030	0.8856	0.8679	0.8502	0.8322	0.8100	0.7200
	0.6200	0.5200	0.4100	0.2900	0.1900	0.0900	0.0200
15	0.3010	0.2967	0.2922	0.2878	0.2833	0.2800	0.2600
	0.2300	0.2000	0.1800	0.1500	0.1100	0.0800	0.0400
16	0.3010	0.2923	0.2836	0.2749	0.2663	0.2600	0.2100
	0.1700	0.1300	0.0900	0.0600	0.0400	0.0200	0.0043
17	0.3010	0.2923	0.2836	0.2749	0.2663	0.2600	0.2100
	0.1700	0.1300	0.0900	0.0600	0.0400	0.0200	0.0043
Theta =	0.0000	0.0100	0.0200	0.0300	0.0400	0.0500	0.1000
	0.1500	0.2000	0.2500	0.3000	0.3500	0.4000	0.4500

Alpha	ln L(alpha,theta)						
1.0000	6.3802	10.6628	12.0897	12.6856	13.0980	13.5284	12.9405
	11.3287	9.7399	7.7597	6.0788	4.3058	2.6710	1.4990
0.9900	15.9676	15.1147	14.3726	14.0113	13.9370	14.0814	13.0356
	11.3281	9.7084	7.7210	6.0407	4.2742	2.6490	1.4855
0.9800	17.2404	16.2882	15.3166	14.7229	14.4638	14.4660	13.1130
	11.3234	9.6755	7.6816	6.0022	4.2423	2.6269	1.4720
0.9700	17.9436	16.9575	15.8964	15.1955	14.8372	14.7529	13.1759

	11.3150	9.6413	7.6416	5.9634	4.2103	2.6047	1.4585
0.9600	18.4120	17.4094	16.3008	15.5383	15.1179	14.9752	13.2264
	11.3030	9.6056	7.6009	5.9243	4.1781	2.5824	1.4449
0.9500	18.7513	17.7390	16.6014	15.7989	15.3361	15.1514	13.2662
	11.2878	9.5687	7.5596	5.8848	4.1458	2.5601	1.4314
0.9400	19.0085	17.9901	16.8330	16.0026	15.5092	15.2928	13.2967
	11.2695	9.5305	7.5177	5.8449	4.1132	2.5376	1.4178
0.9300	19.2087	18.1863	17.0153	16.1646	15.6480	15.4069	13.3190
	11.2484	9.4911	7.4751	5.8048	4.0805	2.5152	1.4041
0.9200	19.3670	18.3419	17.1607	16.2945	15.7599	15.4990	13.3340
	11.2245	9.4505	7.4319	5.7642	4.0476	2.4926	1.3905
0.9100	19.4930	18.4661	17.2771	16.3988	15.8500	15.5730	13.3424
	11.1980	9.4086	7.3881	5.7234	4.0145	2.4699	1.3768
0.9000	19.5933	18.5653	17.3703	16.4824	15.9219	15.6317	13.3448
	11.1691	9.3657	7.3436	5.6821	3.9812	2.4472	1.3631
0.8900	19.6728	18.6440	17.4443	16.5486	15.9787	15.6773	13.3417
	11.1378	9.3215	7.2986	5.6406	3.9477	2.4244	1.3493
0.8800	19.7348	18.7056	17.5023	16.6001	16.0223	15.7115	13.3336
	11.1043	9.2763	7.2529	5.5986	3.9141	2.4015	1.3355
0.8700	19.7820	18.7527	17.5464	16.6389	16.0546	15.7357	13.3208
	11.0686	9.2300	7.2066	5.5564	3.8803	2.3785	1.3217
0.8600	19.8165	18.7872	17.5786	16.6668	16.0769	15.7510	13.3037
	11.0309	9.1825	7.1597	5.5138	3.8463	2.3554	1.3079
0.8500	19.8398	18.8108	17.6004	16.6849	16.0903	15.7584	13.2826
	10.9911	9.1340	7.1122	5.4708	3.8121	2.3323	1.2940
0.8400	19.8533	18.8247	17.6128	16.6943	16.0957	15.7586	13.2577
	10.9494	9.0844	7.0641	5.4274	3.7777	2.3090	1.2802
0.8300	19.8581	18.8299	17.6170	16.6959	16.0939	15.7523	13.2292
	10.9058	9.0338	7.0153	5.3838	3.7431	2.2857	1.2663
0.8200	19.8549	18.8274	17.6137	16.6904	16.0856	15.7399	13.1973
	10.8604	8.9822	6.9660	5.3397	3.7083	2.2623	1.2523
0.8100	19.8446	18.8178	17.6036	16.6785	16.0713	15.7221	13.1622
	10.8133	8.9295	6.9161	5.2953	3.6734	2.2389	1.2383
0.8000	19.8277	18.8018	17.5872	16.6606	16.0514	15.6992	13.1241
	10.7644	8.8758	6.8655	5.2505	3.6382	2.2153	1.2243
0.7900	19.8048	18.7798	17.5650	16.6373	16.0263	15.6714	13.0830
	10.7138	8.8211	6.8144	5.2054	3.6029	2.1916	1.2103
0.7800	19.7763	18.7523	17.5376	16.6088	15.9965	15.6393	13.0392
	10.6615	8.7653	6.7626	5.1599	3.5673	2.1679	1.1963
0.7700	19.7427	18.7197	17.5051	16.5756	15.9621	15.6029	12.9926
	10.6076	8.7086	6.7102	5.1140	3.5316	2.1441	1.1822
0.7600	19.7042	18.6823	17.4680	16.5379	15.9236	15.5625	12.9435
	10.5522	8.6509	6.6572	5.0678	3.4956	2.1202	1.1680
0.7500	19.6611	18.6403	17.4265	16.4960	15.8810	15.5184	12.8918
	10.4951	8.5922	6.6036	5.0212	3.4595	2.0962	1.1539
0.7400	19.6137	18.5941	17.3808	16.4501	15.8345	15.4706	12.8377
	10.4365	8.5325	6.5494	4.9742	3.4232	2.0721	1.1397
0.7300	19.5622	18.5439	17.3312	16.4004	15.7845	15.4195	12.7812
	10.3764	8.4718	6.4945	4.9268	3.3866	2.0480	1.1255
0.7200	19.5068	18.4898	17.2778	16.3470	15.7310	15.3650	12.7224
	10.3148	8.4102	6.4390	4.8791	3.3499	2.0237	1.1113
0.7100	19.4476	18.4320	17.2208	16.2902	15.6741	15.3074	12.6614
	10.2517	8.3475	6.3829	4.8309	3.3129	1.9994	1.0970
0.7000	19.3849	18.3706	17.1603	16.2300	15.6140	15.2467	12.5982
	10.1871	8.2839	6.3262	4.7824	3.2758	1.9749	1.0827
0.6900	19.3187	18.3059	17.0965	16.1665	15.5508	15.1830	12.5328
	10.1211	8.2192	6.2689	4.7335	3.2384	1.9504	1.0684

