GENETIC STUDIES OF BLOOD COAGULATION FACTOR XIII

A thesis submitted

to

THE FACULTY OF GRADUATE STUDIES
UNIVERSITY OF MANITOBA

In partial fulfillment of the requirements for the
DEGREE OF MASTER OF SCIENCE

by

PAUL WONG
DEPARTMENT OF ANATOMY

ISBN Ø-315-33925-X

GENETIC STUDIES OF BLOOD COAGULATION FACTOR XIII

BY

PAUL WONG

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TO MY FAMILY

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to all who have assisted in the completion of this research. I would like to especially thank Dr. P.J.McAlpine for her invaluable guidance and infinite patience throughout this project.

I would like to express my appreciation to the faculty, staff, and students of the Department of Human Genetics for their support and to thank in particular L. Komarnicki, J. Alleslev and J. Knoll for meaningful discussions. In addition I would like to thank S.Philipps for her limitless help and advice.

I would like to thank the following for providing samples and/or phenotypic data used in the present study:

1. M.Lewis, H.Kaita, and S.Philipps: Rh lab, University of Manitoba, Winnipeg.
2. A.E.Chudley, and M.Ray: Department of Human Genetics, University of Manitoba, Winnipeg.
3. M.L.Schroeder: Children's Hospital, Winnipeg.
4. M.Taylor: Canadian Red Cross, Winnipeg.
5. D.W.Cox: Department of Genetics, Hospital for Sick Children, Toronto.
6. H.Hughes: Department of Genetics, Hospital for Sick Children, Toronto.
7. T.Feasby: Department of Clinical Neurological Sciences, University Hospital, London, Ontario.
8. P.W.Allderdice: Faculty of Medicine, Memorial University of Newfoundland, St John's.
9. E.Giblett: Puget Sound Blood Centre, Seattle, Washington.

The work presented in this thesis was supported in part through a studentship funded by the Manitoba Health Research Council as well as funding from the Medical Research Council of Canada (MT6112) and the Children's Hospital of Winnipeg Research Foundation Inc.

ABSTRACT

In the present study the nature and frequency of variation of the mutant genes defining the F13A and F13B loci were investigated in samples of Canadian subpopulations. Both loci were found to be polymorphic. Segregation of F13A phenotypes in all mating types were consistent with Mendelian expectations for two codominant alleles at a single autosomal locus. Segregation of F13B phenotypes in seven of eight mating types screened were consistent with Mendelian expectations for three codominant alleles at a single autosomal locus.

Classical linkage analysis and exclusion mapping were used to define the genetic map positions of F13A and F13B. Linkage studies of F13A with chromosome 6p marker loci confirmed linkage between F13A and HLA and suggested linkage between F13A and GLO. The addition of the present data to those previously reported verify a gene order of 6pter:F13A:HLA:GLO:cen. Close linkage between 42 of the 62 loci tested and F13B was excluded on the basis of male and/or female lod scores; for eleven of these loci (C4A, CMT, FR, GOT2, APOC2, LW, NP, SOD1, WD, WR, AND YT) the data obtained represent the first report of formal exclusion of linkage with F13B. When the present linkage data were considered in relation to the physical locations of the marker loci, F13B could be excluded from approximately 25% of the human genome. Results of exclusion mapping excluded F13B from an additional 1.5% of the genome.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1.000 INTRODUCTION

Human coagulation factor XIII (F13) is one of a set of plasma proteins required for the formation of a stable fibrin clot. The clotting process features elaborate sequential cascades of proenzyme to enzyme conversions as well as other complex biological reactions (Fig.1). Two pathways are known to initiate the steps involved in coagulation. The intrinsic pathway refers to the sequence of reactions as they occur *in vitro* when the exposure of plasma to a negatively charged surface such as glass leads to the activation of factor XII (F12). The extrinsic pathway refers to the sequence of reactions as they occur *in vivo* when the interaction of factor VII(F7) with a phospholipid tissue factor and calcium ions leads to the formation of a complex which activates factor X(F10) (reviewed by Graham *et al.*,1983). The sequence of reactions that occur after the activation of F10 is identical in both pathways. Factor 13 participates in the final reaction of blood coagulation by catalyzing the formation of γ-glutamyl-2-lysl crosslinks between fibrin molecules which stabilize the clot structure.

The blood coagulation process, although extensively investigated, is not fully understood. In addition a genetic contribution to normal blood coagulation has long been appreciated but detailed genetic dissection of this system is incomplete. According to Ratnoff (1978), the earliest record of a familial disorder of hemostasis appears in the Tract Yebamoth of the Babylonian Talmud,

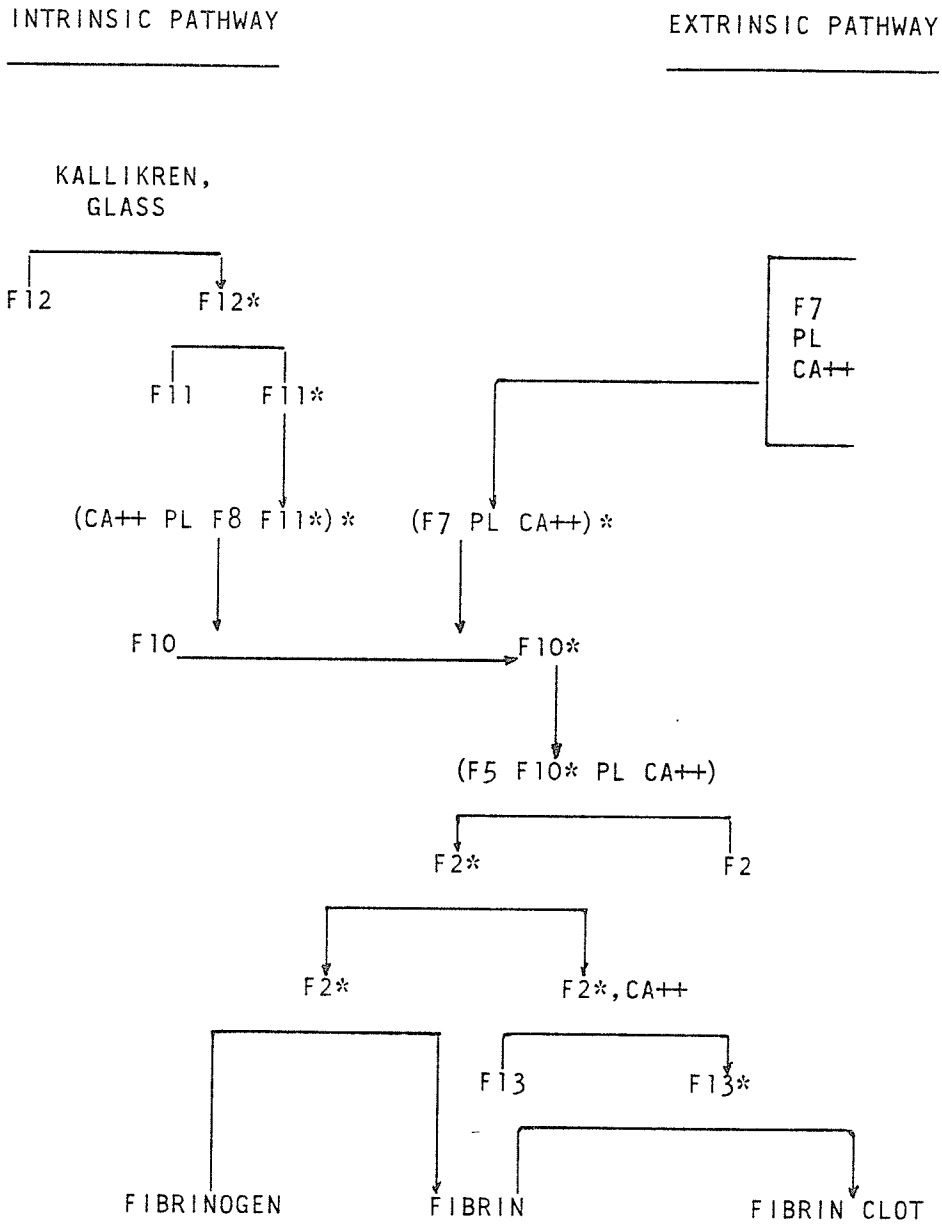

FIG 1. THE BLOOD COAGULATION CASCADE (MODIFIED AFTER GRAHAM ET AL., 1983).

F:FACTOR; *:ACTIVATED; CA++:CALCIUM IONS; PL:PHOSPHOLIPID; ( ):COMPLEX; ->:DIRECTION OF REACTION.

where it is reported that, during the second century A.D., a boy was exempted from circumcision because his brothers had experienced excessive bleeding following this procedure. The understanding of the genetics of blood coagulation, until recently, has relied heavily on the study of such apparent inherited deficiencies of specific factors which resulted in the manifestation of a hemostatic disorder. However, the rarity of these deficiencies together with the lack of appropriate technology to detect normal variation of the blood coagulation proteins have limited the amount of progress made in the understanding of the genetic basis of blood coagulation components.

Nevertheless a number of genes responsible for proteins involved in blood coagulation have been mapped to the human genome by exclusion, family, and molecular studies (Table 1). Still the structural genes coding for factor XI (F11), factor II (F2), factor V (F5), and factor 13B (F13B) among others have yet to be assigned to the genetic map.

Recently new technological innovations have allowed the study of normal variation of several blood coagulation factors such as fibrinogen (F1), factor II (F2), and the components of factor 13 (F13A and F13B) (Olaisen et al., 1982; Board et al., 1982; Board, 1979; Board,1980). In the present study, genetically determined qualitative variation of both components of F13 was investigated to assess the nature and extent of normal allelic variation of the F13 loci in Canadians and to use this variation in an attempt to map these two loci in man.

TABLE 1. TABULATION OF GENE LOCI CODING FOR KNOWN CLOTTING FACTORS WHICH HAVE BEEN MAPPED TO SPECIFIC CHROMOSOMAL REGIONS.

LOCUS	REGIONAL ASSIGNMENT	TYPE OF STUDY	REFERENCES
FGG	4q28-q31	F:linkage studies	Olaisen et al.,1982
F3	1pter-p21	S:somatic cell hybrids	Carson et al.,1985
F7	13q34	E:deletion mapping	Pfeiffer et al.,1982
		E:deletion mapping	deGrouchy et al.,1984
F8C	Xq28	F:linkage studies	Tsevrenis et al.,1979
F8VWF	12pter-p12	M:in situ hybridization	Ginsburg et al.,1985
		M:in situ hybridization	Verweij et al.,1985
F9	Xq26-q27.3	M:somatic cell hybrids	Chance et al.,1983
		M:somatic cell hybrids	Camerino et al.,1984
		M:in situ hybridization	Boyd et al.,1984
		M:in situ hybridization	Purrello et al.,1985
		M:in situ hybridization	Mattei et al.,1985
		M:somatic cell hybrids	Mattei et al.,1985
		M:in situ hybridization	Buckle et al.,1985
F10	13q34	E:deletion mapping	Pfeiffer et al.,1982
		E:deletion mapping	deGrouchy et al.,1984
F12	6p23	E:deletion mapping	Pearson et al.,1981
		E:deletion mapping	Niebuhr et al.,1985
F13A	6p23-qter	F:linkage studies	Olaisen et al.,1985
		E:deletion mapping	Niebuhr et al.,1985

F:family studies; E:exclusion mapping; M:molecular studies; S:somatic cell hybrid studies.

1.100 Structure and Function of F13

Factor 13 is found in plasma, on platelet membranes, and in placental tissue (Barbui et al., 1974; Seelig and Folk, 1980; Kreckel et al., 1982). In plasma, the F13 proenzyme is a glycoprotein consisting of two polypeptide species, F13A and F13B, organized into a tetramer with a molecular formula of A_2B_2 (Loewy et al., 1961; Schwartz et al., 1971; Israels et al., 1973; Schwartz et al., 1973). In this structure the two different polypeptide types are believed to be associated non-covalently (Schwartz et al., 1971; Schwartz et al., et al., 1973). In contrast, platelet and placental F13 exists solely as an A_2 dimer (Seelig and Folk, 1980).

F13A and F13B are genetically distinct glycoproteins which differ in amino acid composition, molecular size and the degree of glycosylation (Schwartz et al., 1971; Schwartz et al., 1973; Board, 1979; Board, 1980). F13A has a molecular weight of 75,000 daltons and negligible amounts of associated carbohydrate while F13B has a molecular weight of 88,000 daltons and most of the carbohydrate associated with the F13 proenzyme (Schwartz et al., 1971; Schwartz et al., 1973). In both cases the gene loci responsible for the structural proteins are autosomal but not linked (Board, 1979; Board, 1980).

Conversion of the plasma F13 proenzyme to the enzymatically active form is regulated by thrombin and calcium ions (Lorand et al., 1974). Thrombin cleaves a 4000 dalton peptide from each of the F13A subunits present in the tetramer (Schwartz et al., 1973). Calcium ions

interact with the modified F13A subunits to unmask a specific cysteine residue which distorts the F13A tertiary structure and in turn leads to the dissociation of the F13B subunits (Lorand et al., 1974). The remaining modified F13A dimer represents the active form of F13 responsible for the transglutaminase activity which crosslinks and stabilizes the fibrin clot. F13B does not seem to have a direct role in the blood coagulation pathway; it may, however, play a secondary role in the protection of F13A subunits from premature degradation and/or regulate further activation of F13 by negative feedback (Schwartz et al., 1973; Seelig and Folk, 1980).

1.200 Genetically Determined Variation

Three different substitutions can be made for any nucleotide in a given DNA sequence so that for a group of three consecutive nucleotides 63 different changes can be made at the DNA level each generating a new mutant triplet. Thus it follows that a very large number of structurally different alleles can be generated by separate mutational events within a single gene. The total potential for genetic variation at the DNA level is, however, never realized at the protein level because alterations in DNA resulting in synonomous mutations and variation in DNA corresponding to those areas which are not translated into protein, such as intervening sequences, will not generate a structurally altered protein.

Changes in the genetic code which are reflected in the structural protein may affect the quality or the quantity of the protein which in turn may result in the formation of a normal or an abnormal variant with pathological and/or clinical consequences.

1.210 Abnormal Phenotypic Variants

Mutant phenotypes which appear clinically as protein deficiency disorders characterized by reduced clotting efficiency have been described for many of the major blood coagulation factors presently known. The most common of these disorders is Hemophilia A, a disorder characterized by a lack of physiologically active antihemophilic factor (factor VIII, procoagulant). In contrast, congenital F13 deficiency appears to be a very rare disorder (Ratnoff, 1978; Graham et al.,1983).

The inheritance of F13 deficiency has been shown to be autosomal recessive (Lorand et al., 1970; McDonagh et al., 1971; Barbui et al., 1978; Fried et al., 1981; Berliner et al., 1984), although a single family has been reported in which X-linked inheritance of F13 deficiency cannot be excluded (Hampton et al., 1966). People who are heterozygous for F13 deficiency alleles have up to 60% of normal F13A activity levels and do not exhibit any bleeding tendencies (Lorand et al., 1970; McDonagh et al., 1971).

People homozygous for F13 deficiency alleles (or compound heterozygotes) lack F13A subunits and have decreased amounts of F13B subunits (Israels et al., 1973; Barbui et al.,1978). Homozygotes experience haemorrhagic episodes after trauma and exhibit slow and poor wound healing (Duckert et al., 1960; Hampton et al., 1966). In these individuals blood coagulation initially appears to function normally after injury. Severe bleeding, however, usually occurs 24-36 hours later and lasts for weeks (Duckert et al., 1960). Clots which form in these individuals are unlike those from unaffected people in that they are very loose and are soluble in both 5 M urea and 1% monochloroacetic acid (Duckert et al., 1960; Ratnoff and Steinberg, 1968). Several other clinical symptoms such as bleeding from the umbilical stump approximately a week after birth and the occurence of intracranial haemorrhage have become associated with F13 deficiency. In addition, a high degree of fetal wastage among F13 deficient women has been reported (Duckert et al., 1960; Fried et al., 1981; Graham et al., 1983).

1.220 Normal Phenotypic Variants

1.221 Detection of qualitative variation after electrophoresis

Electrophoresis combined with detection systems specific for single enzymes and other proteins is a powerful tool for demonstrating genetically determined qualitative variation, but it is not

sufficiently sensitive to differentiate all possible differences that may occur in variant proteins. Theoretically only one third of the possible changes in the net charge of a polypeptide would be reflected by a change in electrophoretic mobility (Harris, 1980). Furthermore, electrophoretic methods do not discriminate between protein variants produced by different amino acid substitutions which result in the same net charge nor do they identify variants arising from a single amino acid substitution in which the original amino acid is substituted by one that is similiarly charged.

1.222 F13A Electrophoretic Variants

After electrophoresis of plasma samples from a group of 179 unrelated Australian blood donors, Board (1979) identified three common phenotypes, designated F13A1, F13A2-1, F13A2 which he attributed to the segregation of two autosomal codominant alleles, F13A*1 and F13A*2, with estimated frequencies of 0.79 and 0.21 respectively. Kreckel et al. (1982) electrophoretically examined platelets from 239 unrelated blood donors from Hessen, Germany for F13A phenotypes and estimated that the F13A*1 and F13A*2 alleles occurred with frequencies of 0.797 and 0.203 respectively in the population examined. From the analysis of 250 unrelated Japanese blood donors, Kera and Nishimukai (1982) estimated that the F13A*1 and F13A*2 alleles occurred with frequencies of 0.90 and 0.10 respectively in their sample population.

Several rare F13A alleles have been reported in the literature. These include a null allele, F13A*0; an allele which gives rise to a subunit with an increased level of transglutaminase activity, F13A*3; and an allele which gives rise to a variant with increased anodal mobility, F13A*4 (Board et al., 1980; Castle et al., 1981; Board and Coggan, 1981). In all cases only phenotypes which represent a heterozygote combination of the rare allele with one of the two common alleles have been observed.

1.223 F13B Electrophoretic Variants

Six common F13B phenotypes, designated F13B1, F13B2, F13B3, F13B2-1, F13B3-1, and F13B3-2, were detected after electrophoresis of plasma samples from 245 unrelated Australian blood donors and attributed to the segregation of three autosomal codominant alleles, F13B*1, F13B*2, and F13B*3 with estimated frequencies of 0.7469, 0.0836 and 0.1693 respectively (Board, 1980; Board, 1984). Similarily, Kreckel and Kuhnl (1982) examined plasma from 178 unrelated blood donors from Hessen, Germany for F13B phenotypes and have estimated that the alleles, F13B*1, F13B*2 and F13B*3 occurred with frequencies of 0.708, 0.109, and 0.183 respectively in the population studied. Two rare phenotypic variants with altered electrophoretic mobility have been observed and attributed to the heterozygous combination of one of two rare alleles (designated as F13B*4 and F13B*5) with one of the common alleles (Board, 1984).

