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The Locus for Combined Factor V–Factor VIII Deficiency (F5F8D) Maps to 18q21, between D18S849 and D18S1103

M. Neerman-Arbez,[1] S. E. Antonarakis,[1,2] J.-L. Blouin,[2] S. Zeinali,[3] M. Akhtari,[4] Y. Afshar,[5] and E. G. D. Tuddenham[6]

[1]Division of Medical Genetics, Department of Genetics and Microbiology, University of Geneva Medical School, and [2]Division of Medical Genetics, Cantonal Hospital of Geneva, Geneva; [3]Pasteur Institute, and [4]Haemophilia Centre and [5]Haematology Department, Imam Khomeini Hospital, Tehran; and [6]MRC Haemostasis Research Group, Royal Postgraduate Medical School, London

Summary

Combined factor V–factor VIII deficiency (F5F8D) is a rare, autosomal recessive coagulation disorder in which the levels of both coagulation factor V and coagulation factor VIII are diminished. In order to map and subsequently clone the gene responsible for this phenotype, DNAs from 19 families (16 from Iran, 2 from Pakistan, and 1 from Algeria) with a total of 32 affected individuals were collected for a genomewide linkage search using genotypes of highly informative DNA polymorphisms. All pedigrees except two contained at least one consanguineous marriage. A maximum LOD score (Z_{max}) of 14.82 for $\theta = .02$ was generated with marker D18S1129 in 18q21; LOD scores >9 were obtained for several other markers—D18S849, D18S1103, D18S64, and D18S862. Multipoint analysis resulted in $Z_{max} = 18.91$ for the interval between D18S1129 and D18S64. Informative recombinants placed the locus for F5F8D between D18S849 and D18S1103, in an interval of ~1 cM. These results are similar to the recently reported linkage of this disease to chromosome 18q in Jewish families (Nichols et al. 1997) and provide evidence that the same gene is responsible for all F5F8D among human populations. The difference in clinical severity of the phenotype in unrelated families, as well as the failure to detect a specific haplotype of DNA polymorphisms in the consanguineous Iranian families, suggests the existence of different molecular defects in the F5F8D gene. There exists an apparently gap-free contig with CEPH YACs linking the two markers on either side of the critical region. Positional cloning efforts are now in progress to clone the F5F8D gene.

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Address for correspondence and reprints: Dr. Stylianos E. Antonarakis, Centre Médical Universitaire, 1 rue Michel Servet, CH-1211 Geneva, Switzerland; E-mail: sea@medsun.unige.ch; or Dr. E. G. D. Tuddenham, MRC Haemostasis Research Group, Clinical Sciences Centre, Royal Postgraduate Medical School, DuCane Road, London W12 ONN, United Kingdom; E-mail: etuddenh@hgmp.mrc.ac.uk