0.6800	19.2491	18.2378	17.0295	16.1000	15.4847	15.1165	12.4653
	10.0536	8.1536	6.2109	4.6842	3.2008	1.9258	1.0541
0.6700	19.1764	18.1665	16.9594	16.0304	15.4155	15.0471	12.3957
	9.9847	8.0870	6.1522	4.6345	3.1630	1.9011	1.0397
0.6600	19.1005	18.0922	16.8862	15.9578	15.3436	14.9751	12.3241
	9.9143	8.0194	6.0929	4.5844	3.1250	1.8763	1.0253
0.6500	19.0215	18.0148	16.8101	15.8824	15.2688	14.9003	12.2505
	9.8425	7.9508	6.0330	4.5339	3.0868	1.8514	1.0108
0.6400	18.9395	17.9344	16.7310	15.8041	15.1914	14.8230	12.1748
	9.7693	7.8813	5.9724	4.4829	3.0484	1.8264	0.9963
0.6300	18.8545	17.8512	16.6491	15.7231	15.1112	14.7430	12.0971
	9.6946	7.8107	5.9112	4.4316	3.0097	1.8014	0.9818
0.6200	18.7667	17.7651	16.5645	15.6394	15.0284	14.6605	12.0175
	9.6185	7.7391	5.8493	4.3799	2.9708	1.7762	0.9673
0.6100	18.6761	17.6762	16.4770	15.5530	14.9431	14.5756	11.9360
	9.5410	7.6664	5.7867	4.3277	2.9317	1.7509	0.9527
0.6000	18.5826	17.5845	16.3869	15.4639	14.8551	14.4881	11.8524
	9.4621	7.5928	5.7234	4.2751	2.8924	1.7256	0.9381
0.5900	18.4863	17.4901	16.2941	15.3722	14.7647	14.3982	11.7670
	9.3817	7.5181	5.6595	4.2221	2.8529	1.7001	0.9235
0.5800	18.3873	17.3930	16.1987	15.2780	14.6717	14.3059	11.6796
	9.2999	7.4424	5.5949	4.1686	2.8131	1.6746	0.9088
0.5700	18.2856	17.2932	16.1006	15.1811	14.5762	14.2111	11.5902
	9.2167	7.3656	5.5295	4.1147	2.7731	1.6489	0.8941
0.5600	18.1811	17.1907	15.9998	15.0817	14.4783	14.1140	11.4990
	9.1320	7.2877	5.4635	4.0604	2.7328	1.6232	0.8794
0.5500	18.0739	17.0855	15.8965	14.9798	14.3778	14.0144	11.4058
	9.0458	7.2088	5.3967	4.0056	2.6923	1.5974	0.8647
0.5400	17.9640	16.9777	15.7906	14.8753	14.2749	13.9124	11.3106
	8.9581	7.1288	5.3293	3.9504	2.6516	1.5714	0.8499
0.5300	17.8514	16.8672	15.6821	14.7683	14.1696	13.8081	11.2135
	8.8690	7.0478	5.2611	3.8947	2.6107	1.5454	0.8351
0.5200	17.7361	16.7541	15.5709	14.6587	14.0617	13.7013	11.1144
	8.7784	6.9656	5.1921	3.8385	2.5695	1.5192	0.8202
0.5100	17.6181	16.6383	15.4571	14.5465	13.9514	13.5921	11.0134
	8.6863	6.8822	5.1224	3.7818	2.5280	1.4930	0.8053
0.5000	17.4973	16.5198	15.3407	14.4318	13.8385	13.4805	10.9104
	8.5926	6.7978	5.0519	3.7247	2.4863	1.4667	0.7904
0.4900	17.3737	16.3985	15.2217	14.3145	13.7232	13.3664	10.8053
	8.4974	6.7122	4.9807	3.6671	2.4444	1.4402	0.7755
0.4800	17.2474	16.2746	15.1000	14.1946	13.6053	13.2499	10.6982
	8.4006	6.6254	4.9087	3.6090	2.4022	1.4137	0.7605
0.4700	17.1183	16.1479	14.9756	14.0721	13.4848	13.1309	10.5891
	8.3022	6.5374	4.8359	3.5505	2.3597	1.3870	0.7455
0.4600	16.9863	16.0184	14.8485	13.9469	13.3618	13.0093	10.4779
	8.2022	6.4482	4.7623	3.4914	2.3170	1.3603	0.7304
0.4500	16.8515	15.8861	14.7186	13.8190	13.2362	12.8853	10.3645
	8.1006	6.3578	4.6878	3.4318	2.2740	1.3334	0.7153
0.4400	16.7138	15.7510	14.5860	13.6884	13.1079	12.7586	10.2491
	7.9973	6.2662	4.6125	3.3717	2.2308	1.3064	0.7002
0.4300	16.5731	15.6130	14.4505	13.5551	12.9770	12.6294	10.1314
	7.8923	6.1732	4.5364	3.3110	2.1873	1.2794	0.6851
0.4200	16.4294	15.4720	14.3122	13.4189	12.8433	12.4974	10.0116
	7.7856	6.0790	4.4594	3.2498	2.1435	1.2522	0.6699
0.4100	16.2826	15.3280	14.1709	13.2799	12.7068	12.3628	9.8895
	7.6771	5.9835	4.3815	3.1881	2.0994	1.2249	0.6547
0.4000	16.1327	15.1810	14.0267	13.1380	12.5675	12.2254	9.7651

	7.5669	5.8866	4.3028	3.1258	2.0551	1.1975	0.6394
0.3900	15.9797	15.0309	13.8794	12.9931	12.4253	12.0853	9.6383
	7.4548	5.7883	4.2231	3.0630	2.0105	1.1700	0.6241
0.3800	15.8234	14.8776	13.7291	12.8452	12.2802	11.9422	9.5092
	7.3408	5.6886	4.1425	2.9996	1.9656	1.1424	0.6088
0.3700	15.6637	14.7211	13.5756	12.6942	12.1321	11.7962	9.3776
	7.2250	5.5875	4.0609	2.9356	1.9204	1.1146	0.5935
0.3600	15.5007	14.5612	13.4188	12.5400	11.9810	11.6473	9.2436
	7.1071	5.4849	3.9784	2.8710	1.8750	1.0868	0.5781
0.3500	15.3342	14.3979	13.2587	12.3826	11.8266	11.4952	9.1069
	6.9873	5.3808	3.8949	2.8058	1.8292	1.0588	0.5627
0.3400	15.1640	14.2311	13.0952	12.2219	11.6690	11.3400	8.9677
	6.8654	5.2752	3.8104	2.7400	1.7831	1.0308	0.5472
0.3300	14.9902	14.0608	12.9282	12.0578	11.5081	11.1816	8.8257
	6.7413	5.1679	3.7248	2.6736	1.7368	1.0026	0.5317
0.3200	14.8126	13.8867	12.7575	11.8901	11.3438	11.0198	8.6809
	6.6151	5.0591	3.6382	2.6065	1.6901	0.9743	0.5162
0.3100	14.6311	13.7087	12.5832	11.7187	11.1760	10.8546	8.5332
	6.4867	4.9485	3.5505	2.5388	1.6431	0.9458	0.5006
0.3000	14.4455	13.5269	12.4050	11.5437	11.0045	10.6859	8.3826
	6.3559	4.8362	3.4616	2.4704	1.5958	0.9173	0.4851
0.2900	14.2557	13.3409	12.2228	11.3647	10.8292	10.5134	8.2289
	6.2227	4.7222	3.3717	2.4013	1.5482	0.8886	0.4694
0.2800	14.0615	13.1507	12.0365	11.1817	10.6500	10.3372	8.0720
	6.0870	4.6063	3.2805	2.3316	1.5002	0.8599	0.4538
0.2700	13.8628	12.9560	11.8459	10.9946	10.4668	10.1570	7.9119
	5.9488	4.4885	3.1881	2.2611	1.4519	0.8310	0.4381
0.2600	13.6594	12.7568	11.6508	10.8031	10.2794	9.9727	7.7483
	5.8078	4.3687	3.0945	2.1899	1.4033	0.8019	0.4223
0.2500	13.4511	12.5528	11.4511	10.6070	10.0875	9.7841	7.5811
	5.6641	4.2469	2.9997	2.1180	1.3544	0.7728	0.4065
0.2400	13.2377	12.3439	11.2465	10.4063	9.8911	9.5911	7.4102
	5.5175	4.1230	2.9034	2.0453	1.3051	0.7435	0.3907
0.2300	13.0189	12.1296	11.0369	10.2006	9.6899	9.3933	7.2353
	5.3679	3.9969	2.8059	1.9718	1.2554	0.7141	0.3749
0.2200	12.7945	11.9099	10.8219	9.9896	9.4836	9.1907	7.0564
	5.2151	3.8685	2.7069	1.8976	1.2054	0.6846	0.3590
0.2100	12.5641	11.6845	10.6013	9.7733	9.2720	8.9829	6.8731
	5.0589	3.7377	2.6065	1.8225	1.1550	0.6549	0.3431
0.2000	12.3275	11.4529	10.3748	9.5511	9.0549	8.7697	6.6853
	4.8992	3.6044	2.5045	1.7465	1.1043	0.6251	0.3271
0.1900	12.0842	11.2148	10.1420	9.3229	8.8318	8.5506	6.4927
	4.7359	3.4685	2.4010	1.6697	1.0532	0.5952	0.3111
0.1800	11.8338	10.9699	9.9025	9.0881	8.6023	8.3254	6.2949
	4.5685	3.3298	2.2958	1.5921	1.0017	0.5651	0.2951
0.1700	11.5760	10.7177	9.6559	8.8464	8.3662	8.0937	6.0917
	4.3970	3.1882	2.1890	1.5135	0.9498	0.5350	0.2790
0.1600	11.3100	10.4575	9.4016	8.5972	8.1228	7.8549	5.8825
	4.2211	3.0436	2.0804	1.4339	0.8975	0.5046	0.2629
0.1500	11.0353	10.1889	9.1391	8.3400	7.8716	7.6085	5.6670
	4.0403	2.8956	1.9699	1.3534	0.8448	0.4742	0.2468
0.1400	10.7511	9.9110	8.8676	8.0741	7.6119	7.3538	5.4446
	3.8543	2.7443	1.8575	1.2718	0.7917	0.4436	0.2306
0.1300	10.4566	9.6231	8.5863	7.7986	7.3430	7.0901	5.2147
	3.6628	2.5892	1.7431	1.1893	0.7382	0.4129	0.2144
0.1200	10.1506	9.3240	8.2941	7.5126	7.0638	6.8164	4.9764
	3.4650	2.4301	1.6265	1.1056	0.6842	0.3820	0.1981