Electrophoretic and isoelectric focusing (IEF) studies of plasma samples from members of Japanese populations have failed to detect phenotypes involving the F13B2 subunit (Kera et al., 1981; Nishigaki and Omato, 1982; Nakamura and Abe, 1982). In the case of the data obtained by IEF analysis, the inability to identify phenotypes involving F13B2 could be technical as IEF does not resolve F13B1 and F13B2 components (Board,1984). However, the failure to detect the F13B2 subunit electrophoretically using methods which are able to differentiate F13B1 from F13B2 suggests that the F13B*2 allele was either absent or existed at a very low frequency in the Japanese population studied.

1.230 Genetic Polymorphisms

The frequencies with which the common F13A and F13B alleles occur in the populations already studied indicate that the loci at which these alleles occur are polymorphic. A genetic polymorphism, according to Ford's definition, exists when individuals of a population can be categorized into two or more distinct phenotypes which are determined by two or more alleles, each occurring at a frequency higher than can be explained by recurrent mutation, at a given gene locus (Cavalli-Sforza and Bodmer, 1971). As it is generally accepted that mutation rates in man do not exceed 10^{-4} mutations per locus per generation, it follows that allele frequencies of 0.01 or greater are not expected to be achieved by recurrent mutation. For practical

purposes it has been customary to classify loci as polymorphic when 2% or more of the population were heterozygous at the locus concerned. More recently the definition of a genetic polymorphism has been revised to describe those loci at which the most common allele has a frequency no greater than 0.99 without any constraint on the composition of the other 1% alleles (Harris, 1980).

1.300 Gene Mapping

Characterization of genetically determined traits should include estimation of the frequency of the mutant alleles that define the locus (loci) to which the trait is attributed as well as determination of the map position(s) of the locus (loci). Mapping of human genes has been accomplished through the use of several different approaches such as classical linkage analysis, gene dosage and exclusion mapping in persons with known aneuploid chromosome constitution, the analysis of somatic cell hybrids, and *in situ* hybridization. These approaches have already been used successfully in the mapping of several blood coagulation factor genes (Table 1). Of particular interest to the present study are the applications of classical linkage analysis and exclusion mapping.

1.310 Linkage Studies

1.311 Theoretical Considerations

Linkage is the occurrence of two gene loci sufficiently close together on the same chromosome so that their alleles do not assort independently. The distance between these two loci can be measured indirectly by the amount of recombination that occurs between them. It is generally assumed that the more distant two loci are the more probable that recombination will occur between them so that the alleles at loci which are far apart on the same chromosome or on different chromosomes tend to assort independently, i.e. show 50% recombination.

The rationale in applying classical linkage analysis to gene mapping is as follows: if a gene, A, is localized to a specific area, W, of chromosome K and it is shown that gene B is closely linked to gene A, then logic dictates that gene B must also be situated near area W of chromosome K.

1.312 Linkage Analysis

The method of linkage analysis adopted in the current study is the lod score method of Morton (1955). Mathematically this method is a sequential test which compares the likelihood of obtaining the observed data under the assumption of various values of the recombination fraction (θ) between two loci with those which would be obtained at a recombination value of 0.50 (i.e. independent assortment).

Linkage data are obtained from family studies and focus on the segregation of alleles at specific loci or physical markers on chromosomes (such as heterochromatic regions and breakpoints of structurally rearranged chromosomes) in relation to other loci (or chromosomal markers). Nonallelic genes which consistently segregate together are probably linked. Matings in which clear segregation of the alleles at two loci is evident provide informative linkage data whereas matings in which clear segregation cannot be observed are not informative. In order to obtain linkage data at least one parent in a given nuclear family must be heterozygous at the two loci of interest. Phenotypes of grandparents, grandchildren and partially examined children are not used directly in the calculation of the lod scores. They are, however, useful in further defining the genotypes of the parents in the nuclear family being examined. Analysis of grandparents are necessary to establish the phase of the doubly heterozygous parent(s). Families in which the phase is known are much more informative than those in which phase is unknown (Maynard-Smith et al., 1961).

For large kindreds the linkage program used in the present study subdivides individuals of that pedigree into nuclear families. For each locus pair the total lod score for the kindred is the sum of the lod scores of each individual nuclear family in the pedigree. Furthermore the total lod scores for a locus pair consists of adding the individual lod score values obtained for each kindred tested. One

of three possible interpretations i.e. 1) linkage exists between the two loci considered, 2) linkage does not exist, or 3) more data are required in order to reach a final conclusion, can be made at all times from the data at hand. In practice, if the total lod score value at any specific θ value exceeds the value of +3 then it is generally accepted that the two loci are linked. If the total lod score is less than -2 at any value of θ (e.g. θ_1), then one generally excludes linkage between the two loci at θ values equal to and less than θ_1 (Morton, 1955).

1.313 Linkage Studies of F13A and F13B

At the beginning of the present study the location of the F13A and F13B loci in the human genome were not known. Shortly thereafter, Keats et al. (1984) presented a maximum lod score of +1.67 at θ=0 between F13A and JK, a blood group locus believed to be on chromosome 2 (Ott, 1974; Keats et al., 1977). Simultaneously Olaisen et al. (1984) reported a maximum paternal lod score of +6.9 at a θ value of 0.08 and a maximum maternal lod score of +0.14 at a θ value of 0.44 between F13A and the HLA gene complex, which maps to 6p21.3 (Robson and Lamm, 1984). On the basis of these data F13A was provisionally assigned to 6p21-qter (Robson and Lamm, 1984); the assignment has since been refined to 6p21-6p24.

No lod scores have since appeared in the literature to support or refute the possible linkage of JK with F13A. A number of studies, however, have provided additional data in support of HLA:F13A linkage (Board et al., 1984; Eiberg et al., 1984; Olaisen et al.,1985) and have examined the relationships of F13A with other marker loci on chromosome 6 such as GLO, a locus located on 6p and closely linked proximally to HLA (Weitkamp and Guttormsen, 1976; Bender and Grzeschik, 1976), and PGM3, a locus situated on 6q but which is loosely linked to HLA (Lamm et al., 1971). In all cases F13A:GLO lod scores did not reach significant levels for the acceptance of linkage; however, the θ value corresponding to the maximum paternal F13A:GLO lod score was larger than that corresponding to the maximum paternal F13:HLA lod score in each study, thus suggesting a gene order of pter:F13A:HLA:GLO:cen (Board et al., 1984; Eiberg et al., 1984; Olaisen et al., 1985). In addition Olaisen et al.(1985) have reported data which indicate that F13A is not linked to PGM3 thus supporting the proposed gene order.

Linkage studies of F13B with other marker loci have not led to its localization within the human genome. The lod scores of F13B with a large number of commonly studied genetic markers have however reached formally significant levels which exclude linkage of these marker loci to F13B (Eiberg et al., 1984). Recently a hint of close linkage (total lod score of +1.71 at θ=0.05) between the cystic fibrosis gene and F13B was reported (Eiberg et al., 1985), but this potential linkage relationship has since been weakened with additional testing (P.J. McAlpine personal communication, 1985).

1.320 Exclusion Mapping

Exclusion mapping involves the analysis of individuals with known autosomal deficiencies. If for example, an individual with a specific autosomal deficiency can be demonstrated to have two alleles at any autosomal locus, then the locus in question can be excluded from the deleted segment. Conversely, if an individual with a specifc autosomal deficiency has only one copy of an autosomal gene, then assignment of that locus to the deleted segment is suggested.

1.400 Specific Aims

In the present study genetically determined qualitative variation of the components of F13 was examined with the following specific aims:

1. to assess the nature and extent of F13A and F13B allelic variation in Canadian population groups

2. to determine if F13A is within measurable recombination distance from one or more marker loci, with particular emphasis on HLA and JK

3. to determine if F13B is in measurable recombination from one or more other marker loci

4. to attempt to identify the chromosomal assignment of the F13B locus from the analysis of F13B phenotypes in individuals with known aneuploid chromosome constitutions.

2.000 MATERIALS AND METHODS

2.100 Plasma Samples

Plasma samples analyzed were obtained from an existing store of blood samples gathered as part of a continuing research project designed to map human genes by family studies. These families were ascertained through a variety of variant phenotypes: blood group antigens, electrophoretically detected variants of enzymes and other proteins, structural chromosomal rearrangements and clinically defined traits. None of the families were known to carry structural rearrangements involving chromosomes 6 or 2.

Plasma samples were isolated from anticoagulated venous blood by centrifugation at 1640 X g for 5 minutes and stored at either -70°C or -20°C until examined. Samples analyzed for F13A were used neat while those analysed for F13B were incubated with neuraminidase (Sigma type V , dissolved in 0.01 M Na_2HPO_4 (which was initially adjusted to pH 4.8 with 40% citric acid) in order to obtain a final concentration of 10 U/ml) in a 1:1 ratio overnight at 37°C prior to analysis.

2.200 Sample Selection

Families analyzed for F13A were initially selected without regard to previously tested loci showing segregation but in the later part of the study were selected if HLA phenotyping had been performed. Families analyzed for F13B were selected on the basis of segregation at gene loci at which allelic variation is generally uncommon or technically difficult to detect. In addition a set of individuals was selected for F13B analysis because they possessed known aneuploid constitutions. Allele frequenciers were estimated from the phenotypes of randomly selected unrelated individuals together with those of the unrelated individuals of the families studied for each marker.

2.300 General electrophoretic conditions

Electrophoresis was carried out on thin layer agarose gels on 12 cm x 12.5 cm glass plates. Sample slots were formed by placing a plastic comb 0.2-0.5 mm above and 2 cm from the potential cathodal end of the glass plate before spreading 30 ml of 1% agarose (w/v) in gel buffer over the glass plate. The gel was allowed to solidify at room temperature after which the plastic comb was removed.

Four µl of a hemoglobin marker (a red blood cell lysate made from freeze thawing packed red blood cells washed with normal saline) were applied to the two outer most sample slots on each gel. Volumes

of up to 13 µl of plasma were then applied to each of the remaining slots.

Electrophoresis was carried out with the gel sandwiched horizontally between cooling plates through which water at 7°C circulated. The gel was connected to the bridge buffer by wicks consisting of eight layers of Whatman chromatography paper 1 (WCP1).

2.310 F13A Electrophoretic conditions

The buffer system utilized was essentially that described by Graham (personal communication, 1983). The stock buffer was TEB pH 8.6 (0.90 M Tris, 0.02 M Na_2EDTA, and 0.50 M Boric acid). The gel and bridge buffers were 1 in 10 and 1 in 4 dilutions, respectively, of the stock buffer. Electrophoresis was carried out at 18 V/cm at 7°C until the hemoglobin marker had migrated 5 cm toward the anode.

2.320 F13B Electrophoretic conditions

The buffer system utilized was a modification of that described by Board (1980). The bridge buffer consisted of 37.7 mM lithium hydroxide and 248.3 mM boric acid, pH 8.1. The gel buffer was prepared by combining 90 volumes of a solution consisting of 7.62 mM citric acid

and 52 mM Tris, pH 8.3, with 10 volumes of bridge buffer. Electrophoresis was carried out at 7°C at 9 V/cm until the hemoglobin marker had run out of the well. The sample slots were then filled with liquid agarose which was allowed to solidify and electrophoresis allowed to continue until the hemoglobin markers had migrated 8 cm towards the anode.

2.400 Detection Systems

2.410 F13A Detection System

F13A activity was demonstrated using a functional detection system in which zones of transglutaminase activity were identified by first overlaying each gel with two layers of WCP1 freshly soaked in a reaction mixture modified after Board (1979) and Graham(1983). This reaction mixture contained:

1. 1 ml, 1 M Tris/HCl pH 8.0
2. 2 ml, 0.05 M CaCl
3. 3 ml, casein (Sigma) 10 mg/ml : disolved in 0.2 M Tris/HCl pH 8.0
4. 1 ml, monodansyl cadaverine (Sigma): 10 mg disolved in 300 µl 0.1 M HCl and then made up to volume with 0.1 M Tris/HCl pH 8.0
5. 200 U, topical thrombin, bovine (Parke-Davis)
6. 20 µl, β-mercaptoethanol

Gels with the reaction mixture overlay were incubated in a humid environment at 37°C for 14-16 hr. During incubation , areas on the gel in which normal F13A protein had been localized catalyze the crosslinking of monodansyl cadaverine, a fluorescent amine, to casein.

After incubation the WCP1 paper overlays were removed and the gels were fixed and washed according to a procedure modified after Graham (1983). All washes were performed at room temperature in plastic containers with gentle agitation. The gels were first rinsed in distilled water and then washed in 200 ml of a 10% Trichloroacetic acid solution for 20 min to precipitate the casein of the reaction mixture onto the gel. After fixation, a number of steps were undertaken to elute free monodansyl cadaverine from the gel. Initially gels were rinsed in distilled water and then subjected to a 20 min wash followed by a 30 min wash in 10 % acetic acid . Subsequently the gels were rinsed in distilled water, and washed in 0.1 M Tris buffer pH 7.5 for 20 min. Areas of F13A transglutaminase activity appeared indirectly as areas of casein-bound monodansyl cadaverine which fluoresced under shortwave UV light.

2.420 F13B Detection System

F13B was detected by an enzyme-linked immunoblotting procedure as described by Board (1984). After electrophoresis, proteins in the gel were passively blotted (transferred) to a nitrocellulose filter which had been soaked (for at least 4 hr) in washing buffer (0.05 M

Tris, 0.15 M NaCl, pH 7.5). Blotting was achieved by placing in succession, the nitrocellulose, a piece of WCP1 soaked in washing buffer, a 1 cm thick layer of absorbent paper, a glass plate and a two kg weight on top of the gel. Blotting was allowed to continue for one hr.

After blotting, the nitrocellulose filters were soaked overnight in 3% bovine serum albumin dissolved in washing buffer inorder to block areas on the nitrocellulose blot where protein had not been transferred. The filters were then soaked in 20 ml of washing buffer containing 2 µl/ml rabbit anti-human factor XIIIB subunit (Calbiochem) for 30 minutes, washed in 200 ml of washing buffer for two hr, soaked for 30 min in 20 ml of washing buffer containing 2 µl/ml of goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) and washed in 200 ml of washing buffer for two hr. All procedures were performed at room temperature in plastic containers with gentle agitation.

At the end of the washes the F13B protein bound to the nitrocellulose filter was indirectly associated with the enzyme alkaline phosphatase. Thus, areas of F13B protein were detected by developing the blot for alkaline phosphatase activity by incubating the filter in 50 ml staining buffer(0.06 M Boric acid, 0.04 M NaOH, pH 9.4) containing 60 mg $MgSO_4$, 25 gm ß-napthyl-phosphate and 25 mg Fast Blue BB salt.

2.500 Linkage Analysis

F13A and F13B segregation data were tested for linkage with other marker loci using the Mark III program of Côté (1975) which utilizes the lod score method of Morton (1955). Lod scores were calculated at θ = 0.05, 0.10, 0.20, 0.30, and 0.40, separated into paternal, maternal and intercross categories according to the sex of the informative parent(s) and summed separately. Maximum lod scores ($\hat{z}$) and corresponding $\hat{\theta}$'s were estimated from graphical presentations of the lod scores. To assist in these estimations lod scores were calculated at additional values of θ. The 95% confidence intervals for $\hat{\theta}$ were defined by the θ's corresponding to the lod scores at ($\hat{z}$-1) (Conneally et al., 1985).

2.600 Nomenclature

The genetic notation used follows the designations outlined by the International Systems for Human Gene Nomenclature (Shows et al., 1979; Shows and McAlpine, 1982; Shows et al., 1984; McAlpine et al., 1985); genetic notation used in the present study which are not found in these publications are listed in the appendix.

3.000 RESULTS

3.100 Description of Phenotypes

3.110 F13A

The F13A electrophoretic patterns of 1887 individual plasma samples after agarose gel electrophoresis appeared to be the same as those described by Board (1979) where the phenotype designated as F13A2 is represented by a single protein band which migrates slightly anodally, the phenotype designated as F13A1 is represented by a single band which migrates more anodally, and the phenotype designated as F13A2-1 is represented by three distinct bands, two of which are the same as the F13A1 and F13A2 protein bands and a third which migrates between the bands of F13A1 and F13A2 phenotypes (Fig. 2).

3.120 F13B

The F13B protein band patterns of 1032 individual plasma samples after agarose gel electrophoresis and immunoblotting were similar to the six phenotypes observed by Kreckel and Kuhnl (1982) where three common phenotypes (designated as F13B1, F13B2, F13B3) each presented with a single intense major protein band and a number of minor protein bands which were fainter and more anodal and three common phenotypes (designated as F13B2-1, F13B3-2, F13B3-1) each showed two intense major

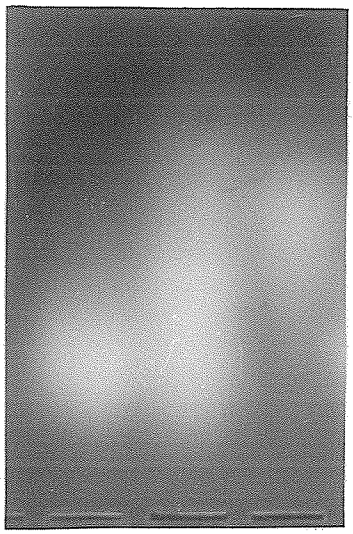

FIG. 2 PHOTOGRAPH SHOWING THE THREE COMMON PHENOTYPES OF F13A.

LANE 1: F13A2
LANE 2: F13A2-1
LANE 3: F13A1

protein bands and a number of minor bands (Fig.3). Of the three phenotypes which exhibited a single major protein band, F13B3 presents with a single protein band which is slightly anodal to the origin, F13B2 presents with a major protein band which migrates more anodally, and the F13B1 phenotype presents with a single major protein band which is the most anodal of the three. The protein bands detected in phenotypes with two major bands, were in all cases present in two of the three phenotypes which present with a single major band. The minor bands are apparently due to posttranslational modifications as many of these can be appreciably removed with prolonged neuraminidase treatment (Kreckel and Kuhnl, 1982).

3.200 Population and Family Studies

3.210 F13A

3.211 Population Studies

The distribution of F13A phenotypes in 338 unrelated Canadians classified into one of four major groups on the basis of ethnic origin is presented in Table 2.