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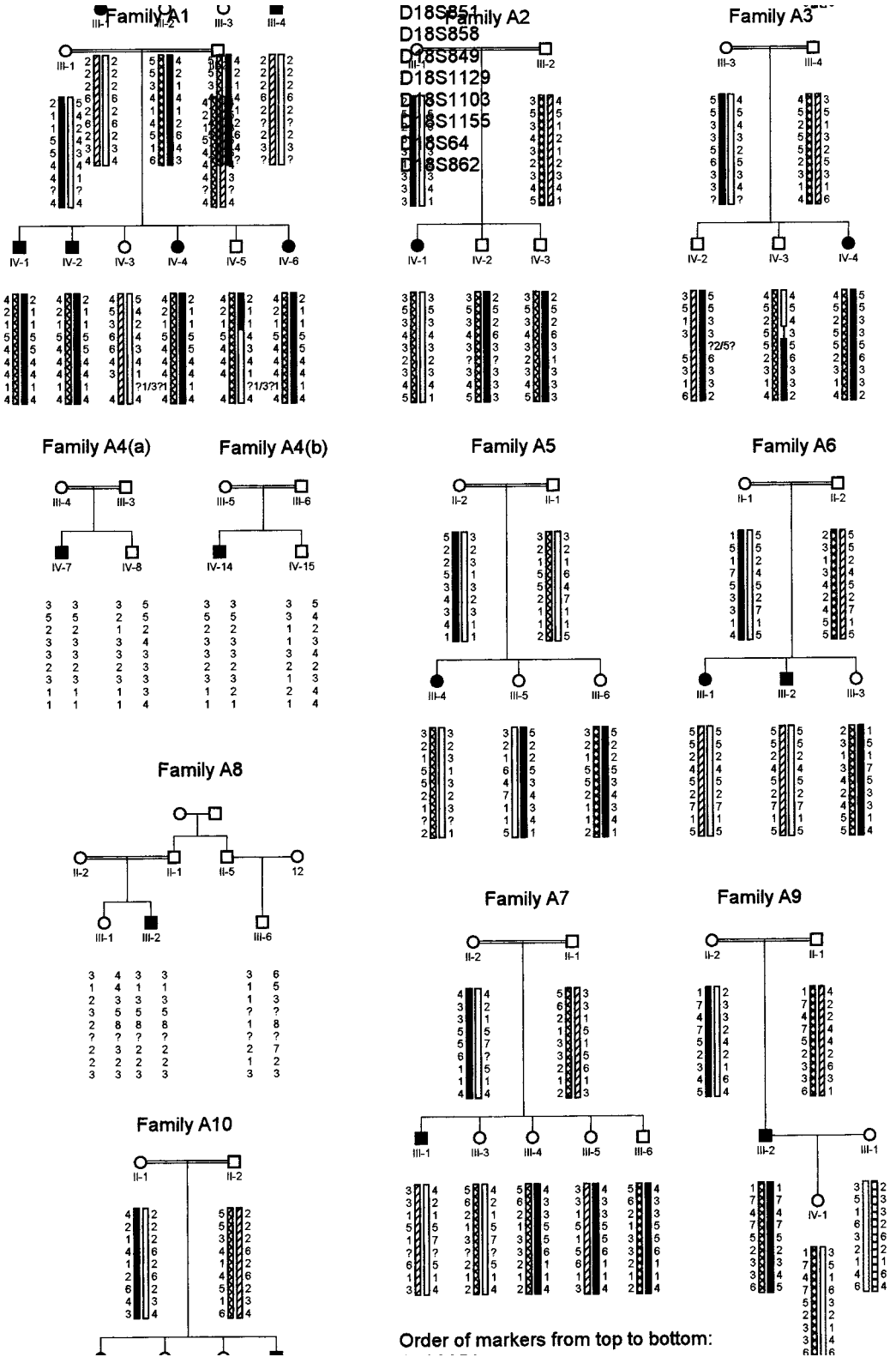
Introduction

Combined factor V–factor VIII deficiency (F5F8D) is a rare, recessive bleeding disorder characterized by reduction in levels of both factor V and factor VIII to the range of 5–20 U/dl (MIM 227310). Fewer than 100 families with this disorder have been described in the literature to date (Seligsohn and Ramot 1969; Mazzone et al. 1982; Seligsohn et al. 1982, 1984; Ozsoylu 1983), but in societies where consanguineous marriages are common, such as those in the Middle East, its frequency is similar to that of other recessive bleeding disorders—for example, factor VII deficiency and Glanzmann thrombasthenia. Clinically, the severity of the bleeding ranges from mild (factor V and factor VIII levels 10–20 U/dl) to moderate (5–10 U/dl) (Seligsohn 1989).

Linkage to the factor V and factor VIII loci has been excluded, as has linkage to components of the protein C pathway, derangement of which might lead to excessive destruction of the two factors deficient in F5F8D (Marlar and Griffin 1980; Canfield and Kisiel 1982; Suzuki et al. 1983; Gardiner and Griffin 1984; Rahim et al. 1985; Zivelin et al. 1995). We collected 16 F5F8D families from Iran, 2 of Pakistani origin, and 1 from Algeria, in order to perform a genomewide linkage analysis as a first step toward the identification of the gene responsible for the phenotype. In the course of collecting the families in Iran and as a result of obtaining detailed clinical histories and factor-assay levels of extended kindreds, it became apparent that severity varies between families but is consistent within a given family. Also, one individual with a severe form of the disorder (factor V and factor VIII levels of 2 U/dl) was encountered. These are new clinical observations, which strongly suggest genetic heterogeneity.