0.1100	9.8320	9.0126	7.9900	7.2148	6.7733	6.5316	4.7288
	3.2605	2.2668	1.5077	1.0209	0.6299	0.3510	0.1818
0.1000	9.4991	8.6871	7.6722	6.9038	6.4698	6.2341	4.4708
	3.0484	2.0988	1.3865	0.9349	0.5750	0.3198	0.1655
0.0900	9.1498	8.3457	7.3388	6.5776	6.1516	5.9223	4.2008
	2.8278	1.9257	1.2628	0.8478	0.5197	0.2885	0.1491
0.0800	8.7814	7.9856	6.9872	6.2337	5.8161	5.5935	3.9168
	2.5976	1.7470	1.1364	0.7594	0.4640	0.2571	0.1327
0.0700	8.3903	7.6032	6.6139	5.8686	5.4600	5.2446	3.6162
	2.3562	1.5622	1.0071	0.6698	0.4078	0.2255	0.1163
0.0600	7.9711	7.1934	6.2137	5.4774	5.0784	4.8707	3.2954
	2.1016	1.3704	0.8748	0.5787	0.3511	0.1937	0.0998
0.0500	7.5160	6.7481	5.7789	5.0526	4.6642	4.4648	2.9489
	1.8311	1.1708	0.7391	0.4863	0.2939	0.1618	0.0833
0.0400	7.0118	6.2544	5.2969	4.5820	4.2054	4.0152	2.5685
	1.5410	0.9622	0.5999	0.3923	0.2362	0.1298	0.0667
0.0300	6.4343	5.6886	4.7446	4.0434	3.6807	3.5011	2.1401
	1.2257	0.7431	0.4568	0.2968	0.1779	0.0976	0.0501
0.0200	5.7304	4.9981	4.0711	3.3890	3.0445	2.8780	1.6368
	0.8762	0.5117	0.3094	0.1996	0.1192	0.0652	0.0334
0.0100	4.7324	4.0182	3.1207	2.4762	2.1637	2.0184	0.9963
	0.4775	0.2652	0.1573	0.1007	0.0599	0.0327	0.0167
Theta =	0.0000	0.0100	0.0200	0.0300	0.0400	0.0500	0.1000
	0.1500	0.2000	0.2500	0.3000	0.3500	0.4000	0.4500

Hypotheses	Max.lnL	Estimates of alpha	theta
H2: linkage, heterogeneity	19.8581	0.8300	0.0000
H1: linkage, homogeneity	13.5284	(1)	0.0500
H0: no linkage	(0)	(0)	(0.5)

Components of chi-square

Source		d.f.	chi-square	p-value
H2 vs. H1	Heterogeneity	1	12.659	0.0002
H1 vs. H0	Linkage	1	27.057	0.0000
H2 vs. H0	Total	2	39.716	0.0000

Approx. 95% confidence intervals:

Alpha	0.4600	0.9800
Theta	0.0000	0.0200

Family no.	Posterior prob. of linkage	Approx.conf.limits lower	upper
1	0.9442	0.7471	0.9941
2	0.9442	0.7471	0.9941
3	0.9442	0.7471	0.9941
4	0.9442	0.7471	0.9941
5	1.0000	0.9997	1.0000
6	0.0008	0.0001	0.0757
7	0.0002	0.0000	0.0403
8	0.9491	0.7649	0.9947
9	0.9513	0.7731	0.9949
10	0.9491	0.7649	0.9947
11	0.9071	0.6301	0.9899
12	0.9071	0.6301	0.9899
13	0.9630	0.8196	0.9962
14	0.9750	0.8720	0.9975
15	0.9071	0.6301	0.9899
16	0.9071	0.6301	0.9899
17	0.9071	0.6301	0.9899

Program HOMOG J. Ott

Heterogeneity: two family types, one linked, the other unlinked

XLAHOMO-19-2

Family	Lod scores						
1	-4.3980	-0.8140	-0.7220	-0.6470	-0.4440	-0.3130	-0.2930
	0.2870	-0.1940	-0.1250	-0.0760	-0.0410	-0.0180	-0.0040
2	0.7270	0.6530	0.6340	0.6150	0.5390	0.4620	0.4430
	0.4230	0.3470	0.2550	0.1680	0.0940	0.0370	0.0040
3	0.5677	0.5357	0.5275	0.5191	0.4845	0.4481	0.4387
	0.4291	0.3895	0.3366	0.2796	0.2180	0.1500	0.0780
4	0.6020	0.5489	0.5350	0.5220	0.4650	0.4070	0.3930
	0.3770	0.3180	0.2430	0.1700	0.1040	0.0490	0.0130
5	-4.3979	-0.8135	-0.7210	-0.6466	-0.4440	-0.3170	-0.2920
	-0.2695	-0.1940	-0.1250	-0.0760	-0.0410	-0.0180	-0.0040
6	0.6020	0.5489	0.5350	0.5220	0.4650	0.4070	0.3930
	0.3770	0.3180	0.2430	0.1700	0.1040	0.0490	0.0130
7	0.3010	0.2660	0.2580	0.2490	0.2150	0.1810	0.1730
	0.1650	0.1340	0.0970	0.0640	0.0370	0.0170	0.0040
8	0.2847	0.2674	0.2630	0.2587	0.2410	0.2230	0.2180
	0.2137	0.1890	0.1650	0.1390	0.1100	0.0770	0.0410
9	1.2040	1.1160	1.0940	1.0710	0.9800	0.8879	0.8650
	0.8410	0.7460	0.6250	0.5030	0.3790	0.2550	0.1290
10	0.6020	0.5665	0.5580	0.5483	0.5110	0.4710	0.4600
	0.4500	0.4080	0.3520	0.2920	0.2280	0.1580	0.0830
11	-3.1100	0.3010	0.3720	0.4230	0.5320	0.5595	0.5590
	0.5560	0.5270	0.4598	0.3680	0.2630	0.1550	0.0620
12	0.6020	0.5665	0.5575	0.5480	0.5110	0.4710	0.4610
	0.4510	0.4080	0.3520	0.2920	0.2280	0.1580	0.0830
13	-3.5600	-0.5300	-0.4420	-0.3720	-0.1880	-0.0810	-0.0620
	-0.0442	0.0100	0.0510	0.0704	0.0730	0.0610	0.0370
14	0.3010	0.2830	0.2790	0.2740	0.2550	0.2355	0.2300
	0.2250	0.2040	0.1760	0.1460	0.1140	0.0790	0.0410
15	-3.7680	-3.4900	-3.2728	-3.0400	-2.2700	-1.7360	-1.6300
	-1.5260	-1.1800	-0.8500	-0.5950	-0.3910	-0.2290	-0.1000
16	0.9030	0.8320	0.8100	0.7950	0.7200	0.6410	0.6210
	0.6010	0.5170	0.4080	0.2980	0.1900	0.0940	0.0250
17	-3.7160	-0.8132	-0.7210	-0.6460	-0.4440	-0.3170	-0.2920
	-0.2690	-0.1940	-0.1250	-0.0760	-0.0410	-0.0180	-0.0040
18	-99.0000	3.2740	3.2960	3.2989	3.2100	3.0367	2.9850
	2.9300	2.6890	2.3450	1.9600	1.5400	1.0700	0.5600
19	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
20	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
21	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
22	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
23	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
24	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
25	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030

	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
26	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
27	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
28	-99.0000	0.3650	0.3640	0.3600	0.3410	0.3140	0.3030
	0.2940	0.2590	0.2120	0.1620	0.1150	0.0700	0.0310
Theta =	0.0000	0.0400	0.0500	0.0600	0.1000	0.1400	0.1500
	0.1600	0.2000	0.2500	0.3000	0.3500	0.4000	0.4500