The distribution of F13A phenotypes in the 188 unrelated Canadians of Western European descent is presented in Table 3. No statistically significant deviation was detected between male and

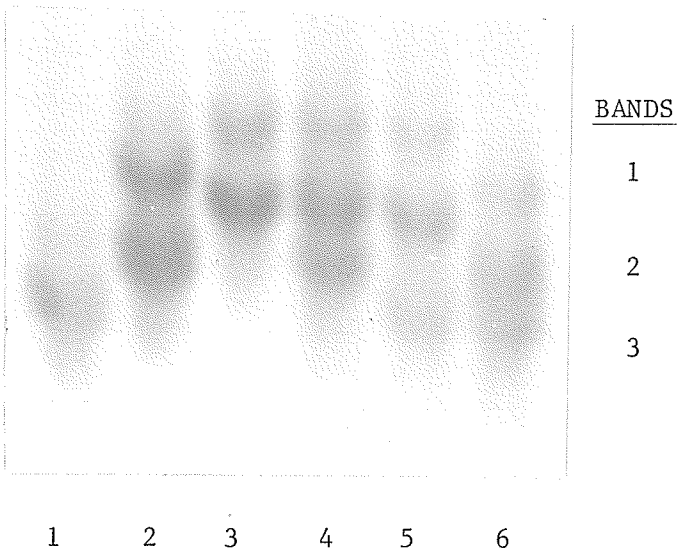

FIG. 3 PHOTOGRAPH SHOWING THE SIX COMMON PHENOTYPES OF F13B.

LANE 1: F13B3
LANE 2: F13B2
LANE 3: F13B3
LANE 4: F13B2-1
LANE 5: F13B3-1
LANE 6: F13B3-2

TABLE 2. THE DISTRIBUTION OF F13A PHENOTYPES IN 338 UNRELATED CANADIANS.

POPULATION GROUPS	PHENOTYPES			
	1	2-1	2	TOTAL
WESTERN EUROPEAN	119	62	7	188
OTHER EUROPEANS	14	5	1	20
NEWFOUNDLAND	72	43	8	123
ASIATIC	7	0	0	7
TOTAL	212	110	16	338

TABLE 3. THE DISTRIBUTION OF F13A PHENOTYPES IN 188 UNRELATED CANADIANS OF WESTERN EUROPEAN DESCENT.

PHENOTYPE	MALES		FEMALES		TOTALS	
	OBS	EXP	OBS	EXP	OBS	EXP
1	56	54.46	63	65.53	119	120.32
2-1	23	25.55	39	34.84	62	60.16
2	4	3.00	3	4.63	7	7.52
TOTALS	83		105		188	

NOTE: EXPECTED NUMBERS WERE CALCULATED ON THE BASIS OF THE FOLLOWING ALLELE FREQUENCIES:

MALE: F13A*1=0.81±0.03; F13A*2=0.19±0.03

FEMALE: F13A*1=0.79±0.03; F13A*2=0.21±0.03

TOTAL: F13A*1=0.80±0.02; F13A*2=0.20±0.02

female distributions of the phenotypes F13A1 and F13A2-1 (X^2_1 =0.112, p>0.500) nor between the distribution of the three phenotypes in the total sample and the distribution expected under Hardy-Weinberg equilibrium (assuming two codominant alleles at a single autosomal locus and using estimated allele frequencies of F13A*1 = 0.80 ± 0.02 and F13A*2 = 0.20 ± 0.02; X^2_2 = 0.185, p>0.250). The distribution of F13A phenotypes in the sample population, therefore, is consistent with an autosomal pattern of inheritance.

The distribution of F13A phenotypes among the 123 unrelated Newfoundlanders is presented in Table 4. No statistically significant deviations were detected between the male and female distributions of the phenotypes F13A1 and F13A2-1 (X^2_1 = 0.749, p>0.250) nor between the distribution of the phenotypes observed in the total sample and the distribution expected under Hardy-Weinberg equilibrium (assuming two codominant alleles at a single autosomal locus and using estimated allele frequencies of F13A*1 = 0.76 ± 0.03 and F13A*2 = 0.24 ± 0.03; X^2_2 =0.210; p>0.750). Thus the F13A distribution in this sample population was also found to be consistent with a pattern of autosomal inheritance.

3.212 Family Studies

The distribution of F13A phenotypes in 200 families is presented in Table 5. Segregation ratios among the children of all mating types did not deviate significantly from those which would be expected for a

TABLE 4. THE DISTRIBUTION OF F13A PHENOTYPES IN 123 UNRELEATED NEWFOUNDLANDERS.

PHENOTYPE	MALES		FEMALES		TOTALS	
	OBS	EXP	OBS	EXP	OBS	EXP
1	31	29.64	41	41.06	72	71.05
2-1	15	17.71	28	27.38	43	44.87
2	4	2.65	4	4.56	8	7.08
TOTAL	50		73		123	

NOTE: EXPECTED NUMBERS WERE CALCULATED ON THE BASIS OF THE FOLLOWING ALLELE FREQUENCIES:

MALE: F13A*1=0.77±0.04; F13A*2=0.23±0.04

FEMALE: F13A*1=0.75±0.04; F13A*2=0.25±0.04

TOTAL: F13A*1=0.76±0.03; F13A*2=0.24±0.03

TABLE 5. THE DISTRIBUTION OF F13A PHENOTYPES IN 200 FAMILIES

PARENTAL PHENOTYPE		TOTAL NO. OF FAMILIES	OFFSPRING PHENOTYPES			
MAT	PAT		1	2-1	2	TOTAL
1	1	69	209	0	0	209
1	2-1	43	59	66	0	125
2-1	1	47	72	69	0	141
1	2	5	0	13	0	13
2	1	6	0	22	0	22
2-1	2-1	21	15	51	22	88
2-1	2	6	0	10	6	16
2	2-1	3	0	8	4	12
2	2	-	-	-	-	-
TOTAL		200	355	239	32	626

MAT:MATERNAL; PAT:PATERNAL.

Mendelian System with two autosomal alleles at a single autosomal locus (F13A1 ♂ x F13A2-1 ♀ : X^2_1 =0.392, p>0.500; F13A2-1 ♂ x F13A1 ♀ : X^2_1 = 0.063, p>0.750; F13A2-1 ♂ x F13A2-1 ♀ : X^2_2 =0.341, p>0.500).

3.220 F13B

3.221 Population Studies

The distribution of F13B phenotypes in 199 unrelated Canadians classified into one of four major groups on the basis of ethnic origin, is presented in Table 6.

The distribution of F13B phenotypes among the 123 unrelated Canadians of Western European descent is presented in Table 7. There were no statisically significant deviations between the male and female distributions of the phenotypes F13B1, F13B3-1, and F13B2-1 (X^2_2 =3.09, p>0.010) nor between observed distribution of the six phenotypes in the total sample and the distribution expected under Hardy-Weinberg equilibrium (X^2_2 =4.994, p>0.050); for the latter calculation estimated allele frequencies for the sample population studied were F13B*1 = 0.74 ± 0.03, F13B*2 = 0.11 ± 0.02, and F13B*3 = 0.15 ± 0.02. The distribution of F13B phenotyopes in the sample population is consistent with an autosomal pattern of inheritance.

TABLE 6. THE DISTRIBUTION OF F13B PHENOTYPES IN 199 UNRELATED CANADIANS.

POPULATION GROUPS	PHENOTYPES						
	1	2	3	3-2	3-1	2-1	TOTAL
WESTERN EUROPEAN	85	1	2	9	31	21	149
OTHER EUROPEANS	6	0	0	0	2	1	9
NEWFOUNDLAND	20	0	0	1	6	3	30
ASIATIC	0	0	4	3	4	0	11
TOTAL	111	1	6	13	43	25	199

TABLE 7. THE DISTRIBUTION OF F13B PHENOTYPES IN 149 UNRELATED CANADIANS OF WESTERN EUROPEAN DESCENT.

	MALES		FEMALES		TOTALS	
PHENOTYPE	OBS	EXP	OBS	EXP	OBS	EXP
1	38	35.81	47	46.36	85	81.59
2	0	0.40	1	1.47	1	1.80
3	1	1.59	1	1.71	2	3.35
3-2	5	1.59	4	3.17	9	4.92
3-1	13	15.08	18	17.78	31	33.08
2-1	5	7.53	16	16.51	21	24.26
TOTAL	62		87		149	

NOTE: THE EXPECTED NUMBERS WERE CALCULATED ON THE BASIS OF THE FOLLOWING ALLELE FREQUENCIES; MALE: F13B*1=0.76 ± 0.04; F13B*2=0.08±0.02; F13B*3=0.16±0.03; FEMALE: F13B*1=0.74±0.03; F13B*2=0.13±0.03; F13B*3=0.14±0.03; TOTAL: F13B*1=0.74±0.03; F13B*2=0.11±0.02; F13B*3=0.15 ±0.02.

3.222 Family Studies

Distribution of F13B phenotypes in 125 families is presented in Table 8. Segregation ratios among the children of all mating types for which further statistical analysis could be performed (except one) indicated no significant deviation from the ratios expected under a Mendelian system with three codominant alleles at a single autosomal locus (F13B1 ♂ x F13B2-1 ♀ :X^2_1 =0.080, p>0.750; F13B1 ♂ x F13B3-1 ♀ : X^2_1 =0.191, p>0.500; F13B3-1 ♂ x F13B1 ♀ :X^2_1 =0.133, p>0.500; F13B1 ♂ x F13B3-2 ♀ :X^2_1 =2.882, p>0.050; F13B3-2 ♂ x F13B1 ♀ :X^2_1 =0.167, p>0.500; F13B2-1 ♂ x F13B3-1 ♀ :X^2_1 =2.048, p>0.100; F13B3-2 ♂ x F13B3-1 ♀ :X^2_2 =3.119, p>0.100). In contrast, the segregation ratio observed in the F13B2-1 ♂ x F13B1 ♀ mating, deviated significantly from the expected ratio (X^2_1 =5.670, p<0.025).

3.300 Gene mapping

3.310 F13A Linkage Studies

Paternal, maternal and intercross lod scores for F13A versus 53 marker loci are presented in Table 9. Close linkage between F13A and chromosome 2 marker loci analyzed (ACP1, IGKC, JK) are unlikely in both males and females, as the lod scores were -2 or less at θ values of 0.05.

TABLE 8. THE DISTRIBUTION OF F13B PHENOTYPES IN 124 FAMILIES.

PARENTAL PHENOTYPE		TOTAL NO. OF	OFFSPRING PHENOTYPES						
PAT	MAT	FAMILIES	1	2	3	3-2	3-1	2-1	TOTAL
1	1	10	37	0	0	0	0	0	37
1	2	1	0	0	0	0	0	3	3
2	1	-	-	-	-	-	-	-	-
1	3	-	-	-	-	-	-	-	-
3	1	-	-	-	-	-	-	-	-
1	2-1	14	26	0	0	0	0	24	50
2-1	1	13	34	0	0	0	0	17	51
1	3-1	13	22	0	0	0	25	0	47
3-1	1	22	58	0	0	0	62	0	120
1	3-2	4	0	0	0	0	12	5	17
3-2	1	7	0	0	0	0	13	11	24
2	2	-	-	-	-	-	-	-	-
2	3	-	-	-	-	-	-	-	-
3	2	-	-	-	-	-	-	-	-
2	3-1	-	-	-	-	-	-	-	-
3-1	2	1	0	0	0	2	0	1	3
2	2-1	-	-	-	-	-	-	-	-
2-1	2	-	-	-	-	-	-	-	-
2	3-2	-	-	-	-	-	-	-	-
3-2	2	-	-	-	-	-	-	-	-
3	3	-	-	-	-	-	-	-	-
3	2-1	-	-	-	-	-	-	-	-
2-1	3	1	0	0	0	3	2	0	5
3	3-2	-	-	-	-	-	-	-	-
3-2	3	-	-	-	-	-	-	-	-
3	3-1	-	-	-	-	-	-	-	-
3-1	3	2	0	0	5	0	2	0	7
2-1	3-1	7	4	0	0	4	5	8	21
3-1	2-1	4	1	0	0	5	3	2	11
2-1	3-2	3	0	3	0	3	2	5	13
3-2	2-1	4	0	2	0	1	5	3	11
2-1	2-1	4	1	2	0	0	0	8	11
3-1	3-1	3	4	0	1	0	4	0	9
3-1	3-2	2	0	0	0	3	1	0	4
3-2	3-1	8	0	0	6	10	6	4	26
3-2	3-2	1	0	0	0	5	0	0	5
TOTAL		124	187	7	12	36	142	91	475

MAT:MATERNAL; PAT:PATERNAL.

TABLE 9. LOD SCORES FOR LINKAGE BETWEEN F13A AND OTHER LOCI

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
1	PGD	M	1	4	4	17	-3.234	-1.682	-0.416	0.049	0.148
	RH	P	–	–	23	72	-9.517	-4.886	-1.391	-0.287	-0.021
		M	3	5	25	75	-9.731	-5.019	-1.371	-0.167	0.096
		T	3	5	48	147	-19.248	-9.905	-2.762	-0.454	0.075
	FUCA1	P	–	–	5	16	-5.047	-3.106	-1.358	-0.530	-0.124
		M	0	2	9	27	-0.482	0.261	0.670	0.521	0.235
		T	0	2	14	43	-5.529	-2.845	-0.688	-0.009	0.111
	UMPK	P	0	2	3	6	0.095	0.281	0.348	0.280	0.157
		M	–	–	1	5	-1.442	-0.887	-0.388	-0.151	-0.035
		T	0	2	4	11	-1.347	-0.606	-0.040	0.129	0.122
	PGM1	P	2	0	25	80	-15.718	-9.179	-3.691	-1.401	-0.387
		M	6	4	31	103	-22.713	-13.591	-5.682	-2.187	-0.568
		I	–	–	1	4	-0.927	-0.458	-0.121	-0.023	-0.001
		T	8	4	57	187	-39.358	-23.228	-9.494	-3.611	-0.956
	AMY2	P	–	–	1	2	0.258	0.215	0.134	0.064	0.017
		M	–	–	1	5	0.535	0.465	0.318	0.170	0.049
		T	–	–	2	7	0.793	0.680	0.452	0.234	0.066
	FY	P	4	0	24	66	-8.765	-4.862	-1.860	-0.794	-0.332
		M	4	3	13	44	-5.087	-2.610	-0.708	-0.118	-0.020
		I	–	–	2	10	0.258	0.356	0.293	0.162	0.048
		T	8	3	39	120	-13.594	-7.116	-2.275	-0.750	-0.304
	AT3	M	–	–	1	3	-0.721	-0.444	-0.194	-0.076	-0.018
	DO	P	–	–	6	21	-1.502	-0.773	-0.226	-0.055	-0.008
		M	2	0	5	16	-3.647	-2.299	-1.110	-0.543	-0.213
		T	2	0	11	37	-5.149	-3.072	-1.336	-0.598	-0.221

TABLE 9. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
2	ACP1	P	–	–	22	81	-8.776	-4.406	-1.133	-0.149	0.024
		M	4	5	17	55	-12.647	-7.532	-3.080	-1.101	-0.208
		T	4	5	39	136	-21.423	-11.938	-4.213	-1.250	-0.184
	IGKC	P	–	–	3	14	-2.884	-1.775	-0.776	-0.303	-0.071
	JK	P	–	–	20	61	-8.733	-4.904	-1.788	-0.584	-0.119
		M	3	5	11	41	-7.023	-3.882	-1.269	-0.256	0.054
		I	–	–	3	13	-0.574	0.064	0.374	0.316	0.167
		T	3	5	34	115	-16.330	-8.722	-2.683	-0.524	0.102
3	TF	P	–	–	9	45	-11.745	-7.113	-3.029	-1.157	-0.267
		M	6	0	9	30	-8.091	-5.290	-2.712	-1.402	-0.588
		T	6	0	18	75	-19.836	-12.403	-5.741	-2.559	-0.855
	AHSG	P	0	2	8	34	-1.460	-0.344	0.358	0.402	0.214
		M	4	1	10	33	-7.254	-4.525	-2.100	-0.963	-0.351
		I	–	–	1	4	-0.244	-0.080	-0.007	0.000	0.000
		T	4	3	19	71	-8.958	-4.949	-1.749	-0.561	-0.137
	3I	P	–	–	4	13	-1.647	-0.901	-0.314	-0.099	-0.019
		M	–	–	3	6	-1.163	-0.633	-0.184	-0.006	0.043
		T	–	–	7	19	-2.810	-1.534	-0.498	-0.105	0.024
4	GC	P	1	1	12	53	-10.044	-6.013	-2.507	-0.944	-0.217
		M	2	0	6	22	-3.834	-2.279	-0.986	-0.449	-0.182
		T	3	1	18	75	-13.878	-8.292	-3.493	-1.393	-0.399
	MN	P	–	–	28	94	-10.499	-4.976	-0.964	0.090	0.114
		M	6	6	30	89	-15.806	-9.034	-3.411	-1.150	-0.237
		I	–	–	4	17	0.798	0.904	0.751	0.466	0.200
		T	6	6	62	200	-25.507	-13.106	-3.624	-0.594	0.077
	4Q2	P	–	–	1	4	-0.721	-0.444	-0.194	-0.076	-0.018

TABLE 9. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
6	HLA	P	–	–	18	56	4.023	4.195	3.317	1.978	0.711
		M	–	–	9	29	-0.503	0.269	0.595	0.420	0.139
		I	–	–	1	7	-0.455	-0.110	0.022	0.010	0.001
		T	–	–	28	92	3.065	4.354	3.934	2.408	0.851
	BF	P	–	–	5	12	0.311	0.416	0.342	0.180	0.050
	GLO1	P	–	–	25	94	-1.936	0.650	1.829	1.346	0.457
		M	6	6	24	84	-18.604	-11.071	-4.548	-1.694	-0.414
		I	–	–	1	5	-0.784	-0.496	-0.222	-0.087	-0.020
		T	6	6	50	183	-21.324	-10.917	-2.941	-0.435	0.023
	PLG	P	–	–	10	41	-9.024	-5.277	-2.064	-0.698	-0.137
		M	1	1	8	27	-3.296	-1.808	-0.648	-0.239	-0.086
		I	–	–	1	6	-0.412	-0.028	0.147	0.106	0.033
		T	1	1	19	74	-12.732	-7.113	-2.565	-0.831	-0.190
7	7P1	P	–	–	1	4	-0.721	-0.444	-0.194	-0.076	-0.018
9	AK1	P	–	–	8	33	-1.504	-0.428	0.202	0.219	0.076
		M	–	–	6	22	-2.554	-1.323	-0.384	-0.080	-0.005
		T	–	–	14	55	-4.058	-1.751	-0.182	0.139	0.071
	ABO	P	6	5	19	73	-9.552	-5.436	-2.101	-0.804	-0.250
		M	2	0	14	74	-7.419	-4.412	-1.810	-0.671	-0.152
		I	–	–	2	10	-0.966	-0.524	-0.201	-0.076	-0.018
		T	8	5	35	157	-17.937	-10.372	-4.112	-1.551	-0.420
	9IV	P	–	–	1	2	0.258	0.215	0.134	0.064	0.017
	9IQ	P	–	–	1	5	-0.464	-0.229	-0.060	-0.011	-0.001
		M	1	1	2	9	-1.442	-0.888	-0.388	-0.152	-0.036
		T	1	1	3	14	-1.906	-1.117	-0.448	-0.163	-0.037
11	UPS	M	–	–	3	10	-1.184	-0.672	-0.254	-0.088	-0.019