During the course of the study, Nichols et al. (1996) reported linkage of a F5F8D locus to an unspecified region of chromosome 18q in nine non-Ashkenazi Jewish families. We therefore concentrated our genotyping efforts on markers on this chromosome. We found linkage of F5F8D on 18q in our families, in an ~1-cM interval between markers D18S849 and D18S1103, suggesting that the same gene is responsible for this disease, across human populations. The difference in clinical severity of the phe-


Family A1
III-1
III-2
III-3
III-4
IV-1
IV-2
IV-3
IV-4
IV-5
IV-6
Family A2
D18S851
D18S858
D18S849
D18S1129
D18S1103
D18S1155
D18S64
D18S862
III-1
III-2
IV-1
IV-2
IV-3
Family A3
III-3
III-4
IV-2
IV-3
IV-4
Family A4(a)
III-4
III-3
IV-7
IV-8
Family A4(b)
III-5
III-6
IV-14
IV-15
Family A5
II-2
II-1
III-4
III-5
III-6
Family A6
II-1
II-2
III-1
III-2
III-3
Family A8
II-2
II-1
II-5
12
III-1
III-2
III-6
Family A7
II-2
II-1
III-1
III-3
III-4
III-5
III-6
Family A9
II-2
II-1
III-2
III-1
IV-1
Family A10
II-1
II-2
Order of markers from top to bottom:

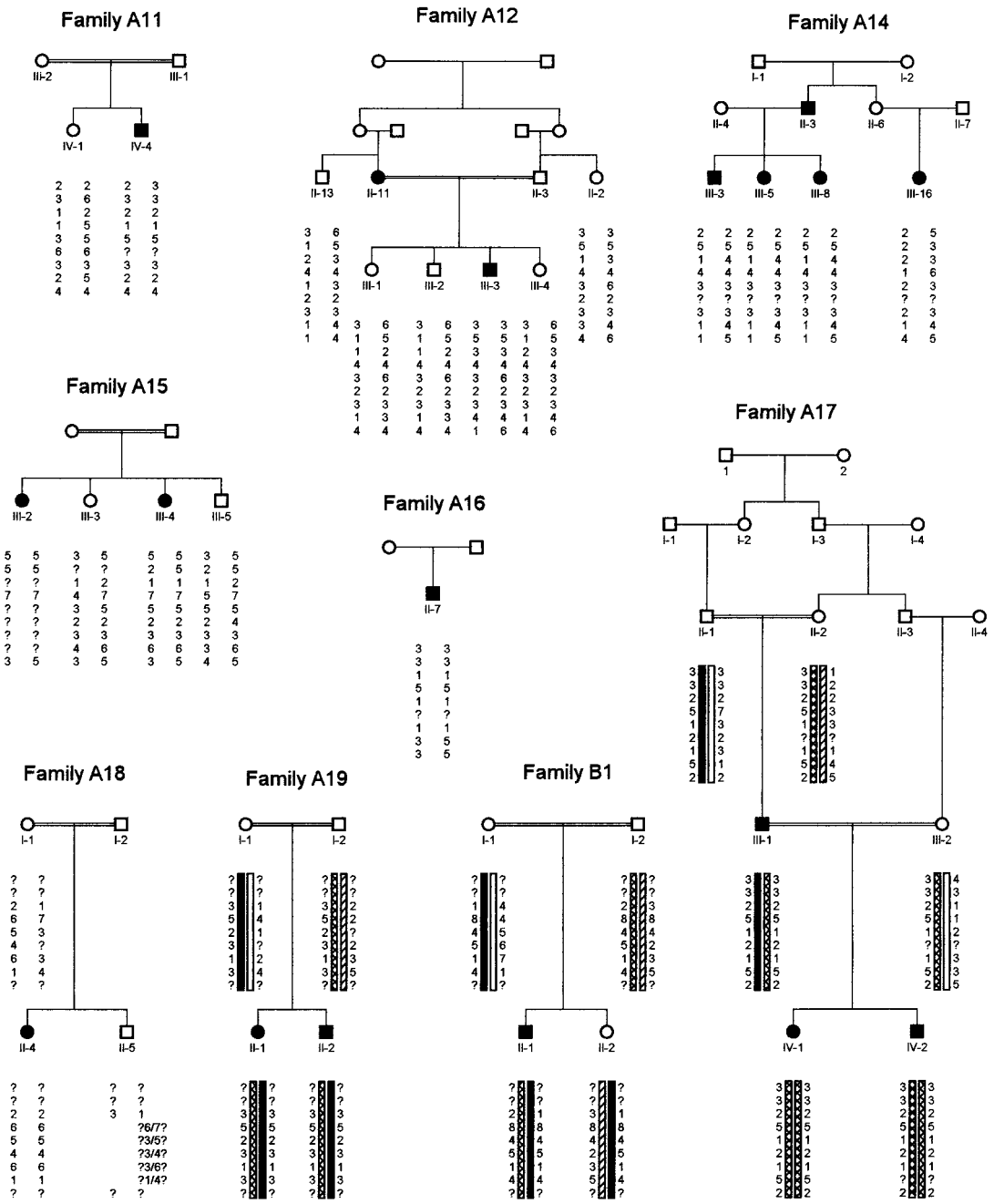


Figure 1 Pedigrees with F5F8D that were used for linkage analysis. Haplotypes for nine markers on 18q, generated by the Cyrillic program, are shown. Representative bars are depicted only for those families in which both parents and children were analyzed and in which haplotypes for polymorphic markers could be constructed. All consanguineous marriages involve first cousins, except for B1 (the mother of I-1 and the father of I-2 are half-sibs). Families 4(a) and 4(b) are also related; the two fathers are brothers, and the two mothers are sisters. The phase of certain genotypes is unknown, and these are indicated by question marks (?) on both sides of the numbered alleles. Question marks without numbers denote unknown genotypes.

notype among families and the lack of a specific haplotype of DNA polymorphisms in the Iranian families suggest allelic heterogeneity; that is, more than one mutation in the F5F8D gene is responsible for the phenotype in different families. Positional cloning efforts to isolate the elusive F5F8D gene are now in progress.

Families, Material, and Methods

Families

Blood was collected from 16 families from Iran (A1–A12 and A14–A17), 2 families of Pakistani origin (A18 and A19), and 1 family of Algerian origin (B1) (fig. 1).

Appropriate informed consent was obtained from all of these families. All families except A14 and A16 were characterized by at least one consanguineous marriage. Factor V and factor VIII assays were performed by a one-stage procedure using congenitally deficient plasma substrates and normal pooled plasma as reference (Dacie and Lewis 1995, p. 329). The levels of factor V and factor VIII measured in affected individuals are shown in table 1.

DNA Polymorphism and Linkage Analysis

Genomic DNA was purified from blood lymphocytes, according to a standard SDS–proteinase K and phenol/chloroform extraction method (Kunkel et al. 1977). DNA polymorphisms were analyzed by PCR amplification of simple sequence repeats. Microsatellite markers located in 18q (D18S535, D18S851, D18S858, D18S849, D18S1129, D18S1103, D18S1155, D18S64, D18S862, D18S1270, and D18S541, ordered from centromere to telomere) were used. Information about the oligonucleotide primers used for the scoring of genotypes of these markers and their mapping position can be obtained from the Genome Database (GDB) (http://gdbwww.gdb.org/) or Cooperative Human Linkage Center (http://www.chlc.org:80/data/CHLCmaps). Oligonucleotides were obtained from Research Genetics. One oligonucleotide primer of each marker pair was labeled with γ^{32}P-ATP by use of T4 polynucleotide kinase. PCR was performed on a Perkin-Elmer thermocycler to amplify genomic DNA in a total volume of 15 μl mixture/reaction, containing 0.4 pM labeled forward primer, 2.6 pM unlabeled reverse primer, 1.3 μM each dNTP, and 0.25 U *Taq* polymerase. PCR products were separated by electrophoresis in a 6% denaturing urea/polyacrylamide gel. Scoring of the polymorphic alleles was performed across families (i.e., the allele numbering was consistent throughout the families), for all markers except D18S849 and D18S541.