Alpha	ln L(alpha,theta)						
1.0000	-99.0000	15.9988	17.2238	18.1729	20.1442	20.2782	20.0341
	21.0850	18.4955	16.1259	13.1639	9.9426	6.5071	3.1568
0.9900	-62.8031	19.4979	20.2011	20.6217	21.1082	20.6048	20.2794
	21.2493	18.5230	16.0867	13.1071	9.8878	6.4648	3.1326
0.9800	-51.1709	20.2021	20.8573	21.2353	21.5226	20.8008	20.4331
	21.3504	18.5342	16.0436	13.0490	9.8325	6.4223	3.1082
0.9700	-44.3822	20.6202	21.2368	21.5850	21.7668	20.9266	20.5327
	21.4109	18.5324	15.9969	12.9898	9.7767	6.3796	3.0837
0.9600	-39.5844	20.9150	21.4973	21.8201	21.9260	21.0081	20.5962
	21.4428	18.5199	15.9470	12.9294	9.7204	6.3366	3.0592
0.9500	-35.8794	21.1386	21.6897	21.9897	22.0336	21.0590	20.6340
	21.4535	18.4984	15.8941	12.8678	9.6636	6.2935	3.0346
0.9400	-32.8665	21.3148	21.8371	22.1161	22.1062	21.0872	20.6522
	21.4478	18.4691	15.8384	12.8051	9.6063	6.2500	3.0098
0.9300	-30.3318	21.4565	21.9520	22.2116	22.1534	21.0980	20.6549
	21.4288	18.4332	15.7800	12.7413	9.5485	6.2064	2.9850
0.9200	-28.1474	21.5714	22.0421	22.2834	22.1810	21.0947	20.6452
	21.3988	18.3913	15.7192	12.6765	9.4902	6.1625	2.9601
0.9100	-26.2308	21.6649	22.1122	22.3364	22.1931	21.0799	20.6250
	21.3596	18.3442	15.6560	12.6106	9.4314	6.1184	2.9352
0.9000	-24.5257	21.7404	22.1659	22.3739	22.1923	21.0553	20.5959
	21.3125	18.2924	15.5907	12.5437	9.3722	6.0740	2.9101
0.8900	-22.9917	21.8007	22.2055	22.3984	22.1807	21.0224	20.5592
	21.2585	18.2362	15.5233	12.4759	9.3124	6.0294	2.8849
0.8800	-21.5994	21.8476	22.2331	22.4115	22.1598	20.9821	20.5156
	21.1983	18.1761	15.4538	12.4070	9.2522	5.9845	2.8596
0.8700	-20.3260	21.8828	22.2499	22.4146	22.1307	20.9354	20.4661
	21.1326	18.1123	15.3825	12.3372	9.1914	5.9394	2.8343
0.8600	-19.1541	21.9074	22.2572	22.4089	22.0944	20.8828	20.4111
	21.0619	18.0451	15.3093	12.2664	9.1302	5.8941	2.8088
0.8500	-18.0698	21.9225	22.2559	22.3952	22.0516	20.8250	20.3512
	20.9867	17.9748	15.2343	12.1946	9.0685	5.8485	2.7833
0.8400	-17.0618	21.9289	22.2466	22.3743	22.0029	20.7624	20.2867
	20.9073	17.9015	15.1576	12.1220	9.0062	5.8026	2.7576
0.8300	-16.1211	21.9272	22.2301	22.3466	21.9488	20.6953	20.2181
	20.8240	17.8253	15.0792	12.0484	8.9435	5.7565	2.7319
0.8200	-15.2400	21.9181	22.2069	22.3126	21.8896	20.6240	20.1455
	20.7371	17.7465	14.9992	11.9739	8.8803	5.7102	2.7060
0.8100	-14.4122	21.9020	22.1774	22.2729	21.8258	20.5489	20.0693
	20.6467	17.6651	14.9176	11.8985	8.8165	5.6636	2.6801
0.8000	-13.6323	21.8794	22.1419	22.2277	21.7575	20.4702	19.9896
	20.5532	17.5813	14.8345	11.8222	8.7523	5.6167	2.6540
0.7900	-12.8958	21.8506	22.1009	22.1774	21.6851	20.3880	19.9066
	20.4565	17.4951	14.7499	11.7450	8.6876	5.5695	2.6278
0.7800	-12.1986	21.8160	22.0545	22.1221	21.6087	20.3024	19.8206
	20.3569	17.4066	14.6638	11.6669	8.6224	5.5221	2.6016

0.7700	-11.5374	21.7758	22.0031	22.0621	21.5286	20.2138	19.7315
	20.2545	17.3160	14.5763	11.5879	8.5566	5.4744	2.5752
0.7600	-10.9092	21.7303	21.9469	21.9977	21.4448	20.1221	19.6395
	20.1494	17.2232	14.4873	11.5080	8.4903	5.4264	2.5487
0.7500	-10.3114	21.6797	21.8860	21.9290	21.3576	20.0275	19.5447
	20.0417	17.1283	14.3969	11.4273	8.4236	5.3782	2.5221
0.7400	-9.7417	21.6241	21.8206	21.8561	21.2670	19.9301	19.4473
	19.9314	17.0314	14.3051	11.3457	8.3563	5.3297	2.4954
0.7300	-9.1981	21.5639	21.7508	21.7791	21.1731	19.8299	19.3472
	19.8186	16.9325	14.2119	11.2632	8.2884	5.2808	2.4686
0.7200	-8.6787	21.4991	21.6769	21.6982	21.0761	19.7270	19.2445
	19.7034	16.8317	14.1173	11.1798	8.2201	5.2317	2.4417
0.7100	-8.1820	21.4298	21.5988	21.6136	20.9760	19.6216	19.1394
	19.5859	16.7289	14.0215	11.0956	8.1512	5.1823	2.4146
0.7000	-7.7064	21.3562	21.5168	21.5252	20.8728	19.5135	19.0318
	19.4660	16.6242	13.9242	11.0104	8.0818	5.1326	2.3875
0.6900	-7.2507	21.2784	21.4309	21.4331	20.7666	19.4030	18.9218
	19.3438	16.5177	13.8256	10.9244	8.0118	5.0826	2.3602
0.6800	-6.8137	21.1964	21.3411	21.3375	20.6575	19.2899	18.8094
	19.2194	16.4093	13.7257	10.8375	7.9413	5.0323	2.3328
0.6700	-6.3943	21.1105	21.2476	21.2384	20.5456	19.1745	18.6947
	19.0928	16.2991	13.6245	10.7497	7.8702	4.9817	2.3053
0.6600	-5.9916	21.0206	21.1504	21.1358	20.4308	19.0565	18.5777
	18.9639	16.1871	13.5219	10.6610	7.7986	4.9308	2.2776
0.6500	-5.6047	20.9268	21.0496	21.0299	20.3131	18.9362	18.4583
	18.8329	16.0732	13.4180	10.5714	7.7264	4.8795	2.2499
0.6400	-5.2328	20.8292	20.9453	20.9205	20.1927	18.8135	18.3367
	18.6997	15.9576	13.3128	10.4809	7.6536	4.8279	2.2220
0.6300	-4.8751	20.7278	20.8373	20.8078	20.0694	18.6885	18.2128
	18.5643	15.8402	13.2062	10.3895	7.5803	4.7760	2.1939
0.6200	-4.5309	20.6226	20.7259	20.6918	19.9434	18.5611	18.0866
	18.4268	15.7209	13.0983	10.2972	7.5064	4.7238	2.1658
0.6100	-4.1998	20.5138	20.6110	20.5725	19.8147	18.4313	17.9582
	18.2871	15.5999	12.9891	10.2040	7.4318	4.6712	2.1375
0.6000	-3.8809	20.4013	20.4926	20.4499	19.6832	18.2991	17.8275
	18.1452	15.4771	12.8785	10.1098	7.3567	4.6183	2.1090
0.5900	-3.5740	20.2852	20.3708	20.3240	19.5489	18.1646	17.6946
	18.0012	15.3525	12.7666	10.0147	7.2810	4.5650	2.0805
0.5800	-3.2784	20.1654	20.2456	20.1949	19.4119	18.0278	17.5594
	17.8551	15.2261	12.6533	9.9186	7.2046	4.5113	2.0518
0.5700	-2.9937	20.0421	20.1170	20.0626	19.2722	17.8886	17.4219
	17.7067	15.0978	12.5386	9.8216	7.1276	4.4573	2.0229
0.5600	-2.7196	19.9151	19.9849	19.9269	19.1297	17.7470	17.2822
	17.5562	14.9678	12.4226	9.7235	7.0500	4.4029	1.9939
0.5500	-2.4556	19.7846	19.8494	19.7881	18.9844	17.6030	17.1402
	17.4035	14.8359	12.3051	9.6245	6.9717	4.3481	1.9648
0.5400	-2.2013	19.6505	19.7105	19.6459	18.8362	17.4566	16.9958
	17.2485	14.7021	12.1863	9.5245	6.8927	4.2929	1.9355
0.5300	-1.9565	19.5127	19.5682	19.5005	18.6853	17.3078	16.8491
	17.0913	14.5665	12.0660	9.4235	6.8131	4.2373	1.9060
0.5200	-1.7209	19.3714	19.4224	19.3517	18.5316	17.1565	16.7001
	16.9319	14.4290	11.9443	9.3215	6.7328	4.1814	1.8764
0.5100	-1.4941	19.2265	19.2732	19.1997	18.3749	17.0028	16.5487
	16.7702	14.2896	11.8212	9.2184	6.6518	4.1250	1.8467
0.5000	-1.2760	19.0779	19.1204	19.0443	18.2154	16.8466	16.3949
	16.6062	14.1483	11.6965	9.1143	6.5700	4.0681	1.8168
0.4900	-1.0662	18.9257	18.9642	18.8855	18.0530	16.6878	16.2387