TABLE 9. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
12	LDHB	P	–	–	1	4	-0.464	-0.229	-0.060	-0.011	-0.001
13	ESD	P	–	–	15	47	-4.829	-2.394	-0.569	-0.035	0.032
		M	3	5	10	31	-7.373	-4.373	-1.726	-0.543	-0.039
		T	3	5	25	78	-12.202	-6.767	-2.295	-0.578	-0.007
14	PI	P	0	2	6	26	-4.161	-2.145	-0.558	-0.015	0.100
		M	1	4	6	22	-2.253	-1.025	-0.090	0.187	0.182
		T	1	6	12	48	-6.414	-3.170	-0.648	0.172	0.282
	IGHG	P	–	–	6	19	-2.832	-1.574	-0.568	-0.186	-0.038
		M	2	0	7	16	-1.154	-0.517	-0.136	-0.094	-0.095
		T	2	0	13	35	-3.986	-2.091	-0.704	-0.280	-0.133
16	PGP	P	1	1	8	28	-4.996	-2.907	-1.150	-0.414	-0.092
		M	1	4	11	40	-7.557	-4.345	-1.582	-0.407	0.041
		I	–	–	1	4	-0.927	-0.458	-0.121	-0.023	-0.001
		T	2	5	20	72	-13.480	-7.710	-2.853	-0.844	-0.052
	HP	P	2	1	13	48	-10.025	-5.982	-2.478	-0.943	-0.249
		M	4	3	17	69	-13.907	-8.452	-3.632	-1.429	-0.370
		I	–	–	3	12	-2.454	-1.431	-0.600	-0.237	-0.056
		T	6	4	33	129	-26.386	-15.865	-6.710	-2.609	-0.675
	16Q	M	0	3	1	3	0.836	0.766	0.612	0.438	0.238
18	PEPA	P	–	–	1	3	0.535	0.465	0.318	0.170	0.049

TABLE 9. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
19	PEPD	M	–	–	1	2	-0.721	-0.444	-0.194	-0.076	-0.018
	C3	P	–	–	8	31	-2.297	-1.110	-0.250	-0.017	0.010
		M	–	–	5	16	-0.835	-0.186	0.188	0.177	0.062
		T	–	–	13	47	-3.132	-1.296	-0.062	0.160	0.072
	LU	P	–	–	5	21	-1.577	-0.666	-0.056	0.060	0.029
		M	0	2	8	26	-3.974	-2.167	-0.681	-0.111	0.067
		T	0	2	13	47	-5.551	-2.833	-0.737	-0.051	0.096
	SE	P	–	–	1	4	-0.178	-0.136	-0.073	-0.032	-0.008
		M	–	–	1	2	0.135	0.121	0.091	0.061	0.031
		T	–	–	2	6	-0.043	-0.015	0.018	0.029	0.023
	19H	P	–	–	1	4	-1.442	-0.887	-0.388	-0.151	-0.035
20	ADA	P	–	–	6	25	-1.155	-0.239	0.329	0.330	0.126
		M	2	2	7	22	-3.812	-2.239	-0.916	-0.368	-0.121
		T	2	2	13	47	-4.967	-2.478	-0.587	-0.038	0.005
22	P1	P	–	–	4	14	-2.695	-1.613	-0.671	-0.251	-0.057
		M	–	–	1	5	-1.442	-0.887	-0.388	-0.151	-0.035
		I	–	–	1	6	-0.443	-0.228	-0.077	-0.026	-0.006
		T	–	–	6	25	-4.580	-2.728	-1.136	-0.428	-0.098
	22M	P	–	–	1	2	-0.721	-0.444	-0.194	-0.076	-0.018
		M	–	–	1	6	-0.721	-0.444	-0.194	-0.076	-0.018
		T	–	–	2	8	-1.442	-0.888	-0.388	-0.152	-0.036

TABLE 9. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
U	CMT	P	–	–	1	2	-0.721	-0.444	-0.194	-0.076	-0.018
		M	–	–	1	7	-1.442	-0.887	-0.388	-0.151	-0.035
		T	–	–	2	9	-2.163	-1.331	-0.582	-0.227	-0.053
	CO	P	–	–	7	19	-2.110	-1.131	-0.374	-0.112	-0.021
		M	–	–	2	5	-0.463	-0.229	-0.060	-0.012	-0.001
		T	–	–	9	24	-2.573	-1.360	-0.434	-0.124	-0.022
	C6	P	–	–	4	16	-2.369	-1.346	-0.508	-0.175	-0.037
		M	–	–	2	9	-1.442	-0.888	-0.388	-0.152	-0.036
		T	–	–	6	25	-3.811	-2.234	-0.896	-0.327	-0.073
	GPT	P	2	2	24	86	-8.260	-3.977	-0.880	-0.042	0.045
		M	3	4	21	68	-11.082	-6.207	-2.226	-0.674	-0.092
		T	5	6	45	154	-19.342	-10.184	-3.106	-0.716	-0.047
	KEL	P	–	–	5	18	-1.835	-0.881	-0.190	-0.004	0.011
		M	2	0	3	9	-4.163	-2.729	-1.378	-0.671	-0.247
		T	2	0	8	27	-5.998	-3.610	-1.568	-0.675	-0.236
	RGS	P	–	–	1	3	0.535	0.465	0.318	0.170	0.049
		M	–	–	1	3	-0.721	-0.444	-0.194	-0.076	-0.018
		T	–	–	2	6	-0.186	0.021	0.124	0.094	0.031
	YT	P	–	–	3	12	0.606	0.701	0.576	0.329	0.097
		M	1	2	1	5	-0.442	-0.189	0.010	0.070	0.061
		T	1	2	4	17	0.164	0.512	0.586	0.399	0.158

C:CHROMOSOME; U:UNMAPPED LOCI; MT:MATING TYPE; R:NUMBER OF RECOMBINANTS; NR:NUMBER OF NONRECOMBINANTS; F:FAMILIES; C:CHILDREN.

* REPRESENT CHROMOSOMAL MARKERS (3I: inv(3)(p25q21) ; 4Q2/7P1: t(4;7)(q21:p15) ; 9IQ: inv ins(9)(q22.1q34.3q34) ; 9IV: 9qh+ ; 19H: 19qh+ ; 22M: 22s+ ; 16Q: 16qh+).

In contrast, positive paternal lod scores were obtained between F13A and three closely linked chromosome 6p loci, HLA, GLO, and BF; maximum positive lod scores ($\hat{z}$), the corresponding recombination fractions ($\hat{\theta}$) and the 95 % confidence intervals (95% CI) were estimated from graphical presentations of these lod scores (Fig. 4). The paternal F13A:HLA lod scores reached formal levels for the acceptance of linkage ($\hat{z}$ = +4.20, $\hat{\theta}$ =0.08, 95% CI: 0.03-0.21), while maternal lod scores for this locus pair, although slightly positive ($\hat{z}$ = 0.60, $\hat{\theta}$ = 0.20), failed to reach a formal level of significance. Neither the paternal F13A:GLO ($\hat{z}$ = +1.82, $\hat{\theta}$ =0.20, 95% CI: 0.10-0.36) nor the paternal F13A:BF ($\hat{z}$ = +0.42, $\hat{\theta}$ =0.12) lod scores reached formal levels for the acceptance of linkage. No informative families were identified to provide maternal F13A:BF lod scores while the maternal lod scores obtained for F13A:GLO were not suggestive of linkage.

Close linkage of F13A to PLG, a marker locus recently assigned to chromosome 6q25-qter (Swisshelm et al., 1985; Murray et al., 1985), was found to be unlikely, lod scores having reached formal levels of exclusion at a θ of 0.20 in males and 0.05 in females.

Lod scores between F13A and marker loci known to be on chromosomes other than 2 and 6 were, in general, not suggestive of linkage. However, slightly positive lod scores ($\hat{z} \leq +0.555$) were obtained at θ=0.05 for F13A with AMY2, 9IV, and PEPA in paternal lod scores and with SE in maternal lod scores, and in addition a peak

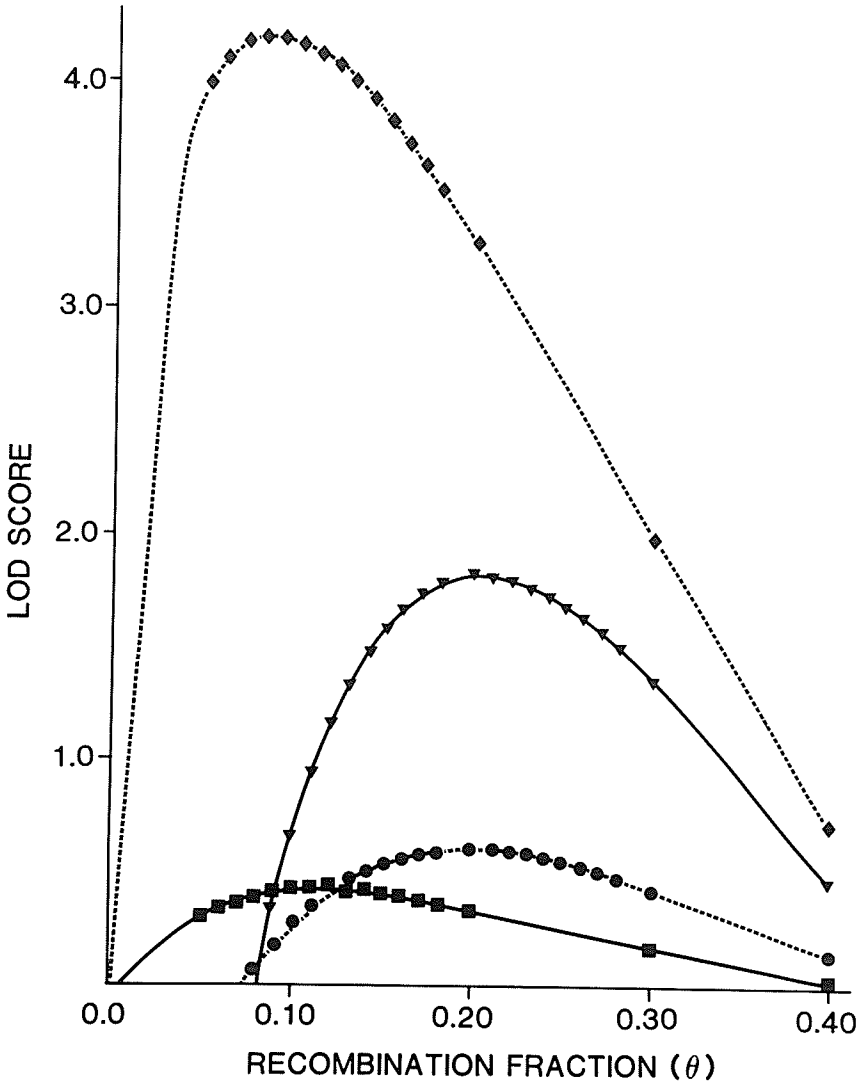

FIG. 4. POSITIVE LOD SCORES AND THEIR CORRESPONDING RECOMBINATION FRACTIONS(θ) BETWEEN F13A AND OTHER LOCI ASSIGNED TO THE SHORT ARM OF CHROMOSOME 6.

◆ PATERNAL F13A:HLA LOD SCORES
● MATERNAL F13A:HLA LOD SCORES
▼ PATERNAL F13A:GLO LOD SCORES
■ PATERNAL F13A:BF LOD SCORES

paternal lod score of +0.348 was detected between F13A and UMPK at $\theta = 0.20$. However, as all of these loci have been assigned to chromosomes other than 2 or 6 and the cytogenetic marker (9IQ) was on chromosome 9, these lod scores probably represent random statistical fluctuations.

Slightly positive lod scores were also detected between F13A and two unmapped loci, RGS and YT ($z = +0.535$, $\theta = 0.05$, and $z = 0.701$, $\theta = 0.10$, respectively). As the number of families contributing to the lod scores was three or less and the number of informative offspring was 12 or less further testing will be required to determine if these potential linkage relationships are significant.

3.320 Linkage Studies on the F13B Locus

Lod scores obtained for F13B with 62 marker loci are presented in Table 10. Marker loci for which F13B linkage information had not been previously reported in other studies include AFA, AT3, C4A, CMT, DI, DIA1, FR, F2, GAA, GOT2, APOC2, APOE, LW, MPI, NFLD, NP, SOD1, WD, WR, and YT. In addition, two unique chromosomal rearrangements 4Q2/7P1 (t(4;7)(q21:p15)) and 9IQ (inv ins(9)(q22.1q34.3q34.1)) have been analyzed for linkage to F13B.

TABLE 10. LOD SCORES BETWEEN F13B AND OTHER LOCI.

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
1	PGD	P	2	0	2	4	-1.742	-1.183	-0.662	-0.380	-0.177
		M	–	–	4	10	-1.648	-0.902	-0.314	-0.099	-0.020
		T	2	0	6	14	-3.390	-2.085	-0.976	-0.479	-0.197
	RH	P	6	7	18	102	-19.649	-11.259	-4.242	-1.378	-0.239
		M	1	2	14	59	-10.125	-5.721	-2.092	-0.658	-0.121
		I	–	–	12	38	-7.397	-3.687	-1.062	-0.282	-0.049
		T	7	9	44	199	-37.171	-20.667	-7.396	-2.318	-0.409
	FUCA1	P	–	–	3	23	-4.698	-2.614	-0.899	-0.244	-0.031
		M	1	1	3	6	-2.163	-1.332	-0.582	-0.228	-0.054
		I	–	–	2	8	0.120	0.241	0.219	0.125	0.038
		T	1	1	8	37	-6.741	-3.705	-1.262	-0.347	-0.047
	UMPK	P	–	–	2	7	-1.184	-0.672	-0.254	-0.087	-0.018
	PGM1	P	11	7	31	115	-23.970	-13.924	-5.415	-1.870	-0.443
		M	10	6	23	79	-21.935	-13.447	-5.908	-2.392	-0.643
		I	–	–	3	7	-4.348	-2.702	-1.234	-0.535	-0.168
		T	21	13	57	201	-50.253	-30.073	-12.557	-4.797	-1.254
	AMY2	P	–	–	1	6	-1.185	-0.673	-0.254	-0.087	-0.018
		M	–	–	3	11	-0.392	0.008	0.198	0.148	0.047
		T	–	–	4	17	-1.577	-0.665	-0.056	0.061	0.029
	FY	P	3	2	20	105	-10.135	-4.554	-0.473	0.543	0.348
		M	3	3	19	83	-12.555	-6.674	-1.961	-0.291	0.093
		I	–	–	2	10	-2.048	-1.119	-0.423	-0.156	-0.036
		T	6	5	41	198	-24.738	-12.347	-2.857	0.096	0.405
	AT3	P	–	–	1	6	0.093	0.276	0.323	0.222	0.076
		M	0	2	1	2	0.558	0.510	0.408	0.292	0.158
		T	0	2	2	8	0.651	0.786	0.731	0.514	0.234
	DO	P	3	6	14	82	-9.696	-5.690	-2.287	-0.862	-0.254
		M	2	0	7	30	-2.595	-1.692	-0.856	-0.443	-0.190
		I	–	–	2	9	0.364	0.361	0.292	0.169	0.051
		T	5	6	23	121	-11.927	-7.021	-2.851	-1.136	-0.393

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
2	ACP1	P	2	0	24	107	-18.452	-10.468	-3.927	-1.362	-0.348
	IGKC	P	–	–	2	6	0.093	0.276	0.323	0.222	0.076
		I	–	–	1	2	-0.040	-0.036	-0.024	-0.012	-0.003
		T	–	–	3	8	0.053	0.240	0.299	0.210	0.073
	JK	P	7	2	22	119	-17.246	-9.752	-3.603	-1.189	-0.266
		M	5	4	16	65	-13.155	-7.841	-3.279	-1.276	-0.333
		I	–	–	5	14	-2.024	-0.634	0.336	0.546	0.401
		T	12	6	43	198	-32.425	-18.227	-6.546	-1.919	-0.198
3	TF	P	3	3	7	29	-2.741	-1.302	-0.260	0.014	0.025
		M	1	1	9	21	-3.295	-1.804	-0.628	-0.199	-0.040
		I	–	–	3	11	-1.244	-0.414	0.067	0.110	0.041
		T	4	4	19	61	-7.28	-3.520	-0.821	-0.075	0.026
	AHSG	P	0	3	8	41	-6.531	-3.496	-1.091	-0.248	-0.020
		M	–	–	4	24	-3.699	-1.916	-0.503	-0.030	0.041
		T	0	3	12	65	-10.230	-5.412	-1.594	-0.278	0.021
4	GC	P	2	5	14	71	-9.971	-5.183	-1.449	-0.167	0.134
		M	3	5	15	46	-8.029	-4.422	-1.478	-0.354	0.023
		I	–	–	4	14	-8.440	-5.190	-2.333	-1.007	-0.320
		T	5	10	33	131	-26.440	-14.795	-5.260	-1.528	-0.163
	MN	P	2	2	30	134	-26.316	-14.805	-5.250	-1.483	-0.153
		M	3	4	21	83	-15.822	-9.095	-3.450	-1.167	-0.267
		I	–	–	11	35	-14.086	-8.495	-3.638	-1.461	-0.415
		T	5	6	62	252	-56.224	-32.395	-12.338	-4.111	-0.835
	4Q2	P	–	–	1	4	0.814	0.720	0.517	0.298	0.094