Family information and marker genotypes were stored in pedigree computer program Cyrillic™ (Cherwell Scientific). Analysis was performed by use of the ILINK, MLINK, and LINKMAP programs of the LINKAGE version 5.2 (Lathrop et al. 1984) and FASTLINK version 3.0 (Cottingham et al. 1993) software packages. Multipoint linkage analysis was performed with the help of the computer facility of the U.K. Human Genome Mapping Program resource centre (http://www.hgmp.mrc.ac.uk/). A Z_{max} was calculated for each marker locus by assuming both autosomal recessive mode of inheritance with 100% penetrance and a mutant gene frequency of .00001. For all markers, the allele frequencies were kept equal. Allele-frequency data from the GDB (collected from Europe and the northern United States) were not used, because our study population was mainly Iranian. In addition, slight differences in allele frequency did not significantly alter the estimated LOD scores.

Results

Results of pairwise linkage analyses between the phenotype of F5F8D families A1–A19 and B1 and 11 18q markers are shown in table 2. $Z_{max} = 14.82$ was obtained for marker D18S1129 at recombination fraction $\theta = .02$. Several other markers in this chromosomal region (18q21) also yielded Z_{max} values >9.00 for $\theta < .05$. Results of multipoint linkage analysis for markers D18S849, D18S1129, and D18S64 are shown in figure 2, indicating $Z_{max} = 18.91$ in the interval between D18S1129 and D18S64. The chromosomal order of markers used had been determined elsewhere, either by linkage mapping (Murray et al. 1994; Sheffield et al. 1995; GDB at http://gdbwww.gdb.org or CHLC at http://www.chlc.org:80/data/CHLCmaps) or by physical mapping using sequence-tagged sites on YACs (Cohen et al.1993; Hudson et al. 1995; MIT at http://www-genome.wi.mit.edu/). Their order is schematically shown in figure 3.

Since most of the families are consanguineous, it would be expected that the markers close to the F5F8D locus would show homozygosity for their alleles. For marker D18S1129 there are only two families (A3 and A5) without homozygosity in the affected individuals. For marker D18S1103 there are four such families (A1, A5, A7, and A12). Similarly, for marker D18S849 the families with heterozygosity are A2, A3, A5, and B1.

The haplotypes generated by the Cyrillic program, for nine markers, are depicted in figure 1. Representative bars are shown only for those families in which both parents and children were analyzed and in which haplotypes therefore could be unequivocally constructed. Informative recombinants were observed in a number of families. Of particular importance were two crossovers observed in families A1 and A3. Recombination was found for individual A1-IV5, placing the F5F8D locus distal to D18S849 (fig. 1), and for individual A3-IV3, placing this locus proximal to D18S1103 (unfortunately, marker D18S1129 was uninformative in this family). The region between the two markers D18S849 and D18S1103 on chromosome 18q21 corresponds to a distance of ~1 cM and is entirely covered by YAC contig WC18.4 (Hudson et al. 1995; MIT web page at http://www-genome.wi.mit.edu/). Between them, YACs 789F3, 962C11, 715H10, 949B6, and 826H9 contain the entire critical region as defined by our linkage data (fig. 3).

Discussion

We have described here the mapping of a locus for F5F8D to an ~1-cM region of 18q21. The Z_{max} obtained by use of DNAs from the members of 19 families of Ira-

Table 1

Severity of Bleeding, and Factor V and Factor VIII Values, in Affected Members of F5F8D Families