	16.4399	14.0050	11.5704	9.0090	6.4876	4.0109	1.7867
0.4800	-0.8646	18.7697	18.8044	18.7233	17.8876	16.5265	16.0801
	16.2712	13.8597	11.4428	8.9027	6.4044	3.9532	1.7564
0.4700	-0.6710	18.6101	18.6410	18.5576	17.7192	16.3626	15.9189
	16.1001	13.7125	11.3136	8.7953	6.3204	3.8950	1.7260
0.4600	-0.4851	18.4467	18.4740	18.3885	17.5477	16.1960	15.7552
	15.9266	13.5632	11.1828	8.6867	6.2357	3.8364	1.6954
0.4500	-0.3068	18.2795	18.3033	18.2158	17.3732	16.0268	15.5889
	15.7505	13.4118	11.0504	8.5769	6.1501	3.7772	1.6646
0.4400	-0.1359	18.1084	18.1289	18.0395	17.1955	15.8548	15.4201
	15.5720	13.2583	10.9164	8.4660	6.0638	3.7176	1.6337
0.4300	0.0277	17.9335	17.9508	17.8596	17.0147	15.6801	15.2485
	15.3909	13.1027	10.7808	8.3538	5.9766	3.6575	1.6026
0.4200	0.1841	17.7546	17.7688	17.6760	16.8306	15.5026	15.0743
	15.2073	12.9449	10.6435	8.2404	5.8885	3.5969	1.5712
0.4100	0.3335	17.5716	17.5829	17.4886	16.6432	15.3222	14.8973
	15.0209	12.7848	10.5044	8.1258	5.7995	3.5357	1.5397
0.4000	0.4760	17.3846	17.3931	17.2973	16.4524	15.1388	14.7175
	14.8318	12.6225	10.3635	8.0098	5.7097	3.4739	1.5080
0.3900	0.6116	17.1935	17.1992	17.1022	16.2581	14.9525	14.5348
	14.6400	12.4579	10.2209	7.8925	5.6189	3.4116	1.4761
0.3800	0.7405	16.9981	17.0012	16.9030	16.0603	14.7630	14.3492
	14.4453	12.2908	10.0764	7.7738	5.5271	3.3487	1.4440
0.3700	0.8627	16.7983	16.7990	16.6998	15.8589	14.5705	14.1605
	14.2477	12.1214	9.9300	7.6537	5.4344	3.2852	1.4117
0.3600	0.9783	16.5942	16.5925	16.4924	15.6538	14.3747	13.9688
	14.0471	11.9494	9.7817	7.5322	5.3406	3.2211	1.3791
0.3500	1.0874	16.3855	16.3815	16.2807	15.4450	14.1756	13.7739
	13.8434	11.7749	9.6313	7.4091	5.2457	3.1563	1.3464
0.3400	1.1899	16.1721	16.1661	16.0647	15.2322	13.9731	13.5757
	13.6366	11.5977	9.4789	7.2845	5.1498	3.0909	1.3134
0.3300	1.2860	15.9541	15.9460	15.8441	15.0154	13.7672	13.3742
	13.4266	11.4178	9.3243	7.1583	5.0527	3.0248	1.2802
0.3200	1.3755	15.7311	15.7212	15.6189	14.7945	13.5576	13.1692
	13.2132	11.2351	9.1676	7.0305	4.9544	2.9579	1.2468
0.3100	1.4587	15.5031	15.4914	15.3889	14.5694	13.3444	12.9607
	12.9964	11.0495	9.0086	6.9009	4.8549	2.8903	1.2131
0.3000	1.5354	15.2699	15.2566	15.1540	14.3399	13.1273	12.7485
	12.7761	10.8610	8.8472	6.7695	4.7541	2.8219	1.1792
0.2900	1.6056	15.0313	15.0166	14.9140	14.1058	12.9062	12.5325
	12.5520	10.6693	8.6834	6.6363	4.6519	2.7526	1.1450
0.2800	1.6693	14.7873	14.7712	14.6687	13.8671	12.6811	12.3125
	12.3242	10.4745	8.5171	6.5011	4.5483	2.6826	1.1105
0.2700	1.7265	14.5375	14.5202	14.4180	13.6235	12.4517	12.0885
	12.0924	10.2763	8.3481	6.3640	4.4433	2.6116	1.0758
0.2600	1.7771	14.2817	14.2634	14.1617	13.3748	12.2179	11.8603
	11.8565	10.0746	8.1764	6.2247	4.3367	2.5397	1.0409
0.2500	1.8211	14.0198	14.0006	13.8994	13.1209	11.9796	11.6276
	11.6163	9.8694	8.0017	6.0831	4.2284	2.4669	1.0056
0.2400	1.8583	13.7515	13.7314	13.6311	12.8616	11.7364	11.3903
	11.3716	9.6603	7.8241	5.9392	4.1185	2.3930	0.9701
0.2300	1.8887	13.4765	13.4558	13.3564	12.5965	11.4882	11.1482
	11.1223	9.4473	7.6433	5.7929	4.0067	2.3180	0.9342
0.2200	1.9123	13.1944	13.1732	13.0749	12.3254	11.2347	10.9010
	10.8681	9.2302	7.4591	5.6439	3.8930	2.2420	0.8981
0.2100	1.9287	12.9049	12.8835	12.7865	12.0481	10.9758	10.6485
	10.6087	9.0086	7.2714	5.4921	3.7772	2.1647	0.8616