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
6	F13A	P	2	0	11	47	-7.366	-4.258	-1.698	-0.667	-0.222
		M	2	3	3	11	-2.349	-1.305	-0.438	-0.092	0.025
		I	-	-	1	2	-0.464	-0.229	-0.060	-0.011	-0.001
		T	4	3	15	60	-10.179	-5.792	-2.196	-0.770	-0.198
	HLA	P	-	-	12	56	-8.621	-4.584	-1.399	-0.295	-0.009
		M	-	-	8	25	-4.995	-2.906	-1.149	-0.415	-0.092
		I	-	-	1	7	1.247	1.254	0.945	0.513	0.140
		T	-	-	21	88	-12.369	-6.236	-1.603	-0.197	0.039
	C4A	P	-	-	3	27	-3.091	-1.566	-0.381	-0.012	0.030
	GLO1	P	2	3	20	98	-15.759	-8.781	-3.058	-0.857	-0.094
		M	4	3	23	96	-17.084	-9.554	-3.357	-0.942	-0.082
		I	-	-	6	16	-2.644	-1.371	-0.530	-0.273	-0.144
		T	6	6	49	210	-35.487	-19.706	-6.945	-2.072	-0.320
	PLG	P	0	3	17	80	-6.627	-2.718	0.167	0.863	0.563
		M	1	1	10	33	-8.136	-4.894	-2.060	-0.780	-0.178
		I	-	-	8	29	-3.065	-0.914	0.354	0.435	0.213
		T	1	4	35	142	-17.828	-8.526	-1.539	0.518	0.598
7	7P1	P	-	-	1	4	0.814	0.72	0.517	0.298	0.094

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
9	AK1	P	–	–	2	5	-1.442	-0.888	-0.388	-0.152	-0.036
		M	–	–	3	11	-1.648	-0.902	-0.314	-0.099	-0.019
		I	–	–	1	7	-1.341	-0.805	-0.339	-0.129	-0.030
		T	–	–	6	23	-4.431	-2.595	-1.041	-0.380	-0.085
	ABO	P	7	5	16	85	-20.161	-12.030	-4.982	-1.893	-0.492
		M	–	–	7	27	-3.256	-1.717	-0.521	-0.123	-0.013
		I	–	–	6	18	-3.041	-1.774	-0.733	-0.287	-0.068
		T	7	5	29	130	-26.458	-15.521	-6.236	-2.303	-0.573
	ORM	M	–	–	1	6	0.093	0.276	0.323	0.222	0.076
		I	–	–	3	11	-0.700	-0.194	-0.018	-0.051	-0.029
		T	–	–	4	17	-0.607	0.082	0.305	0.171	0.047
	9IQ	P	–	–	1	2	0.258	0.215	0.134	0.064	0.017
13	ESD	P	2	1	14	70	-14.742	-8.631	-3.427	-1.228	-0.294
		M	3	4	16	63	-13.763	-7.944	-3.019	-0.968	-0.150
		I	–	–	1	5	-0.927	-0.458	-0.121	-0.023	-0.001
		T	5	5	31	138	-29.432	-17.033	-6.567	-2.219	-0.445
14	PI	P	1	1	15	51	-10.733	-6.295	-2.564	-0.985	-0.263
		M	5	0	14	51	-13.801	-8.406	-3.726	-1.645	-0.609
		I	–	–	4	10	-3.833	-2.273	-0.966	-0.406	-0.135
		T	6	1	33	112	-28.367	-16.974	-7.256	-3.036	-1.007
	NP	P	5	2	2	5	-4.442	-2.985	-1.582	-0.818	-0.327
	IGHG	P	5	3	8	32	-9.139	-5.599	-2.478	-1.042	-0.323
		M	–	–	9	38	-6.810	-3.749	-1.290	-0.374	-0.063
		I	–	–	2	8	-1.112	-0.432	0.023	0.112	0.077
		T	5	3	19	78	-17.061	-9.780	-3.745	-1.304	-0.309

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
15	MPI	P	–	–	2	5	-1.464	-0.928	-0.458	-0.233	-0.098
16	PGP	P	4	5	13	57	-14.813	-8.864	-3.661	-1.329	-0.263
		M	–	–	9	33	-3.346	-1.424	-0.042	0.271	0.151
		I	–	–	2	9	-2.538	-1.494	-0.609	-0.225	-0.051
		T	4	5	24	99	-20.697	-11.782	-4.312	-1.283	-0.163
	GOT2	P	–	–	2	18	-2.627	-1.560	-0.642	-0.238	-0.053
		M	–	–	1	4	-0.464	-0.229	-0.060	-0.011	-0.001
		T	–	–	3	22	-3.091	-1.789	-0.702	-0.249	-0.054
	HP	P	6	7	18	81	-12.277	-6.423	-1.757	-0.150	0.159
		M	0	6	23	81	-0.791	1.183	2.068	1.666	0.848
		I	–	–	3	11	-1.380	-0.560	-0.081	-0.002	0.001
		T	6	13	44	173	-14.448	-5.800	0.230	1.514	1.008
17	GAA	P	–	–	2	5	-1.442	-0.888	-0.388	-0.152	-0.036
		M	2	1	3	9	-1.927	-1.157	-0.518	-0.245	-0.099
		T	2	1	5	14	-3.369	-2.045	-0.906	-0.397	-0.135

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
19	PEPD	M	–	–	1	2	-0.721	-0.444	-0.194	-0.076	-0.018
	C3	P	–	–	6	24	-3.740	-1.997	-0.638	-0.167	-0.025
		M	1	1	6	18	-4.069	-2.449	-1.030	-0.391	-0.091
		I	–	–	1	2	-0.464	-0.229	-0.060	-0.011	-0.001
		T	1	1	13	44	-8.273	-4.675	-1.728	-0.569	-0.117
	LE	P	–	–	1	6	-1.185	-0.673	-0.254	-0.087	-0.018
		M	–	–	1	4	-0.721	-0.444	-0.194	-0.076	-0.018
		T	–	–	2	10	-1.906	-1.117	-0.448	-0.163	-0.036
	LU	P	–	–	1	6	-1.185	-0.673	-0.254	-0.087	-0.018
		M	–	–	3	7	-2.163	-1.332	-0.582	-0.228	-0.054
		T	–	–	4	13	-3.348	-2.005	-0.836	-0.315	-0.072
	SE	P	–	–	6	46	-2.278	-1.402	-0.596	-0.222	-0.051
		M	–	–	4	16	-0.786	-0.435	-0.139	-0.035	-0.006
		I	–	–	4	14	-0.295	-0.147	-0.007	0.023	0.010
		T	–	–	14	76	-3.359	-1.984	-0.742	-0.234	-0.047
	APOC2	P	1	1	4	12	-3.605	-2.219	-0.970	-0.379	-0.089
		M	3	0	4	9	-3.205	-2.111	-1.120	-0.614	-0.275
		T	4	1	8	21	-6.810	-4.330	-2.090	-0.993	-0.364
	APOE	P	–	–	1	2	0.258	0.215	0.134	0.064	0.017
		M	–	–	1	2	-0.670	-0.421	-0.187	-0.073	-0.017
		T	–	–	2	4	-0.412	-0.206	-0.053	-0.009	0.000
	LW	P	3	2	2	18	-6.769	-4.248	-1.949	-0.827	-0.238
20	ADA	P	–	–	4	24	-5.419	-3.059	-1.094	-0.320	-0.049
		M	5	4	7	24	-4.441	-2.405	-0.786	-0.221	-0.061
		T	5	4	11	48	-9.860	-5.464	-1.880	-0.541	-0.110
21	SOD1	M	4	5	3	9	-2.606	-1.520	-0.572	-0.157	0.008

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
22	DIA1	P	1	5	2	6	0.394	0.578	0.622	0.509	0.299
		M	–	–	2	6	-1.185	-0.673	-0.254	-0.087	-0.019
		T	1	5	4	12	-0.791	-0.095	0.368	0.422	0.280
	P1	P	–	–	8	35	-2.608	-1.366	-0.413	-0.096	-0.011
		M	–	–	10	45	-6.399	-3.576	-1.287	-0.406	-0.078
		I	–	–	2	7	-0.087	0.030	-0.005	-0.066	-0.054
		T	–	–	20	87	-9.094	-4.912	-1.705	-0.568	-0.143
U	AFA	P	–	–	2	9	1.431	1.232	0.835	0.453	0.137
		M	1	2	2	7	0.093	0.276	0.323	0.222	0.076
		T	1	2	4	16	1.524	1.508	1.158	0.675	0.213
	CMT	P	2	4	4	11	-4.884	-3.173	-1.572	-0.747	-0.265
		M	3	1	3	10	-2.327	-1.264	-0.368	-0.010	0.088
		T	5	5	7	21	-7.211	-4.437	-1.940	-0.757	-0.177
	CO	P	–	–	6	36	-5.304	-2.736	-0.674	0.044	0.172
		M	–	–	4	23	-5.320	-3.244	-1.386	-0.530	-0.121
		T	–	–	10	59	-10.624	-5.980	-2.060	-0.486	0.051
	C6	P	–	–	2	11	-3.606	-2.218	-0.969	-0.378	-0.088
		M	–	–	4	21	-3.045	-1.897	-0.840	-0.330	-0.078
		I	–	–	1	3	-0.549	-0.302	-0.109	-0.036	-0.007
		T	–	–	7	35	-7.200	-4.417	-1.918	-0.744	-0.173

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
U	DI	P	–	–	2	6	-1.184	-0.672	-0.254	-0.087	-0.018
		I	–	–	1	5	-0.927	-0.458	-0.121	-0.023	-0.001
		T	–	–	3	11	-2.111	-1.130	-0.375	-0.110	-0.019
	F2	P	–	–	1	5	-0.186	0.022	0.124	0.095	0.031
		M	1	1	2	5	-1.442	-0.888	-0.388	-0.152	-0.036
		T	1	1	3	10	-1.628	-0.866	-0.264	-0.057	-0.005
	FR	P	–	–	1	2	-0.721	-0.444	-0.194	-0.076	-0.018
		M	–	–	2	10	-2.627	-1.560	-0.642	-0.239	-0.054
		T	–	–	3	12	-3.348	-2.004	-0.836	-0.315	-0.072
	GPT	P	12	14	25	113	-21.972	-12.525	-4.617	-1.412	-0.189
		M	1	4	16	61	-6.106	-2.758	-0.346	0.276	0.237
		I	–	–	4	15	-1.134	-0.344	0.094	0.122	0.045
		T	13	18	45	189	-29.212	-15.627	-4.869	-1.014	0.093
	KEL	P	–	–	7	38	-7.790	-4.409	-1.616	-0.516	-0.098
		M	–	–	7	36	-6.603	-3.731	-1.349	-0.405	-0.066
		T	–	–	14	74	-14.393	-8.14	-2.965	-0.921	-0.164
	NFLD	P	–	–	1	14	-1.513	-0.544	0.149	0.276	0.140
	SPB	P	1	1	4	12	-2.884	-1.776	-0.776	-0.304	-0.072
		M	–	–	3	10	-2.884	-1.775	-0.776	-0.303	-0.071
		T	1	1	7	22	-5.768	-3.551	-1.552	-0.607	-0.143
	WD	P	–	–	2	18	-4.533	-2.676	-1.090	-0.401	-0.090
	WR	P	–	–	1	10	-2.627	-1.560	-0.642	-0.238	-0.054

TABLE 10. (CONTINUED)

C	LOCI	SEGREGATION INFORMATION MT	R	NR	NUMBER OF F	C	ASSUMED RECOMBINATION FRACTION 0.05	0.10	0.20	0.30	0.40
U	YT	P	–	–	2	16	-3.812	-2.232	-0.895	-0.326	-0.072
	—	M	2	1	2	11	-2.906	-1.816	-0.846	-0.385	-0.133
		I	–	–	1	2	-0.698	-0.429	-0.187	-0.073	-0.017
		T	2	1	5	29	-7.416	-4.477	-1.928	-0.784	-0.222

C:CHROMOSOME; U:UNMAPPED LOCI; MT:MATINGTYPE; R:NUMBER OF RECOMBINANTS; NR:NUMBER OF NONRECOMBINANTS; F:FAMILIES; C:CHILDREN.

* REPRESENT CHROMOSOMAL MARKERS (4Q2/7P1: t(4;7)(q21;p15) ; 9IQ: inv ins (9)(q22.1q34.3q34.1).

Paternal and maternal lod scores for F13B with the 62 marker loci analyzed have not reached formal levels of significance for accepting linkage. There are however several positive peak lod scores, any one of which could indicate a potential linkage,but more testing will be required to determine if any of these will reach formal levels of significance.

A number of these potential linkage relationships at this point are unlikely because they can be nullified or weakened appreciably when the lod scores of the present study are added to those of Eiberg et al. (1984) as in the case of the paternal lod scores between F13B and FY, IGKC, TF, GC, PLG, HP, CO, and the maternal lod scores for AMY2, ACP1, GC, ORM, PGP, HP, AHSG, and GPT.

The somewhat positive lod scores obtained for F13B with AT3, 4Q2, 7P1, 9IQ, DIA1, AFA, F2, APOE and NFLD could be consistent with close to moderate linkage between F13B and any of these loci. As the detection of allelic variation at these "loci" is generally rare the number of informative families contributing to these lod scores was limited; in all cases the number of sibships was two or less while the total number of offspring varied from two to 14.

In contrast, formal levels for exclusion of linkage in male and/or female lod scores between F13B and 32 marker loci with known chromosomal assignments (RH, FUCA1, PGM1, FY, DO, ACP1, JK, TF, AHSG,

GC, MN, F13A, HLA, C4A, GLO1, PLG, ABO, ESD, NP, PI, IGHG, PGP, GOT2, HP, C3, LW, APOC2, LU, SE, ADA, SOD1 and P1) and 10 loci for which definite chromosomal assignments have not been reported (CO, C6, FR, CMT, GPT, KEL, SPB, WD, WR, YT) have been reached at θ values of 0.20 or less (Fig. 5).

In addition the total lod scores for F13B with four marker loci (MPI, GAA, DI, and F2), tested for the first time in the present study, were negative at all values of θ. Total lod scores between F13B and NFLD, another locus tested for the time for linkage to F13B, were negative at θ values less than 0.10. Although these lod scores did not reach formal levels for the exclusion of linkage, very close linkage between F13B and these five loci is unlikely as at least one recombinant has been detected in each case.

F13B:F13A lod scores were negative at all θ values less than 0.30 thereby providing additional evidence that the F13 A and B polypeptides are coded by genetically distinct loci. Although the F13A and F13B loci are not closely linked they may still be homologous in origin.

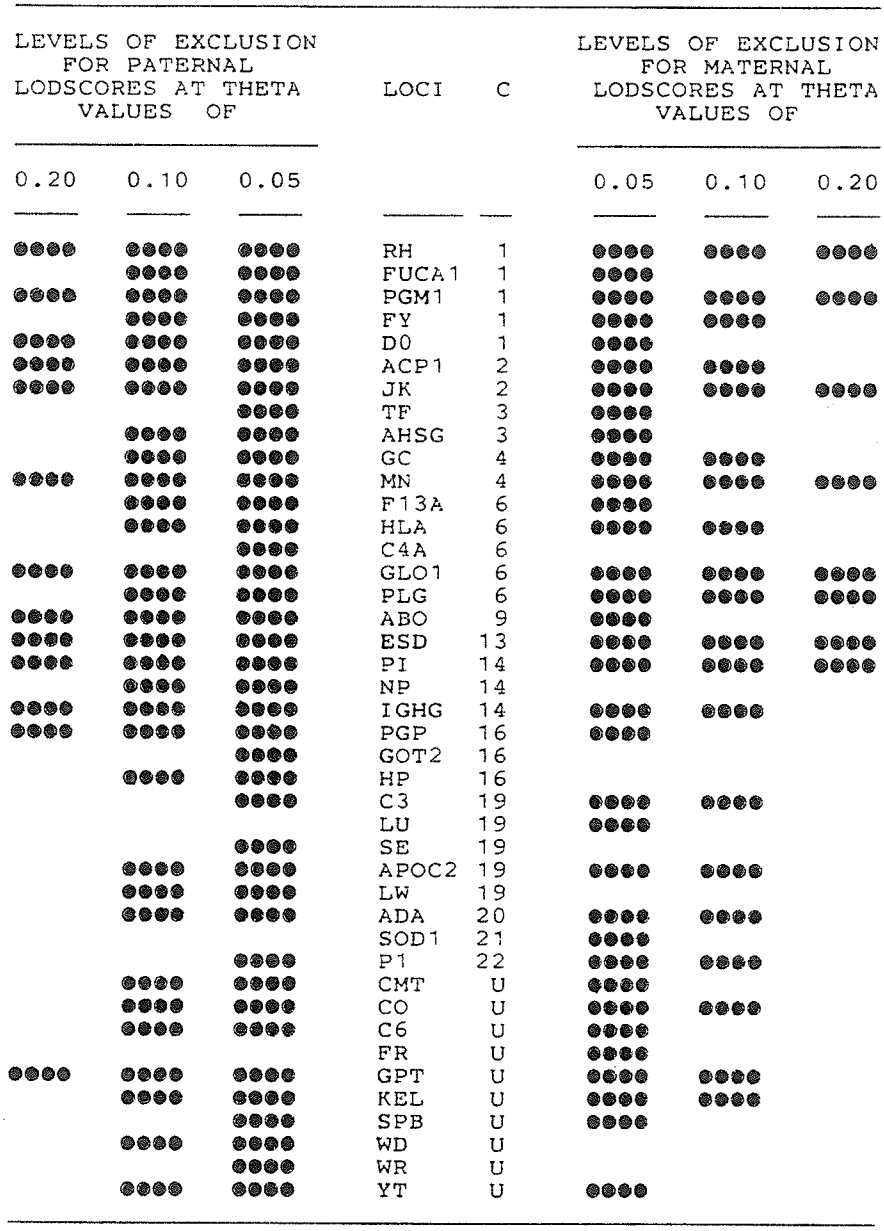

FIG 5. LEVELS OF EXCLUSION FOR LINKAGE BETWEEN F13B AND OTHER MARKER LOCI(LOD SCORES≤-2).

C:CHROMOSOME U:UNASSIGNED LOCI

3.330 F13B Exclusion mapping

Ten plasma samples from individuals with specific chromosomal deletions were phenotyped for F13B (Table 11). Four of the samples exhibited heterozygous phenotypes and excluded F13B from 1p36, 4pter-p16, 4q25-q27, 4q35-qter, 17pter-p13, and 17q25-qter (Fig.6). The exact breakpoints for an 18q- individual who was heterozygous for F13B were not available and thus exact regions of the genome could not be excluded from the analysis of this samples.