0.2000	1.9380	12.6077	12.5862	12.4906	11.7641	10.7110	10.3905
	10.3439	8.7825	7.0800	5.3374	3.6592	2.0861	0.8248
0.1900	1.9399	12.3024	12.2809	12.1869	11.4731	10.4400	10.1264
	10.0733	8.5513	6.8845	5.1794	3.5389	2.0062	0.7877
0.1800	1.9342	11.9883	11.9671	11.8750	11.1746	10.1625	9.8561
	9.7966	8.3150	6.6847	5.0180	3.4159	1.9249	0.7502
0.1700	1.9208	11.6650	11.6443	11.5543	10.8683	9.8780	9.5790
	9.5134	8.0730	6.4803	4.8530	3.2903	1.8421	0.7124
0.1600	1.8993	11.3319	11.3118	11.2242	10.5535	9.5861	9.2947
	9.2232	7.8250	6.2709	4.6838	3.1616	1.7576	0.6742
0.1500	1.8696	10.9882	10.9690	10.8839	10.2297	9.2861	9.0026
	8.9253	7.5704	6.0560	4.5103	3.0297	1.6714	0.6356
0.1400	1.8312	10.6330	10.6151	10.5328	9.8960	8.9773	8.7021
	8.6193	7.3086	5.8351	4.3318	2.8942	1.5833	0.5966
0.1300	1.7837	10.2654	10.2490	10.1698	9.5515	8.6590	8.3923
	8.3042	7.0390	5.6076	4.1479	2.7547	1.4932	0.5573
0.1200	1.7269	9.8842	9.8696	9.7937	9.1953	8.3301	8.0723
	7.9792	6.7606	5.3726	3.9580	2.6107	1.4009	0.5174
0.1100	1.6601	9.4880	9.4754	9.4033	8.8260	7.9895	7.7409
	7.6431	6.4724	5.1292	3.7611	2.4618	1.3061	0.4772
0.1000	1.5828	9.0749	9.0647	8.9967	8.4421	7.6357	7.3966
	7.2944	6.1729	4.8762	3.5563	2.3073	1.2088	0.4365
0.0900	1.4944	8.6426	8.6354	8.5718	8.0414	7.2667	7.0375
	6.9313	5.8605	4.6120	3.3422	2.1462	1.1086	0.3953
0.0800	1.3941	8.1883	8.1844	8.1258	7.6212	6.8799	6.6612
	6.5512	5.5328	4.3344	3.1172	1.9776	1.0052	0.3537
0.0700	1.2811	7.7080	7.7078	7.6546	7.1780	6.4719	6.2641
	6.1508	5.1865	4.0405	2.8788	1.8001	0.8983	0.3115
0.0600	1.1541	7.1960	7.2002	7.1530	6.7064	6.0377	5.8412
	5.7252	4.8171	3.7263	2.6240	1.6120	0.7874	0.2688
0.0500	1.0120	6.6440	6.6533	6.6127	6.1987	5.5697	5.3852
	5.2669	4.4176	3.3855	2.3479	1.4108	0.6720	0.2256
0.0400	0.8531	6.0388	6.0538	6.0205	5.6419	5.0555	4.8838
	4.7638	3.9763	3.0081	2.0435	1.1931	0.5516	0.1817
0.0300	0.6754	5.3559	5.3775	5.3524	5.0127	4.4725	4.3144
	4.1938	3.4727	2.5766	1.6987	0.9541	0.4253	0.1373
0.0200	0.4765	4.5430	4.5722	4.5562	4.2599	3.7711	3.6283
	3.5086	2.8622	2.0550	1.2915	0.6861	0.2921	0.0922
0.0100	0.2529	3.4408	3.4780	3.4724	3.2273	2.8021	2.6785
	2.5642	2.0169	1.3492	0.7738	0.3761	0.1509	0.0464
Theta =	0.0000	0.0400	0.0500	0.0600	0.1000	0.1400	0.1500
	0.1600	0.2000	0.2500	0.3000	0.3500	0.4000	0.4500

Hypotheses	Max.lnL	Estimates of alpha	theta
H2: linkage, heterogeneity	22.4146	0.8700	0.0600
H1: linkage, homogeneity	21.0850	(1)	0.1600
H0: no linkage	(0)	(0)	(0.5)

Components of chi-square

Source		d.f.	chi-square	p-value
H2 vs. H1	Heterogeneity	1	2.659	0.0515
H1 vs. H0	Linkage	1	42.170	0.0000
H2 vs. H0	Total	2	44.829	0.0000

Approx. 95% confidence intervals:

Alpha	0.5200	1.0000
Theta	0.0400	0.1600

Family no.	Posterior prob. of linkage	Approx.conf.limits lower	upper
1	0.6014	0.1475	1.0000
2	0.9650	0.8229	1.0000
3	0.9567	0.7849	1.0000
4	0.9570	0.7878	1.0000
5	0.6016	0.1477	1.0000
6	0.9570	0.7878	1.0000
7	0.9223	0.6624	1.0000
8	0.9239	0.6650	1.0000
9	0.9875	0.9300	1.0000
10	0.9594	0.7966	1.0000
11	0.9466	0.6928	1.0000
12	0.9594	0.7964	1.0000
13	0.7397	0.2497	1.0000
14	0.9263	0.6731	1.0000
15	0.0061	0.0004	1.0000
16	0.9766	0.8749	1.0000
17	0.6019	0.1478	1.0000
18	0.9999	0.9995	1.0000
19	0.9388	0.7147	1.0000
20	0.9388	0.7147	1.0000
21	0.9388	0.7147	1.0000
22	0.9388	0.7147	1.0000
23	0.9388	0.7147	1.0000
24	0.9388	0.7147	1.0000
25	0.9388	0.7147	1.0000
26	0.9388	0.7147	1.0000
27	0.9388	0.7147	1.0000
28	0.9388	0.7147	1.0000

APPENDIX 6 INPUT AND OUTPUT FILES OF LIPED FOR THE ANALYSIS OF cpX73, 19.2, pXG12 AND S21 DATA IN THE FAMILIES WITH X-LINKED SEVERE COMBINED IMMUNODEFICIENCY

```
 1 0010 0.00001      SCID Linkage Study  (cpX73)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0500 0500
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
SCID    A    a aff n.a
    0001     9999
   A    A 1 0
   A    a 0 1
   a    a 0 1
cpx     U    L  UU  UL  LL
    6700     3300
   U    U 1 0 0
   U    L 0 1 0
   L    L 0 0 1
  17    0Bejarano/Garcia/Gundin(family 16)
 1.1             m n.a
 1.2             f n.a
 2.1             m n.a  UU
 2.2 1.1 1.2     f n.a  UL
 2.3             m n.a  LL
 2.4 1.1 1.2     f n.a  UL
 2.5             m n.a  UU
 2.6 1.1 1.2     f n.a  UL
 3.1 2.1 2.2     m n.a  UU
 3.2 2.1 2.2     m n.a  UU
 3.3 2.1 2.2     m aff
 3.4 2.3 2.4     f n.a  UL
 3.5 2.3 2.4     m n.a  UU
 3.6 2.5 2.6     f n.a  UU
 3.7 2.5 2.6     m n.a  UU
 3.8 2.5 2.6     m aff
 3.9 2.5 2.6     m aff
7000
  12    0Tebbe/Koenitz/Moller(family 17)
 1.1             m n.a  LL
```

```
 1.2             f n.a  UL
 2.1             m n.a  UU
 2.2 1.1 1.2     f n.a  UL
 2.3             m n.a  LL
 2.4 1.1 1.2     f n.a  LL
 2.5 1.1 1.2     m aff
 2.6 1.1 1.2     m aff
 2.7 1.1 1.2     m n.a  UU
 3.1 2.1 2.2     m n.a
 3.2 2.3 2.4     f n.a  LL
 3.4 2.3 2.4     m aff
7000
  10   OKhan(family 18)
 1.1             m n.a  LL
 1.2             f n.a  UU
 2.1             m n.a
 2.2 1.1 1.2     f n.a  UL
 2.3 1.1 1.2     m aff
 2.4 1.1 1.2     m aff
 2.5 1.1 1.2     m aff
 2.6 1.1 1.2     m aff  UU
 2.7 1.1 1.2     m n.a  UU
 3.1 2.1 2.2     m aff  UU
9000
```

```
Program LIPED  Version for IBM PC   March 1987    J. Ott
-------------------------------------------------------

 PROBLEM  1   SCID Linkage Study
 **********

 PEDIGREE  1   Bejarano/Garcia/Gundin
 -----------
                17 PERSONS

 LOCUS  0   SCID     VS.     LOCUS  1   cpx
 ----------------------------------------
 Mutation rate at current main locus =   .000010000000

 GENE FREQUENCIES FOR  0   SCID   .0001   .9999
 GENE FREQUENCIES FOR  1   cpx    .6700   .3300

  R MALE  R FEM.   LOG10(L(R))   LOD-SCORE
   .5000  .5000       -9.42633      .00000
   .0000  .0000       -8.67245      .75388
   .0010  .0010       -8.67451      .75181
   .0100  .0100       -8.69314      .73319
   .0500  .0500       -8.77633      .65000
   .1000  .1000       -8.88103      .54530
   .1500  .1500       -8.98592      .44041
   .2000  .2000       -9.08969      .33664
   .2500  .2500       -9.18990      .23643
   .3000  .3000       -9.28227      .14406
   .3500  .3500       -9.36019      .06614
   .4000  .4000       -9.41502      .01131
   .4500  .4500       -9.43843     -.01210

 PEDIGREE  2   Tebbe/Koenitz/Moller
 -----------
                12 PERSONS

 LOCUS  0   SCID     VS.     LOCUS  1   cpx
 ----------------------------------------
 Mutation rate at current main locus =   .000010000000

  R MALE  R FEM.   LOG10(L(R))   LOD-SCORE
   .5000  .5000       -7.72359      .00000
   .0000  .0000       -7.29764      .42595
   .0010  .0010       -7.29872      .42487
   .0100  .0100       -7.30852      .41507
   .0500  .0500       -7.35254      .37105
   .1000  .1000       -7.40833      .31526
   .1500  .1500       -7.46433      .25926
   .2000  .2000       -7.51948      .20411
   .2500  .2500       -7.57233      .15126
```

.3000	.3000	-7.62093	.10266
.3500	.3500	-7.66290	.06069
.4000	.4000	-7.69556	.02803
.4500	.4500	-7.71641	.00718