4.000 DISCUSSION

4.100 Qualitative variation

In the present study, electrophoresis was used to detect normal qualitative variation of F13A and F13B. Analysis of 1887 individual plasma samples by electrophoresis demonstrated the three known common phenotypes of F13A while a survey of 1032 individual plasma samples demonstrated the six known common phenotypes of F13B in individuals of the Canadian population.

TABLE 11. F13B PHENOTYPES OF TEN ANEUPLOID INDIVIDUALS.

I	TYPE OF DISRUPTION	CHROMOSOMES INVOLVED.	REGIONS DELETED	F13B. PHENOTYPE
1	T	1,15	1pter-p36	3-1
2	D	1	1p32	1
3	D	4	4q25-q27	3-1
4	R	4	4pter-p16 4q35-qter	2-1
5	D	7	7q21-q22	1
6	R	15	15pter-p11 15q26-qter	1
7	R	17	17pter-p13 17q25-qter	2-1
8	R	18	18pter-p11 18qter-q23	1
9	D	18	18q21-q22	1
10	D	18	---------	3-1

I:individuals; T:translocation; D:deletion; R:ring chromosome.

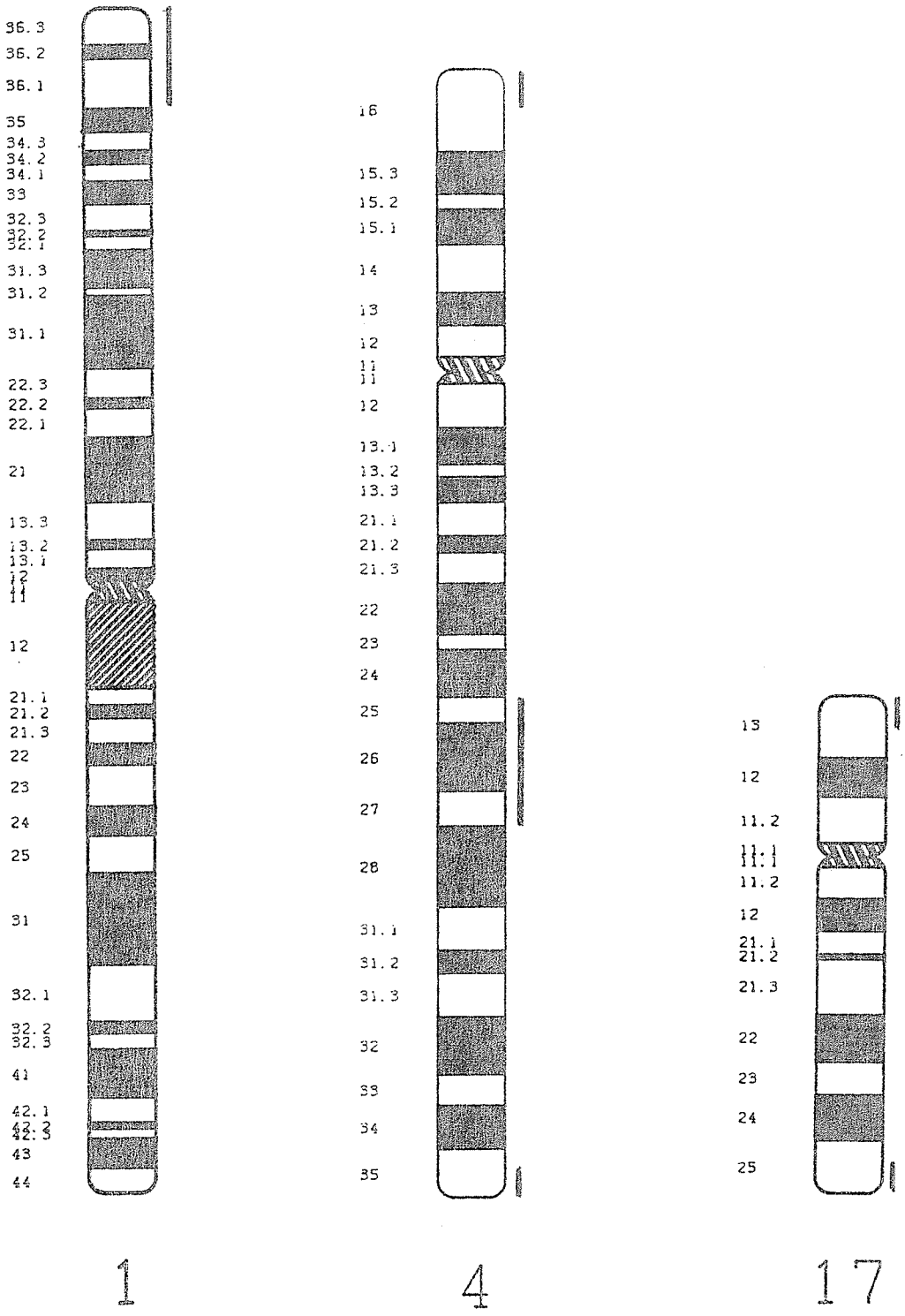

FIG. 6 CHROMOSOMAL REGIONS FOR WHICH THE LOCALIZATION OF F13B HAS BEEN EXCLUDED ON THE BASIS OF THE ANALYSIS OF PLASMA SAMPLES FROM INDIVIDUALS WITH KNOWN ANEUPLOIDY. (———): REGIONS EXCLUDED.

The appearance of an additional protein band in the F13A heterozygous phenotype, which is not in either of the presumed F13A homozygous phenotypes, suggests that the molecular structure of the F13A protein detected could be a dimer. In such a system, homozygous phenotypes would be represented as functional homodimers formed from a polypeptide pool consisting of a single monomeric species while the heterozygous phenotype would consist of three structurally distinct dimers (two different homodimers and one heterodimer) formed from a pool of two distinct monomeric species.

Conversely, the lack of additional bands in the F13B heterozygous phenotypes which are not present in the homozygous F13B phenotypes, suggests that the molecular structure of the F13B subunits detected may be monomeric. Homozygotes show a single major intense band because only one monomeric species is present while heterozygous phenotypes show two major intense bands because two distinct monomeric species are present.

The generation of protein variants with altered electrophoretic mobility could arise through the substitution, at some point, in a given amino acid sequence of a neutral amino acid by a basic or acidic amino acid or vice versa or the substitution of a basic for an acidic amino acid or vice versa (Harris and Hopkinson, 1976). Thus the F13A2 polypeptide could have arisen from the F13A1 polypeptide through the substitution of a neutral amino acid by a basic amino acid or an acidic amino acid by a neutral amino acid. Conversely the

F13A1 polypeptide could have arisen from F13A2 through a basic to neutral or a neutral to acidic amino acid substitution. A similar argument could be used to explain the origin of the three monomeric species of F13B polypeptides. For example, it is possible that the F13B3 polypeptide could have arisen from the F13B1 polypeptide through the replacement of an acidic with a basic amino acid while F13B2 could have been generated through an acidic to neutral amino acid substitution in the F13B1 protein. In total there are eight other possible ways in which single amino acid substitutions could give rise to the three common variant F13B polypeptides. Such specific alterations could occur at the DNA level through point mutations. However, in order to determine whether the electrophoretic variants are indeed the results of single amino acid substitutions the polypeptides of each F13A variant and F13B variant will have to be sequenced and compared.

The minor bands inherent to the F13B phenotypes are likely due to varying extents of posttranslational modifications. Mechanisms by which such non-genetic variation can be introduced into a protein include the deamidation of glutamine or asparagine residues, acetylation, oxidation of sulfhydryls, addition of phosphate groups, additon of carbohydrate groups, molecular aggregation, and the cleavage of a given polypeptide by proteolytic enzymes (Harris and Hopkinson, 1976). In the case of F13B much of the heterogeneity in the banding patterns of neat plasma can be attributed to the extent of

oligosaccharide association with a given F13B subunit type as treatment with neuraminidase, an enzyme which removes the acidic monosaccharide sialic acid, removes many of the minor variant bands seen in the F13B phenotypes.

4.200 Population and Family Studies

4.210 F13A

The allelic frequencies estimated from the distribution of F13A phenotypes in 188 unrelated Canadians of Western European descent (F13A*1 = 0.80 ± 0.02, F13A*2 = 0.20 ± 0.02) as well as from the distribution in 123 unrelated Newfoundlanders (F13A*1 = 0.76 ± 0.03; F13A*2 = 0.24 ± 0.03) are in agreement with allelic frequencies estimated in a sample of the Australian population (F13A*1 = 0.79 and F13A*2 = 0.21) and a sample of the German population (F13A*1 =0.797, F13A*2 = 0.203) (Board, 1979; Kreckel et al., 1982). Distribution of the F13A phenotypes in the two sample populations of this study were indicative of an autosomal basis of inheritance, as no statistically significant deviations were detected between the phenotypic distributions in males and females. Furthermore the phenotypic

distributions for the total samples of both populations were found to be in Hardy-Weinberg equilibrium on the assumption that the phenotypes are attributed to two codominant alleles at a single autosomal locus. The distribution of F13A phenotypes in 626 offfspring from 200 nuclear families did not deviate significantly from those expected for a Mendelian system of two codominant alleles at a single autosomal locus.

4.220 F13B

The allele frequencies estimated from the F13B phenotypes in 149 unrelated Canadians of Western European descent (F13B*1 = 0.74 ± 0.03, F13B*2 = 0.11 ± 0.02, F13B*3 = 0.15 ± 0.02) are in agreement with the allele frequencies estimated in a sample of the Australian population (F13B*1 = 0.7469, F13B*2 = 0.0836, F13B*3 = 0.1693) and a sample of the German population (F13B*1 = 0.708, F13B*2 = 0.109, F13B*3 = 0.183) (Board, 1980; Kreckel and Kuhnl, 1982).

The distribution of the F13B phenotypes in the sample population of Canadians of Western European descent was indicative of an autosomal basis of inheritance, as no statistically significant deviations were noted between male and female distributions in several phenotypic classes. Furthermore phenotypic distributions for the total sample were found to be in Hardy-Weinberg equilibrium on the assumption that

the phenotypes are attributed to three codominant alleles at a single autosomal locus. Except for the offspring of the F13B2-1 ♂ x F13B1 ♀ matings the distributions of phenotypes of 475 offspring from 124 families , representing seven mating types, analyzed were in agreement with those expected for a trait attributed to three codominant alleles at a single autosomal locus. The cause of the statistically significant excess of F13B1 offspring over F13B2-1 offspring detected in the offspring of the F13B2-1 ♂ x F13B1 ♀ matings is not known and has not been previously noted in the literature. It is possible that the deviation detected is a reflection of the small number of families of this type available for testing or perhaps a bias in the ascertainment of families studied. It is also possible, however, that there is some adverse direct or indirect maternal effect against sperm or zygotes carrying the F13B*2 allele when this allele is absent from the maternal genotype. A more extensive analysis of the segregation of F13B in families will be required to determine the biological significance, if any, of this observation.

4.300 Gene mapping

4.310 Linkage studies

4.311 F13A

Close linkage between F13A and JK, as suggested by Keats et al. (1984), who obtained a maximum total lod score of +1.67 at θ=0, is unlikely based on the results of the present study in which a total F13A:JK lod score of -2.683 at θ=0.20 was obtained. It is difficult to compare the combined lod scores of the present study with those of Keats et al.(1984) because the latter did not present a complete set of lod scores. It is evident, however, that the positive data generated by Keats et al. (1984) could be negated by the large negative scores (male lod scores of -8.733 and female lod scores of -7.023 at a θ value of 0.05) obtained at θ values indicative of close linkage in the present study.

Linkage of F13A and HLA was initially reported by Olaisen et al (1984) and several other studies have presented data in support of this relationship (Eiberg et al., 1984; Board et al., 1984; Olaisen et al., 1985). In the present study, positive paternal lod scores have been obtained between F13A and HLA as well as BF and GLO (two loci known to be closely linked to HLA) but only the paternal lod scores obtained for F13A:HLA have reached levels for acceptance of linkage ($\hat{z}$= +4.20, $\hat{\theta}$ = 0.08). The present study, thus provides additional data which support Olaisen et al's initial finding.

Summaries of the paternal and maternal lod scores of Eiberg et al.(1984), Board et al.(1984), Olaisen et al.(1985) and the present study for F13A:HLA and F13A:GLO are presented in Table 12. The maximum total paternal lod score for F13A: HLA, as estimated in Fig. 7 from the combined lod scores of Table 12, was +20.10, $\hat{\theta}$ = 0.15 (95% CI:0.11-0.21) which is well above the formal level for acceptance of linkage. Paternal F13A:GLO lod scores in each independent study, have been consistent in that peak lod scores were positive but less than +3 and occurred at values which were greater than the $\hat{\theta}$ value at which the paternal F13A:HLA lod scores peaked. Because GLO is closely linked proximally to HLA (Weitkamp and Guttormsen, 1976; Bender and Grzeschik, 1976), the F13A:HLA and F13A:GLO data have always been interpreted as indicating that F13A was further from GLO than HLA, placing it distal to HLA. F13A:GLO lod scores, in each independent study, however, were not sufficiently positive to justify a F13A:GLO linkage relationship. The results of Eiberg et al.(1984), Board et al. (1984) and Olaisen et al. (1985) taken in total give a maximum positive paternal lod score of 4.00, $\hat{\theta}$ = 0.23 (95% CI: 0.16-0.22). The addition of the F13A:GLO paternal lod scores obtained in the present study raises the maximum lod score to 5.80, $\hat{\theta}$ = 0.21 (95% CI: 0.15-0.29), thus solidifying the linkage relationship and verifying the gene order of pter:F13A:HLA:GLO:cen on the short arm of chromosome 6. The combined maternal lod scores for both F13A:HLA and F13A:GLO are negative at all tested θ values less than 0.30 and, thus, do not indicate close linkage in either of these relationships.

TABLE 12. SUMMARY OF LOD SCORES FOR F13A AND KNOWN MARKER LOCI ON THE SHORT ARM OF CHROMOSOME 6.

		NUMBER OF		LODSCORES AT RECOMBINATION FRACTIONS OF					
LOCI	MT	F	C	0.05	0.10	0.20	0.30	0.40	S
HLA:F13A	P	25	96	0.98	4.14	5.10	3.72	1.55	1
— —	P	32	108	1.78	6.16	7.50	5.60	2.49	2
	P	9	36	3.16	3.66	3.36	2.41	1.16	3
	P	18	56	4.02	4.19	3.31	1.97	0.71	4
		84	296	9.94	18.15	19.27	13.70	5.91	
HLA:F13A	M	25	94	-12.64	- 6.26	-1.46	-0.04	0.11	1
— —	M	33	120	-27.01	-14.35	-4.31	-0.77	0.17	2
	M	5	20	- 3.98	- 2.17	-0.70	-0.15	0.02	3
	M	9	29	- 0.50	0.26	0.59	0.42	0.13	4
		72	263	-44.13	-22.52	-5.88	-0.54	0.43	
GLO:F13A	P	11	46	-4.35	-1.54	0.36	0.67	0.34	1
— —	P	18	54	-0.67	1.49	2.36	1.72	0.61	2
	P	8	30	-0.14	0.69	1.11	0.95	0.55	3
	P	25	94	-1.93	0.65	1.82	1.34	0.45	4
		62	224	-7.09	1.29	5.65	4.68	1.95	
GLO:F13A	M	8	29	- 2.65	- 1.02	0.08	0.27	0.12	1
— —	M	15	48	-12.12	- 6.49	-2.00	-0.42	-0.01	2
	M	5	20	- 1.51	- 0.54	0.15	0.31	0.22	3
	M	24	84	-18.60	-11.07	-4.54	-1.69	-0.41	4
		52	181	-34.88	-19.12	-6.31	-1.53	-0.08	

NOTE: S=SOURCE 1.EIBERG ET AL.,1984 2.OLAISEN ET AL.,1985
3.BOARD ET AL.,1984 4.THE PRESENT STUDY
P=PATERNAL M=MATERNAL F=FAMILIES C=CHILDREN

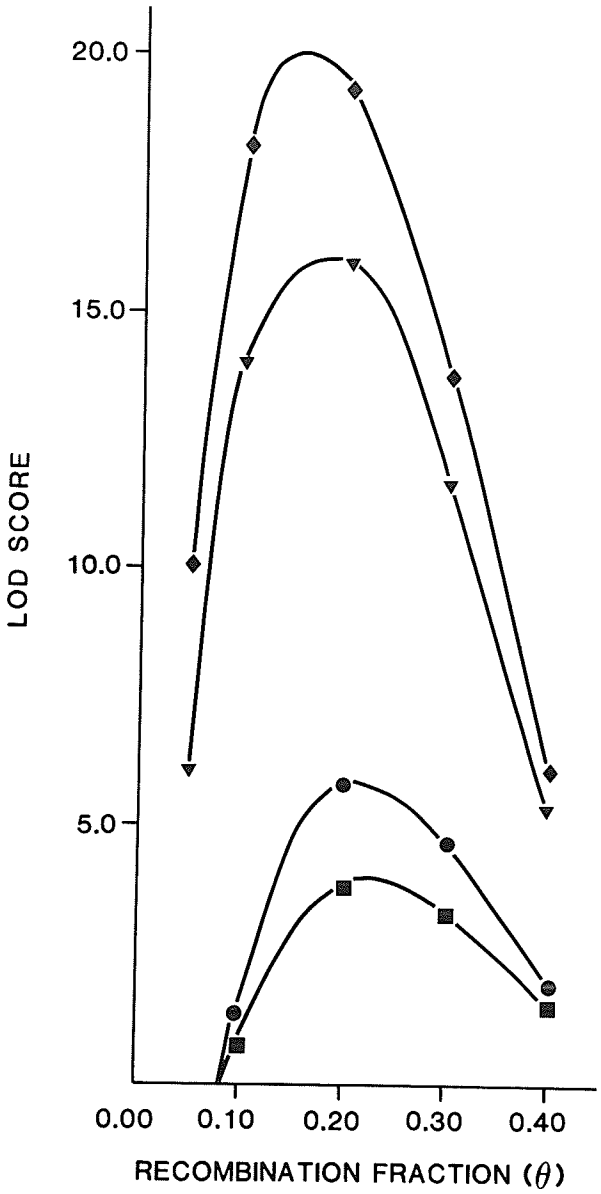

FIG. 7. LOD SCORES AND THEIR CORRESPONDING RECOMBINATION FRACTIONS (θ) FOR F13A:HLA AND F13A:GLO USING THE COMBINED PATERNAL LOD SCORES OF TABLE 12.

◆ F13A:HLA * ▼ F13A:HLA +
● F13A:GLO * ■ F13A:GLO +

+ COMBINED PATERNAL LOD SCORES OF EIBERG et al., 1984; BOARD et al., 1984; OLAISEN et al., 1985.
* PRESENT STUDY TOGETHER WITH PREVIOUSLY PUBLISHED DATA AS ABOVE.

Slightly positive lod scores were obtained between F13A and a number of loci with known chromosomal assignments (AMY2, PEPA, SE, UMPK) and the cytogenetic marker(9IQ); as these loci have been assigned to chromosomes other than 2 or 6 the lod scores obtained here probably represent random statistical fluctuations. Further testing could be carried out in order to ensure that there are indeed no linkage relationships between F13A and these markers. Slightly positive lod scores were also detected between F13A and two unassigned loci, RGS and YT(peak paternal lod scores of +0.535 at θ =0.05 and +0.701 at θ =0.10 respectively). Additional testing of these two unassigned loci with F13A as well as other 6p21 marker loci is important in that it will undoubtedly lead to either the localization or exclusion of these markers from this region of the human genome.

4.312 Linkage F13B

Lod scores for F13B and 62 genetic markers did not reach sufficient levels for the formal acceptance of linkage. Lod scores for F13B with AT3, 4Q2/7P1, 9IQ, DIA1, AFA, F2, APOE, and NFLD, however, were slightly positive and could suggest potential linkage relationships; in all cases the genetic markers involved are markers for which detection of genetic variation is generally rare. Mathematically, the possibility of linkage between F13B and each of these marker "loci" exists, but the probability of obtaining a false

linkage is increased because of the small positive lod scores. In all cases, further testing is required to determine if the lod scores could be raised to such a level, +3 or greater, so that mathematically the probability of identifying a true linkage relationship is 0.95 or greater and the probability of obtaining a false linkage is 0.05 or less.

The most promising potential linkage relationship is between F13B and AFA which has a total maximum lod score of +1.544 at a θ value of 0.07 (Fig. 8). AFA represents the locus coding for a newly described autosomal dominant syndrome that appears to show complete penetrance (Hughes et al., 1985).

The human autosomal haploid genome has been estimated to be 33 Morgans although it has been noted that this figure may be 50% larger in females than in males (reviewed by Renwick, 1969). The percentage contribution of each autosome to the total haploid autosomal complement has been summarized by Maynard-Smith et al.(1961). If the recombination fraction (θ) at which exclusion of linkage between F13B and other loci is taken to be equivalent to the map interval in terms of Morgans , which is approximately true for short intervals (Renwick, 1969; Botstein et al., 1980), then an approximation can be made of the extent of the genome which can be excluded as the location of the F13B locus relative to individual chromosomes and to the total haploid autosomal complement.

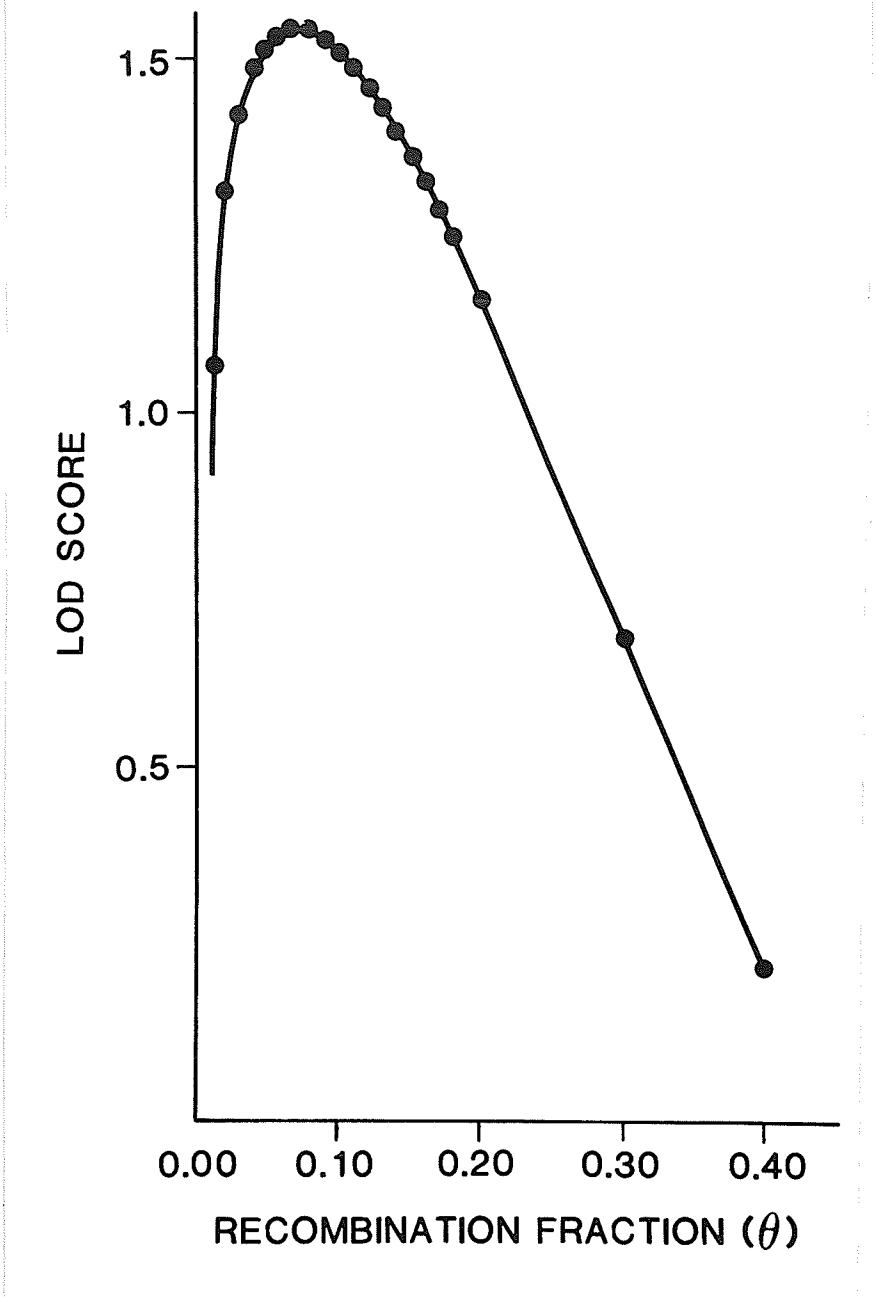

FIG. 8. ESTIMATION OF THE MAXIMUM TOTAL LOD SCORE BETWEEN F13B AND AFA ($\hat{z}$= +1.544 @ $\hat{\theta}$ = 0.07)

Lod scores for 41 of the 62 markers loci analyzed for linkage with F13B, which reached formal levels for the exclusion of close linkage ($\leq$-2) in male and/or female data, can be used to estimate the proportion of the human genome from which F13B can be excluded. The number of Morgans which can be excluded from each autosome on the basis of the negative linkage data is presented in Table 13 where it can be seen that a total of 8.60 Morgans can be excluded from specific autosomes as the location of F13B on the basis of the male lod scores while as much as 5.35 Morgans can be excluded on the basis of the female lod scores. In these calculations known linkage groups such as RH:FUCA1 and F13A:HLA:GLO have been taken into account in approximating the number of Morgans to be excluded. In addition for the purposes of these calculations unmapped loci are assumed not to be linked to any other locus of known chromosomal location and thus are interpreted for the time being, as being situated on unique areas of the gene map. Under these assumptions an additional 1.80 Morgans can be excluded from the male map and 1.10 Morgans from the female map as the location of F13B. Thus, the total map length that can be excluded as the location of F13B based on the lod scores obtained in this study are 8.60 Morgans in males and 6.45 Morgans in females. Taken as a whole the male lod scores presented in this study exclude F13B from an estimated 27% of the human autosome complement while the female lod scores exclude approximately 20% of the human autosome complement.

The proportion of each autosome excluded as well as the area covered by the unmapped loci, expressed as a percentage of the total haploid genome, is presented in Fig. 9. In the data from males the

TABLE 13. TABULATION OF THE NUMBER OF MORGANS EXCLUDED FROM SPECIFIC CHROMOSOMES ON THE BASIS OF LINKAGE STUDIES BETWEEN F13B AND OTHER MARKER LOCI.

CHROMOSOME	NUMBER OF MORGANS EXCLUDED ON THE BASIS OF	
	MALE LOD SCORES	FEMALE LOD SCORES
1	1.40	1.10
2	0.80	0.60
3	0.30	0.20
4	0.60	0.60
6	0.70	0.85
9	0.40	0.10
13	0.40	0.40
14	0.60	0.40
16	0.70	0.10
19	0.60	0.50
20	0.20	0.20
21	----	0.10
22	0.10	0.20
SUBTOTAL	6.80	5.35
U	1.80	1.10
TOTAL	8.60	6.45

U: UNASSIGNED MARKER LOCI.

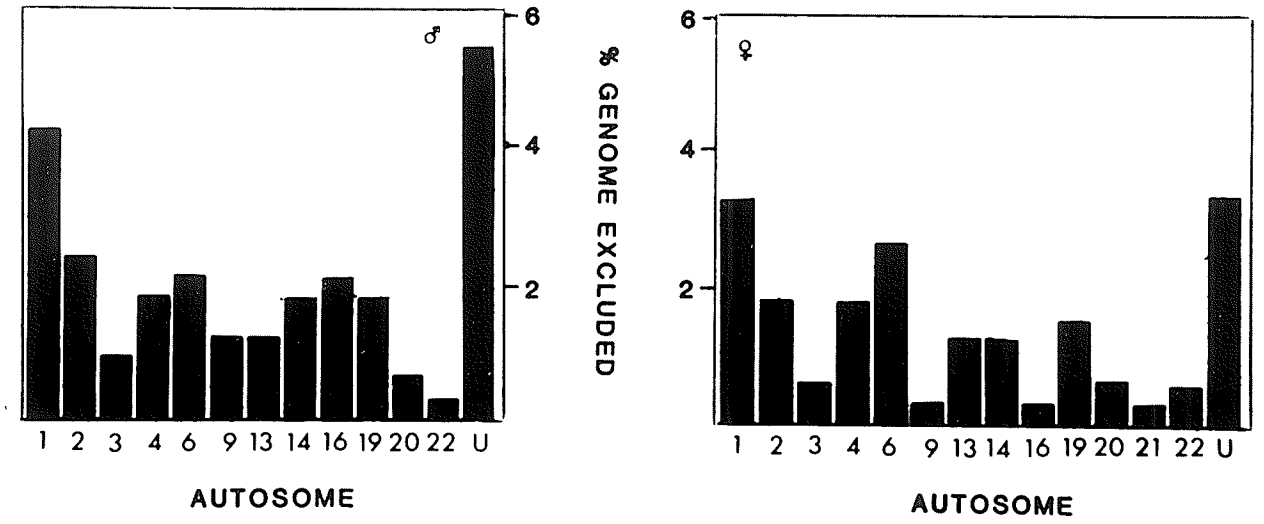

FIG. 9. DIAGRAM SHOWING THE EXTENT OF THE AREA IN WHICH THE LOCATION OF F13B HAS BEEN EXCLUDED FROM SPECIFIC AUTOSOMES IN TERMS OF THE PERCENTAGE OF THE TOTAL HAPLOID AUTOSOME COMPLEMENT (TOTAL GENOME LENGTH ASSUMED TO BE 33 MORGANS IN MALES AND FEMALES).

greatest proportion of the genome excluded as the site of F13B is the area assumed to contain the loci of unknown chromosomal assignments with the next largest percentage exclusion being for chromosome 1. In the data for females, the proportion of the genome excluded by non-linkage with chromosome 1 loci and unassigned loci are approximately equal. The number of Morgans (net length) on each chromosome is not the same and thus to be able to make inter-chromosomal comparisons the percentage of each autosome excluded is presented in Fig. 10 where it can be seen that more than half of each of chromosomes 14,16,and 19 can be excluded based on the data from males while in the female data chromosome 19 is the only autosome for which at least 50% of its length can be excluded.

4.312 F13B Exclusion mapping

The analysis of samples from individuals with known aneuploidy provide information leading to the exclusion of 4pter-p16, 4q25-q27, 4pter-p35, 17pter-p13 and 17q25-qter as potential areas for the location of F13B. These five regions which were not known to be excluded on the basis of linkage analysis represent a maximum of 1.5% of the human haploid autosome genome. In addition the exclusion of band 1p36 is consistent with the previous finding of non-linkage of F13B with PGD, a locus known to be located in 1p36.

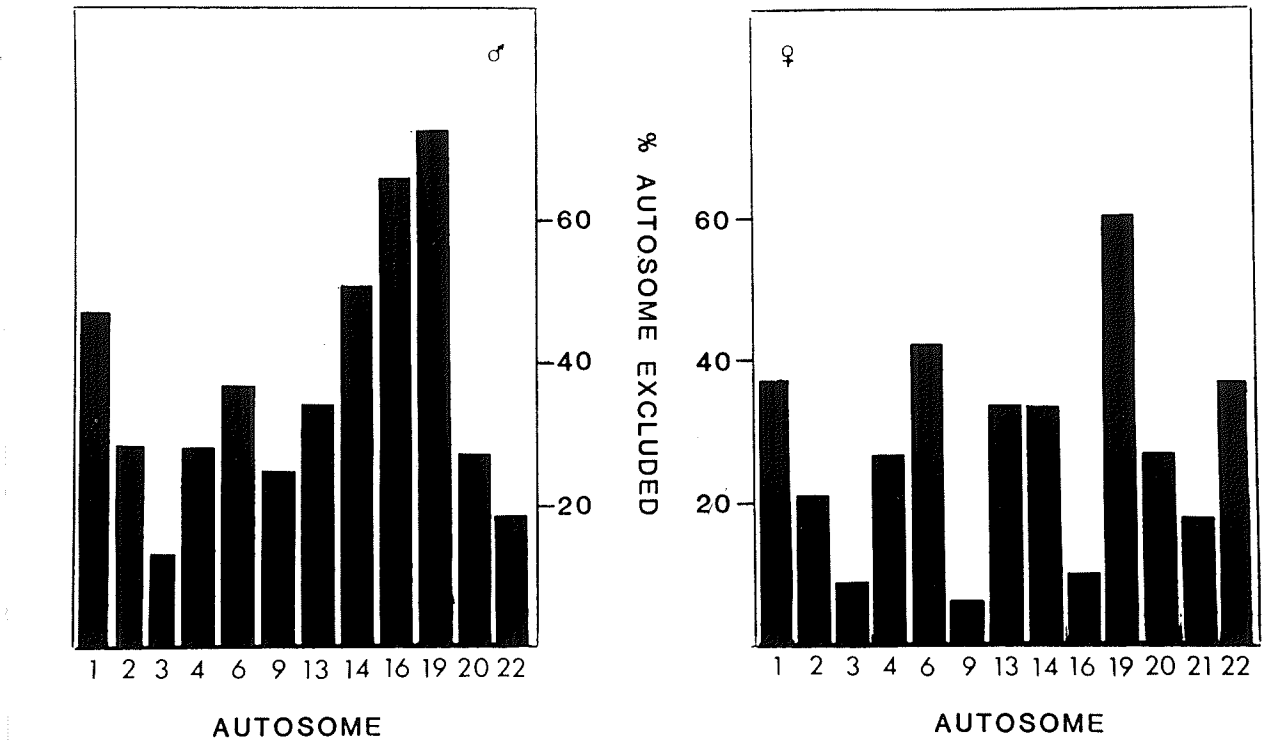

FIG.10 DIAGRAM SHOWING THE PERCENTAGE OF SPECIFIC AUTOSOMES IN WHICH THE LOCATION OF F13B HAS BEEN EXCLUDED ON THE BASIS OF LINKAGE ANALYSIS (TOTAL GENOME LENGTH ASSUMED TO BE 33 MORGANS IN MALES AND FEMALES).

5.000 SUMMARY

5.100 F13A

Population and family studies

1. Two specific Canadian populations were studied for the distribution of F13A phenotypes. Three distinct phenotypes which could be attributed to all possible combinations of two common alleles, F13A*1 and F13A*2, at the F13A locus were observed. Frequencies of F13A*1 and F13A*2 in a sample population of 338 unrelated Canadians of Western European descent were estimated to be 0.80 ± 0.02 and 0.20 ± 0.02 respectively. Frequencies of F13A*1 and F13A*2 in a sample population of 123 unrelated Newfoundlanders were estimated to be 0.76 ± 0.03 and 0.24 ± 0.03 respectively. In both populations surveyed the phenotypic distributions did not significantly deviate from the distributions expected under Hardy-Wienberg Equilibrium.

2. Segregation analysis of F13A in 626 offspring of 200 families indicated that the mode of inheritance of F13A in man followed the pattern expected for a system featuring two codominant alleles at a single autosomal locus.

Linkage studies

1. Results of linkage studies indicate that F13A is not linked to JK as previously proposed by Keats et al.(1984), F13A:JK lod scores having reached a value of -2.683 @ θ =0.20.

2. Results of the present study support a linkage relationship between F13A and HLA, as initially proposed by Olaisen et al. (1984). The maximum F13A:HLA paternal lod score obtained in the present study was +4.20, $\hat{\theta}$ =0.08. When the results of the present study are combined with the previously published data (Eiberg et al.(1984), Board et al.(1984),and Olaisen et al.(1985)) the peak F13A:HLA lod score for male data is 20.1 at $\hat{\theta}$ =0.15

3. The F13A:GLO paternal lod scores presented in this study solidify the loose linkage relationship between F13A and GLO. The maximum F13A:GLO lod score obtained in the present study was +1.82 , $\hat{\theta}$ =0.20. These results alter the former peak lod scores generated by Eiberg et al.(1984), Board et al.(1984), and Olaisen et al,(1985) from $\hat{z}$= +4.0, $\hat{\theta}$ =0.23 to $\hat{z}$= +5.8, $\hat{\theta}$ =0.21. Given that HLA and GLO are closely linked, the data verify the suggested gene order of 6pter:F13A:HLA:GLO:cen.