PEDIGREE 3 Khan

10 PERSONS

LOCUS 0 SCID VS. LOCUS 1 cpx

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.93665	.00000
.0000	.0000	-6.63563	.30102
.0010	.0010	-6.63606	.30058
.0100	.0100	-6.63999	.29665
.0500	.0500	-6.65791	.27874
.1000	.1000	-6.68139	.25526
.1500	.1500	-6.70621	.23044
.2000	.2000	-6.73254	.20411
.2500	.2500	-6.76056	.17608
.3000	.3000	-6.79053	.14612
.3500	.3500	-6.82271	.11394
.4000	.4000	-6.85747	.07918
.4500	.4500	-6.89526	.04139

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	1.48085
.0010	.0010	1.47726
.0100	.0100	1.44491
.0500	.0500	1.29979
.1000	.1000	1.11581
.1500	.1500	.93011
.2000	.2000	.74486
.2500	.2500	.56377
.3000	.3000	.39284
.3500	.3500	.24077
.4000	.4000	.11851
.4500	.4500	.03646

```
 1 0010 0.00001     SCID Linkage Study  (19.2)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0500 0500
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
SCID   A   a aff n.a
    0001    9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
19-2   U   L  UU  UL  LL
    3800    6200
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
  17   0Bejarano/Garcia/Gundin
 1.1            m n.a
 1.2            f n.a
 2.1            m n.a  LL
 2.2 1.1 1.2    f n.a  UL
 2.3            m n.a  LL
 2.4 1.1 1.2    f n.a  UU
 2.5            m n.a  UU
 2.6 1.1 1.2    f n.a  UL
 3.1 2.1 2.2    m n.a  LL
 3.2 2.1 2.2    m n.a  UU
 3.3 2.1 2.2    m aff
 3.4 2.3 2.4    f n.a  UL
 3.5 2.3 2.4    m n.a  UU
 3.6 2.5 2.6    f n.a  UU
 3.7 2.5 2.6    m n.a  UU
 3.8 2.5 2.6    m aff
 3.9 2.5 2.6    m aff
7000
  12   0Tebbe/Koenitz/Moller
 1.1            m n.a  UU
 1.2            f n.a  UL
 2.1            m n.a  LL
 2.2 1.1 1.2    f n.a  UL
```

```
 2.3             m n.a  LL
 2.4 1.1 1.2     f n.a  UU
 2.5 1.1 1.2     m aff
 2.6 1.1 1.2     m aff
 2.7 1.1 1.2     m n.a  LL
 3.1 2.1 2.2     m n.a
 3.2 2.3 2.4     f n.a  UL
 3.4 2.3 2.4     m aff
7000
  15   OKhan
 1.1             m n.a  LL
 1.2             f n.a  UL
 2.1 1.1 1.2     m aff
 2.2             m n.a  LL
 2.3 1.1 1.2     f n.a  UL
 2.4 1.1 1.2     m n.a  LL
 2.5 1.1 1.2     m n.a  LL
 3.1             m n.a
 3.2 2.2 2.3     f n.a  UL
 3.3 2.2 2.3     m aff  UU
 3.4 2.2 2.3     m n.a  LL
 3.5 2.2 2.3     m aff
 3.6 2.2 2.3     m aff
 3.7 2.2 2.3     m aff
 4.1 3.1 3.2     m aff  UU
9000
```

Program LIPED Version for IBM PC March 1987 J. Ott

PROBLEM 1 SCID Linkage Study
****

PEDIGREE 1 Bejarano/Garcia/Gundin
---

17 PERSONS

LOCUS 0 SCID VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

GENE FREQUENCIES FOR 0 SCID .0001 .9999
GENE FREQUENCIES FOR 1 19-2 .3800 .6200

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-9.92197	.00000
.0000	.0000	-14.29197	-4.37000
.0010	.0010	-11.59405	-1.67208
.0100	.0100	-10.61249	-.69052
.0500	.0500	-9.99343	-.07146
.1000	.1000	-9.79516	.12681
.1500	.1500	-9.72471	.19726
.2000	.2000	-9.70758	.21439
.2500	.2500	-9.71958	.20239
.3000	.3000	-9.74892	.17305
.3500	.3500	-9.78830	.13367
.4000	.4000	-9.83250	.08947
.4500	.4500	-9.87777	.04420

PEDIGREE 2 Tebbe/Koenitz/Moller
---

12 PERSONS

LOCUS 0 SCID VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-7.39454	.00000
.0000	.0000	-6.96859	.42595
.0010	.0010	-6.96967	.42487
.0100	.0100	-6.97947	.41507
.0500	.0500	-7.02349	.37105
.1000	.1000	-7.07928	.31526
.1500	.1500	-7.13528	.25926
.2000	.2000	-7.19043	.20411
.2500	.2500	-7.24328	.15126

.3000	.3000	-7.29188	.10266
.3500	.3500	-7.33385	.06069
.4000	.4000	-7.36651	.02803
.4500	.4500	-7.38736	.00718

PEDIGREE 3 Khan

15 PERSONS

LOCUS 0 SCID VS. LOCUS 1 19-2

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-9.85966	.00000
.0000	.0000	-8.05352	1.80614
.0010	.0010	-8.05656	1.80310
.0100	.0100	-8.08407	1.77559
.0500	.0500	-8.20939	1.65027
.1000	.1000	-8.37322	1.48643
.1500	.1500	-8.54520	1.31446
.2000	.2000	-8.72515	1.13451
.2500	.2500	-8.91229	.94737
.3000	.3000	-9.10491	.75475
.3500	.3500	-9.30010	.55955
.4000	.4000	-9.49373	.36593
.4500	.4500	-9.68126	.17840

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	-2.13791
.0010	.0010	.55588
.0100	.0100	1.50013
.0500	.0500	1.94986
.1000	.1000	1.92850
.1500	.1500	1.77098
.2000	.2000	1.55300
.2500	.2500	1.30102
.3000	.3000	1.03045
.3500	.3500	.75391
.4000	.4000	.48342
.4500	.4500	.22978

```
 1 0010 0.00001     SCID Linkage Study  (pXG 12)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0500 0500
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
SCID   A   a aff n.a
    0001    9999
   A   A 1 0
   A   a 0 1
   a   a 0 1
XG12   U   L  UU  UL  LL
    6500    3500
   U   U 1 0 0
   U   L 0 1 0
   L   L 0 0 1
  17   OBejarano/Garcia/Gundin
 1.1            m n.a
 1.2            f n.a
 2.1            m n.a
 2.2 1.1 1.2    f n.a  UU
 2.3            m n.a
 2.4 1.1 1.2    f n.a  UU
 2.5            m n.a
 2.6 1.1 1.2    f n.a  UU
 3.1 2.1 2.2    m n.a
 3.2 2.1 2.2    m n.a
 3.3 2.1 2.2    m aff
 3.4 2.3 2.4    f n.a
 3.5 2.3 2.4    m n.a
 3.6 2.5 2.6    f n.a
 3.7 2.5 2.6    m n.a
 3.8 2.5 2.6    m aff
 3.9 2.5 2.6    m aff
7000
  12   OTebbe/Koenitz/Moller
 1.1            m n.a  LL
 1.2            f n.a  UU
 2.1            m n.a  UU
 2.2 1.1 1.2    f n.a  UL
```

```
 2.3             m n.a  LL
 2.4 1.1 1.2     f n.a  UL
 2.5 1.1 1.2     m aff
 2.6 1.1 1.2     m aff
 2.7 1.1 1.2     m n.a  UU
 3.1 2.1 2.2     m n.a
 3.2 2.3 2.4     f n.a  UL
 3.4 2.3 2.4     m aff
7000
  15   OKhan
 1.1             m n.a
 1.2             f n.a  UL
 2.1 1.1 1.2     m aff
 2.2             m n.a
 2.3 1.1 1.2     f n.a  LL
 2.4 1.1 1.2     m n.a  UU
 2.5 1.1 1.2     m n.a  UU
 3.1             m n.a
 3.2 2.2 2.3     f n.a  LL
 3.3 2.2 2.3     m aff
 3.4 2.2 2.3     m n.a
 3.5 2.2 2.3     m aff
 3.6 2.2 2.3     m aff
 3.7 2.2 2.3     m aff
 4.1 3.1 3.2     m aff
9000
```

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Program LIPED  Version for IBM PC   March 1987    J. Ott
-------------------------------------------------------