5.200 F13B

Population and family studies

1. Six distinct phenotypes which could be attributed to all possible combinations of three common alleles at the F13B locus were observed in a survey of Canadian individuals. The frequencies of F13B*1, F13B*2, F13B*3, in a sample population of 199 unrelated Canadians of Western European descent were 0.74 ± 0.01, 0.11 ± 0.01 and 0.15 ± 0.01 respectively. Phenotypic distributions in this population were not found to deviate statistically from the distribution expected under Hardy-Weinberg Equilibrium.

2. Segregation analysis of F13B phenotypes of 424 offspring from 111 families, representing seven mating types, suggested that the mode of inheritance of F13B in man followed the pattern expected for a system featuring three codominant alleles at a single autosomal locus. A significant deficiency of F13B2-1 offspring relative to F13B1 offspring was however found for the F13B2-1 ♂ x F13B1 ♀ mating type.

Mapping studies of the F13B locus

1. Linkage studies of F13B with 62 marker loci did not reach sufficient levels for the formal acceptance of linkage. There were, however, several positive lod scores suggestive of potential linkage

relationships with the most promising of these being the F13B:AFA lod score which peaked at +1.544 with a corresponding $\hat{\theta}$ value of 0.07.

2. Lod scores for 42 of the 62 marker loci analyzed for linkage with F13B reached formally significant levels for the exclusion of close linkage (lod scores ≤ -2) in male and/or female lod scores. Lod scores between F13B and eleven of these loci (C4A, CMT, FR, GOT2, APOC2, LW, NP, SOD1, WD, WR, and YT) represent the first time in which formal levels for the exclusion of linkage to F13B have been obtained. In addition, the total lod scores for F13B and five marker loci (MPI, GAA, DI, F2, and NFLD),which were tested for linkage to F13B for the first time in this present study, were not suggestive of close linkage. Although formal levels for the exclusion of linkage were not reached in these cases, at least one recombinant was detected between F13B and each of these marker loci.

3. Analysis of the F13B linkage data obtained in the present study allowed the exclusion of approximately 27% of the autosomal human haploid complement on the basis of paternal lod scores and 20% on the basis of maternal lod scores as potential sites for the F13B locus.

4. Analysis of plasma samples from individuals with known chromosomal deficiencies did not lead to the localization of the F13B locus, but did exclude five chromosomal regions 4pter-p16, 4q25-q27, 4q35-qter, 17pter-p13,and 17q25-qter, which were not known

to be excluded on the basis of linkage studies as the site of the F13B locus. These results allowed the exclusion of an additional 1.5% of the haploid autosome complement as the site of F13B.

6.000 REFERENCES

Barbui, T., Cartei, G., Chisesi, T., and Dini, E. (1974). Electroimmunoassay of plasma subunits-A and -S in a case of congenital fibrin stabilizing factor deficiency. Thrombos Diathesis Haemorrh. 32:124-131.

Barbui, T., Rodeghiero, F., Dini, E., Mariani, G., Papa, M.L., DeBiasi, R., Murillo, R.C., and Umana, C.M. (1978). Subunits A and S inheritance in four families with congenital Factor XIII deficiency. Br. J. Haemotol. 38:267-271.

Bender, K. and Grzeschik, K.H. (1976). Possible assignment of the glyoxalase 1 (GLO) gene to chromosome 6 using man-mouse somatic hybrids. Hum. Genet. 31:341-345.

Berliner, S.H., Lusky, A., Zivelin, A., Modan, M., and Seligsohn, U. (1984). Hereditary factor XIII deficiency: report of four families and definition of the carrier state. Br. J. Haemotol. 56:495-505.

Board, P.G. (1979). Genetic polymorphism of the A subunit of human coagulation factor XIII. Am. J. Hum. Genet. 31:116-124.

Board, P.G. (1980). Genetic polymorphism of the B subunit of human coagulation factor XIII. Am. J. Hum. Genet. 32:348-353.

Board, P.G. (1984). Genetic heterogeneity of the B subunit of coagulation factor XIII: resolution of type 2 . Ann. Hum. Genet. 48:223-228.

Board, P.G., Coggan, M., and Hamer, J. (1980). An electrophoretic and quantitative analysis of coagulation factor XIII in normal and deficient subjects. Br. J. Haematol. 45:633-640.

Board, P.G., and Coggan, M. (1981). Polymorphism of the A subunit of cagulation Factor XIII in the Pacific Region. Description of new phenotypes. Hum. Genet. 59:135-136.

Board, P.G., Coggan, M., and Pidcock, M.E. (1982). Genetic heterogeneity of human prothrombin (FII). Ann. Hum. Genet. 46:1-9.

Board, P.G., Reid, M., and Serjeantson, S. (1984). The gene for coagulation factor XIIIa subunit (F13A) is distal to HLA on chromosome 6. Hum. Genet. 67:406-408.

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. (1980). Constuction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Boyd, Y., Buckle, V.J., Munro, E.A., Choo, K.H., Migeon, B.R., and Craig, I.W. (1984). Assignment of the haemophilia B gene (Factor IX) locus to the q26-qter region of the X chromosome. Ann. Hum. Genet. 48:145-152.

Buckle, V., Craig, I.W., and Edwards, J.H. (1985). Fine assignment of the coagulation factor IX gene. Cytogenet. Cell. Genet. 40:593-594.

Camerino, G., Grzeschik, K.H., Jaye, M., DeLaSalle, H., Tolstoshev, P., Lecocq, J.P., Helig, R., and Mandel, J.L. (1984). Regional localization on the human X chromosome and polymorphism of the coagulation factor IX gene (hemophilia B locus). Proc. Natl. Acad. Sci., USA. 81:498-502.

Carson, S.D., Henry, W.M., Haley, L., Byers, M., and Shows, T. (1985). The gene for tissue factor(coagulation factor III) is localized on human chromosome 1pter-1p21. Cytogenet. Cell. Genet. 40:600.

Castle, S.L., Board, P.G., and Anderson, R.A.M. (1981). Genetic heterogeneity of Factor XIII deficiency: first description of unstable A subunits. Br. J. Haematol. 48:337-342.

Cavalli-Sforza, L.L., and Bodmer, W.F. (1971). The genetics of human populaitons. W.H. Freeman and Company, San Francisco.

Chance, P.F., Dryer, K.A., Kurachi, K., Yoshitaka, S., Ropers, H.H., Wieacker, P., and Gartler, S.M. (1983). Regional localization of the human factor IX gene by molecular hybridization. Hum. Genet. 65:207-208.

Conneally, P.M., Edwards, J.H., Kidd, K.K., Lalovel, J.M., Morton, N.E., Ott, J., and White, R. (1985). Report of the committee on methods of linkage analysis and reporting. Cytogenet. Cell. Genet. 40:356-360.

Cote, G.B. (1975). Centromeric linkage in man. Ph.D. thesis, University of Birmingham.

deGrouchy, J., Dautzenberg, M., Turleau, C., Beguin, S., Chavin-Colin, F. (1984). Regional mapping of clotting factor VII and X to 13q34. Expression of factor VII through chromosome 8. Hum. Genet. 66:230-233.

Duckert, F., Jung, E., and Shmerling, D.H. (1960). A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. Thromb. Diath. Haemorrhag. 5:179-187.

Eiberg. H., Mohr, J., and Nielson, L.S. (1984). Linkage relationships of human coagulation factor XIIIB. Cytogenet. Cell. Genet. 37:463.

Eiberg, H., Schmiegelow, K., Koch, C., Mohr, J., Schwartz, M., and Niebuhr, E. (1985). Cystic fibrosis; hint of linkage with F13B. Clin. Genet. 27:206.

Eiberg, H., Nielsen, L.S., and Mohr, J. (1984). Confirmation of F13A assignment and sequence information concerning F13A-HLA-GLO. Clin. Genet. 26:385-388.

Fried, K., Kaufman, S., and Beer,S. (1981). Factor XIII deficiency. Clin. Genet. 20:455-457.

Ginsburg, D., Handin, R.I., Bonthron, D.T., Donlon, T.A., Bruns, G.A.P., Latt, S.A., and Orkin, S.H. (1985). Human von Willebrand Factor (VWF): Isolation of complementary DNA(cDNA) clones and chromosomal localization. Science. 228:1401-1406.

Graham, J.B., Barrow, E.S., Reisner, H.M., and Edgell, C. (1983). The genetics of blood coagulation. Advances in Human Genetics. 13:1-81.

Graham, J.B. (1983) personal communication

Hampton. J.W., Cunningham, G.R., and Bird, R.M. (1966). The pattern of inheritance of defective fibrinase (factor XIII). J. Lab. and Clin. Med. 67:914-921.

Harris, H. (1980). The principles of human biochemical genetics, 3rd ed. North-Holland Publishing Company, New York.

Harris, H., and Hopkinson, D.A. (1976). Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam.

Hughes, H., McAlpine, P.J., Cox, D.W., and Philipps, S. (1985). An autosomal dominant syndrome with 'acromegaloid' features and thickened oral mucosa. J. Med. Genet. 22:119-125.

Israels, E.D., Paraskevas, F., and Israels, L.G. (1973). Immunological studies of coagulation factor XIII. J. Clin. Invest. 52:2398-2403.

Keats, B.J.B., Morton, N.E., and Rao, D.C. (1977). Possible linkages (lod score over 1.5) and a tentative map of the JK-Km linkage group. Human Gene Mapping 4. pp. 304-308.

Keats, B.J.B., Wilson, A.F., and Elston, R.C. (1984). Linkage relations among 34 genetic markers. Cytogenet. Cell. Genet. 37:507-508.

Kera, Y., Nishimukai, H., and Yamasawa, K. (1981). Genetic polymorphism of the B subunit of human coagulation factor XIII: another classification. Hum. Genet. 59: 360-364.

Kera, Y., and Nishimukai, H. (1982). Genetic polymorphism of the A subunit of human coagulation factor XIII in Japanese. Hum. Hered. 32:216-218.

Kreckel, P. and Kuhnl, P. (1982). Improved coagulation factor XIII B (FXIIIB) phenotyping after neuraminidase treatment of plasma and first description of the FXIIIB2 phenotype. Blut. 45:337-345.

Kreckel, P., Kuhnl, P., and Spielmann, W. (1982). Human coagulation factor XIIIA (FXIIIA) phenotyping by immunofixation agarose gel electrophresis (IAGE). Blut. 44:309-314.

Lamm, L.U., and Olaisen, B. (1985). Report of the committee on the genetic constitution of chromosomes 5 and 6. Cytogenet. Cell. Genet. 40:128-155.

Lamm, L.U., Svejgaard, A., and Kissmeyer-Nielson, F. (1971). PGM3:HLA is another linkage in man. Nature new Biol. 231:109-110.

Loewy, A.G., Dunathan, K., Kriel, R., and Wolfinger, H.L.Jr. (1961). Fibrinase. J. Biol. Chem. 236:2625-2655.

Lorand, L., Urayama, T., Atencio, A.C., and Hsia, D.Y. (1970). Inheritance of deficiency of fibrin-stabilizing factor(factor XIII). Am. J. Hum. Genet. 22:89-95.

Lorand, L., Gray, A.J., Brown, K., Credo, R.B., Curtiss, C.G., Domanik, R.A., and Stenberg, P. (1974). Dissociation of the subunit structure of fibrin stabilizing factor during activiation of the zymogen. Biochem. Biophys. Res. Commun. 56:914-922.

Mattei, M.G., Baeteman, M.A., Heilig, R., Oberle, I., Davies, K., Maendel, J.L., Mattei, J.F. (1985). Localization by in situ hybridization of the coagulation factor IX gene of two polymorophic DNA probes with respect to the fragile X site. Human Genetics. 69:327-331.

Maynard-Smith, S., Penrose, L.S., and Smith,C.A.B. (1961). Mathematical tables for research workers in human genetics. J. and A. Churchill Ltd, London.

McAlpine, P.J. (1985) personal communication.

McAlpine, P.J., Shows, T.B., Miller, R.L.,and Pakstis, A.J. (1985). The 1985 catalogue of mapped genes and report of the nomenclature committee. Cytogenet. Cell. Genet. 40:8-66.

McDonagh, J., McDonagh, R.P.Jr., and Duckert, F. (1971). Genetic aspects of factor XIII deficiency. Ann. Hum. Genet. 35:197-206.

Morton, N.E. (1955). Sequential tests for the detection of linkage. Am. J. Hum. Genet. 7:277-318.

Murray, J.C., Sadler, E., Eddy, R.L., and Shows, T.B. (1985). Evidence for assignment of plasminogen (PLG) to chromosome 6, not chromosome 4. Cytogenet. Cell. Genet. 40:709.

Nakamura, S., and Abe, K. (1982). Genetic polymorphism of coagulation factor XIIIB subunit in Japanese. Ann. Hum. Genet. 46:203-207.

Niebuhr, E., Eiberg, H., and Schousboe, I. (1985). Localization of human F12, F13A, and ACP1. Cytogenet. Cell. Genet. 40:714.

Nishigaki, T. and Omoto, K. (1982). Genetic polymorphism of the B subunit of human coagulation factor XIII in Japanese. Jap. J. Hum. Genet. 27:265-270.

Olaisen, B., Teisberg, P., and Gedde-Dahl, T.Jr. (1982). Fibinogen γ chain locus is on chromosome 4 in man. Hum. Genet. 61:24-26.

Olaisen, B., Siverts, A., and Gedde-Dahl, T.Jr. (1984). Linkage data for the coagulation factor genes FXIIIA and FXIIIB. Cytogenet. Cell. Genet. 37:560.

Olaisen, B., Gedde-Dahl. T.Jr., Teisberg, P., Thorsby, E., Siverts,A., Jonassen, R., and Wilhelmy, M.C. (1985). A structural locus for coagulation factor XIIIA (F13A) is located distal to the HLA region on chromosome 6p in man. Am. J. Hum. Genet. 37:215-220.

Ott, J., Schrott, H.G., Goldstein, J.L., Hazzard, W.R., Allen, F.H., Falk, C.T., and Motulsky, A.G. (1974). Linkage studies in a large kindred with familial hypercholesterolemia. Am. J. Hum. Genet. 26:598-603.

Pearson, P.L., VanDerKamp, J., and Veldtkamp, J. (1981). Reduced Hageman factor level in a 6p- patient. Cytogenet. Cell. Genet. 32:309.

Pfeiffer, R.A., Ott, R., Gilgenkrantz, S., and Alexandre, P. (1982). Deficiency of coagulation of factors VII and X associated with deletion of a chromosome 13(q34). Evidence from two cases with 46,XY, t(13:Y)(q11:q34). Hum. Genet. 62:358-360.

Purrello, M., Alhadeff, B., Esposito, D., Szabo, P., Rocchi, M., Truett, M., Masiarz, F., and Siniscalco, M. (1985). The human genes for hemophilia A and hemophilia B flank the X chromosome fragile site at Xq27.3. EMBO. 4:725-729.

Ratnoff, O.D. (1978). Hereditary disorders of hemostasis. in The metabolic basis of inherited disease. Edited by J.B.Stanbury, J.B.Wyngaarden, D.S.Fredrickson. (pp1755-1791). McGraw-Hill Book Company, New York.

Ratnoff, O.D., and Steinberg, A.G. (1968). Inheritance of fibrin stabilizing-factor deficiency. Lancet. 1:25-26.

Renwick, J.H. (1969). Progress in mapping human autosomes. Br. Med. Bull. 25:65-73.

Robson, E.B., and Lamm, L.U. (1984). Report of the committee on the genetic constitution of chromosome 6. Cytogenet. Cell. Genet. 37:47-70.

Schwartz, M.L., Pizzo, S.V., Hill, R.L., and McKee, P.A. (1971). The subunit structures of human plasma and platelet factor XIII (fibrin-stabilizing factor). J. Biol. Chem. 246:5851-5854.

Schwartz, M.L., Pizzo, S.V., Hill, R.L., and McKee, P.A. (1973). Human factor XIII from plasma and platelets. J. Biol. Chem. 248:1395-1407.

Seelig, G.F., and Folk, J.E. (1980). Noncatylytic subunits of human blood plasma coagulation factor XIII. J. Biol. Chem. 255:8881-8886.

Shows, T.B., Alper, C.A., Bootsma, D., Dore, M., Douglas, T., Huisman, T., Kit, S., Klinger, H.P., Kozak, C., Lalley, P.A., Lindsley, D., McAlpine, P.J., McDougall, J.K., Khan, P.M., Meisler,M., Morton, N.E., Opitz, J.M., Partridge, C.W., Payne, R., Roderick, T.H., Rubinstein, P., Ruddle, F.H., Shaw, M., Spranger, J.W., and Weiss, K. (1979). International system for human gene nonmenclature(1979) ISGN(1979). Cytogenet. Cell Genet. 25:96-116.

Shows, T,B., and McAlpine, P.J. (1982). The 1981 catalogue of assigned human genetic markers and report of the nomenclature committee. Cytogenet. Cell. Genet. 32:221-245.

Shows, T.B., McAlpine, P.J. and Miller, R.L. (1984). The 1983 catalogue of mapped human genetic markers and report of the nomenclature comittee. Cytogenet. Cell. Genet. 37:340-393.

Swisshelm, K., Dyer, K., Sadler, E., and Disteche, C. (1985). Localization of the plasminogen gene (PLG) to the distal portion of the long arm of human chromosome 6 by in situ hybridization. Cytogenet. Cell. Genet. 40:756.

Tsevrenis, H., Mandalaki, T., Volkers, W.S., and Khan, P.M. (1979). Close linkage between G6PD and hemophilia-A gene (HEMA) confirmed in Greek families. Cytogenet. Cell. Genet. 25:213.

Verweij, C.L., de Vries, C.J.M., Pannekoek, H., Westerveld, A., and Geurts van Kessel, A. (1985). Localization of the human von Willebrand factor gene on chromosome 12. Cytogenet. Cell. Genet. 40:771.

Weitkamp, L.R. and Guttormsen, S.A. (1976). Genetic linkage of a locus for erythrocyte glyxalase(GLO) with HLA and Bf. Cytogenet. Cell. Genet. 16:364-366.

7.000 APPENDIX

Table of genetic notations used in the present study which are not listed by the International Systems for Human Gene Nomenclature (Shows et al., 1979; Shows and McAlpine, 1982; Shows et al., 1984; McAlpine et al., 1985)

LOCUS SYMBOL	MARKER NAME
AFA	Acromegaloid facial appearance
DI	Diego blood group
F2	Coagulation factor II
FR	Froese blood group
NFLD	Newfoundland blood group
RGS	Reiger Syndrome
SPB	Sinking pre β-lipoprotein (possibly identicle to lipoprotein Lp)
WD	Waldner blood group
WR	Wright blood group
YT	Yt blood group