 PROBLEM  1   SCID Linkage Study
 **********

 PEDIGREE  1   Bejarano/Garcia/Gundin
 -----------
                17 PERSONS

 LOCUS  0   SCID     VS.     LOCUS  1   XG12
 ----------------------------------------
 Mutation rate at current main locus =   .000010000000

 GENE FREQUENCIES FOR  0   SCID   .0001   .9999
 GENE FREQUENCIES FOR  1   XG12   .6500   .3500

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000       -6.73873       .00000
   .0000   .0000       -6.72189       .01684
   .0010   .0010       -6.72196       .01677
   .0100   .0100       -6.72254       .01618
   .0500   .0500       -6.72504       .01369
   .1000   .1000       -6.72788       .01085
   .1500   .1500       -6.73039       .00833
   .2000   .2000       -6.73259       .00614
   .2500   .2500       -6.73445       .00427
   .3000   .3000       -6.73599       .00274
   .3500   .3500       -6.73718       .00154
   .4000   .4000       -6.73804       .00069
   .4500   .4500       -6.73855       .00017

 PEDIGREE  2   Tebbe/Koenitz/Moller
 -----------
                12 PERSONS

 LOCUS  0   SCID     VS.     LOCUS  1   XG12
 ----------------------------------------
 Mutation rate at current main locus =   .000010000000

  R MALE   R FEM.    LOG10(L(R))    LOD-SCORE
   .5000   .5000       -7.10338       .00000
   .0000   .0000       -7.10338       .00000
   .0010   .0010       -7.10338       .00000
   .0100   .0100       -7.10338       .00000
   .0500   .0500       -7.10338       .00000
   .1000   .1000       -7.10338       .00000
   .1500   .1500       -7.10338       .00000
   .2000   .2000       -7.10338       .00000
   .2500   .2500       -7.10338       .00000
```

.3000	.3000	-7.10338	.00000
.3500	.3500	-7.10338	.00000
.4000	.4000	-7.10338	.00000
.4500	.4500	-7.10338	.00000

PEDIGREE 3 Khan

15 PERSONS

LOCUS 0 SCID VS. LOCUS 1 XG12

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-9.16738	.00000
.0000	.0000	-8.56533	.60205
.0010	.0010	-8.56664	.60074
.0100	.0100	-8.57843	.58895
.0500	.0500	-8.63210	.53528
.1000	.1000	-8.70201	.46537
.1500	.1500	-8.77469	.39269
.2000	.2000	-8.84933	.31805
.2500	.2500	-8.92435	.24303
.3000	.3000	-8.99712	.17026
.3500	.3500	-9.06358	.10380
.4000	.4000	-9.11816	.04922
.4500	.4500	-9.15454	.01284

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	.61888
.0010	.0010	.61751
.0100	.0100	.60513
.0500	.0500	.54897
.1000	.1000	.47622
.1500	.1500	.40102
.2000	.2000	.32419
.2500	.2500	.24730
.3000	.3000	.17299
.3500	.3500	.10534
.4000	.4000	.04990
.4500	.4500	.01301

```
 1 0010 0.00001      SCID Linkage Study  (S21)
(20A4)
(2A4,10F2.0)
(20A4)
                m
 2 2
 2 3
 0-1
 9
 0000 0000
 0010 0010
 0100 0100
 0500 0500
 1000 1000
 1500 1500
 2000 2000
 2500 2500
 3000 3000
 3500 3500
 4000 4000
 4500 4500
60000
SCID    A    a aff n.a
    0001     9999
   A    A 1 0
   A    a 0 1
   a    a 0 1
S21     U    L  UU  UL  LL
    6500     3500
   U    U 1 0 0
   U    L 0 1 0
   L    L 0 0 1
  17    0Bejarano/Garcia/Gundin
 1.1              m n.a
 1.2              f n.a
 2.1              m n.a  UU
 2.2 1.1 1.2      f n.a  UL
 2.3              m n.a  UU
 2.4 1.1 1.2      f n.a  UL
 2.5              m n.a  LL
 2.6 1.1 1.2      f n.a  UL
 3.1 2.1 2.2      m n.a  UU
 3.2 2.1 2.2      m n.a  UU
 3.3 2.1 2.2      m aff
 3.4 2.3 2.4      f n.a  UU
 3.5 2.3 2.4      m n.a  LL
 3.6 2.5 2.6      f n.a  UL
 3.7 2.5 2.6      m n.a  UU
 3.8 2.5 2.6      m aff
 3.9 2.5 2.6      m aff
7000
  12    0Tebbe/Koenitz/Moller
 1.1              m n.a
 1.2              f n.a  UU
 2.1              m n.a
 2.2 1.1 1.2      f n.a  UU
```

```
 2.3             m n.a
 2.4 1.1 1.2     f n.a   UU
 2.5 1.1 1.2     m aff
 2.6 1.1 1.2     m aff
 2.7 1.1 1.2     m n.a   UU
 3.1 2.1 2.2     m n.a
 3.2 2.3 2.4     f n.a   UU
 3.4 2.3 2.4     m aff
7000
  15   OKhan
 1.1             m n.a
 1.2             f n.a   UU
 2.1 1.1 1.2     m aff
 2.2             m n.a
 2.3 1.1 1.2     f n.a   UU
 2.4 1.1 1.2     m n.a
 2.5 1.1 1.2     m n.a
 3.1             m n.a
 3.2 2.2 2.3     f n.a   UL
 3.3 2.2 2.3     m aff
 3.4 2.2 2.3     m n.a
 3.5 2.2 2.3     m aff
 3.6 2.2 2.3     m aff
 3.7 2.2 2.3     m aff
 4.1 3.1 3.2     m aff   UU
9000
```

Program LIPED Version for IBM PC March 1987 J. Ott

PROBLEM 1 SCID Linkage Study
****

PEDIGREE 1 Bejarano/Garcia/Gundin
---

17 PERSONS

LOCUS 0 SCID VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

GENE FREQUENCIES FOR 0 SCID .0001 .9999
GENE FREQUENCIES FOR 1 S21 .6500 .3500

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-9.41471	.00000
.0000	.0000	-9.24058	.17412
.0010	.0010	-9.23985	.17486
.0100	.0100	-9.23398	.18073
.0500	.0500	-9.22159	.19312
.1000	.1000	-9.22907	.18563
.1500	.1500	-9.25331	.16139
.2000	.2000	-9.28789	.12682
.2500	.2500	-9.32740	.08731
.3000	.3000	-9.36649	.04821
.3500	.3500	-9.39955	.01516
.4000	.4000	-9.42108	-.00637
.4500	.4500	-9.42677	-.01207

PEDIGREE 2 Tebbe/Koenitz/Moller
---

12 PERSONS

LOCUS 0 SCID VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-6.07757	.00000
.0000	.0000	-6.07757	.00000
.0010	.0010	-6.07757	.00000
.0100	.0100	-6.07757	.00000
.0500	.0500	-6.07757	.00000
.1000	.1000	-6.07757	.00000
.1500	.1500	-6.07757	.00000
.2000	.2000	-6.07757	.00000
.2500	.2500	-6.07757	.00000

.3000	.3000	-6.07757	.00000
.3500	.3500	-6.07757	.00000
.4000	.4000	-6.07757	.00000
.4500	.4500	-6.07757	.00000

PEDIGREE 3 Khan

15 PERSONS

LOCUS 0 SCID VS. LOCUS 1 S21

Mutation rate at current main locus = .000010000000

R MALE	R FEM.	LOG10(L(R))	LOD-SCORE
.5000	.5000	-8.32866	.00000
.0000	.0000	-8.02764	.30102
.0010	.0010	-8.02808	.30058
.0100	.0100	-8.03201	.29665
.0500	.0500	-8.04992	.27874
.1000	.1000	-8.07340	.25526
.1500	.1500	-8.09822	.23044
.2000	.2000	-8.12455	.20411
.2500	.2500	-8.15258	.17608
.3000	.3000	-8.18254	.14612
.3500	.3500	-8.21472	.11394
.4000	.4000	-8.24948	.07918
.4500	.4500	-8.28727	.04139

SUMMARY

R MALE	R FEM.	SUM OF LODS
.0000	.0000	.47514
.0010	.0010	.47544
.0100	.0100	.47738
.0500	.0500	.47186
.1000	.1000	.44089
.1500	.1500	.39183
.2000	.2000	.33093
.2500	.2500	.26339
.3000	.3000	.19433
.3500	.3500	.12909
.4000	.4000	.07280
.4500	.4500	.02932

PERSONAL PUBLICATIONS RELATED TO THIS THESIS

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