Family and Individual	Severity	Factor V[a]	Factor VIII[a]	S1129–S1103 Haplotype[b]
A1:				
IV1	Moderate	8	5.5	5 4/5 5
IV4	Moderate	7	14	5 4/5 5
IV6	Moderate	11	9	5 4/5 5
A2:				
IV1	Moderate	7.5	7	4 3/4 3
A3:				
IV4	Mild	20	16	5 5/3 5
A4:				
IV7	Moderate	10.5	11	3 3/3 3
A5:				
III4	Moderate	5	10	5 5/1 3
A6:				
III1	Moderate	12	12.5	4 5/4 5
III2	Moderate	12	14	4 5/4 5
A7:				
III1	Moderate	7	7	5 1/5 7
A8:				
III2	Moderate/severe	5	6	5 8/5 8
A9:				
III2	Moderate	18	8	7 5/7 5
A10:				
III1	Moderate	12	15	6 2/6 2
III4	Moderate	19	16	6 2/6 2
A11:				
IV4	Mild	18	17	1 5/1 5
A12:				
III3	Severe	2.5	2.2	4 3/4 6
A14:				
III3	Moderate	10	6	4 3/4 3
III5	Moderate	10	13.5	4 3/4 3
III8	Moderate	10	6	4 3/4 3
III16	Moderate	10	21	1 2/6 3
A16:				
II7	Mild	9	13.5	5 1/5 1
A17:				
III1	Moderate	5	7.5	5 1/5 1
IV1	Moderate	34	14.5	5 1/5 1
IV2	Moderate	10	8	5 1/5 1
A18:				
II3	Moderate	10	15	6 5/6 5
A19:				
II1	Mild	14	14	5 2/5 2
II2	Mild	14	14	5 2/5 2
B1:				
II1	Mild	24	15	8 4/8 4

[a] Assays were performed according to standard procedures (see Families, Material, and Methods).
[b] For markers D18S1129 and D18S1103, which are closely linked to the F5F8D locus.

nian, Pakistani, and Algerian origin was 14.82, for marker D18S1129. Linkage of the F5F8D locus to chromosome 18q in nine Jewish families has been reported recently (Nichols et al. 1996, 1997). Our data are in agreement with those obtained by this latter study, implying that the disease is genetically homogeneous (i.e., the same gene is involved) among different human populations. However, both the observation of different haplotypes of polymorphic sites in the affected individuals and the variable clinical phenotype suggest allelic heterogeneity in which different mutations in the same gene may account for these findings.

Table 2

Two-Point LOD Scores for All F5F8D Families Studied, for 11 18q Polymorphic markers

	LOD Score at θ =								
Locus	.0	.001	.01	.05	.1	.2	.3	.4	Z_{max} (θ)[a]
D18S535	−∞	−13.69	−5.3	.06	1.51	1.71	1.06	.41	1.82 (.155)
D18S851	.90	3.80	7.14	8.40	7.74	5.44	3.10	1.21	8.41 (.046)
D18S858	−∞	2.84	6.37	7.77	7.24	5.11	2.88	1.08	7.77 (.05)
D18S849	−∞	8.37	10.83	11.26	10.08	6.98	4.00	1.59	11.42 (.031)
D18S1129	11.59	13.17	14.65	14.30	12.66	8.78	5.05	2.01	14.82 (.02)
D18S1103	−∞	4.62	8.03	9.26	8.53	5.97	3.33	1.20	9.27 (.045)
D18S1155	−∞	4.69	6.41	6.83	6.14	4.19	2.32	.91	6.88 (.035)
D18S64	8.50	9.90	11.41	11.31	10.03	6.97	4.05	1.65	11.64 (.023)
D18S862	4.04	6.24	8.69	9.36	8.49	5.94	3.43	1.39	9.43 (.037)
D18S1270	−15.88	−9.57	−3.17	.87	1.90	1.83	1.11	.44	2.04 (.138)
D18S541	−∞	−3.84	.64	2.99	3.16	2.35	1.38	.56	3.19 (.082)

Note.—Linkage analysis was performed with consanguinity loops included, by use of the MLINK and ILINK programs of the LINKAGE software package. For all markers, the allele frequencies were kept equal.

[a] Calculated under the assumption of an autosomal recessive mode of inheritance with 100% penetrance.

One observation of interest concerns the presence of two different alleles for the closely linked polymorphic loci in affected individuals from consanguineous marriages involving first cousins (e.g., for marker D18S1129, individual A5-III4). For a rare, autosomal recessive phenotype and markers so close to the disease locus, this is unexpected. In the linkage analysis, the LOD scores in this family do not maximize at θ = 0, although there are no apparent recombinants between the phenotype and the markers studied. One plausible explanation for this phenomenon is that the linkage program scores the presence of historical recombi-

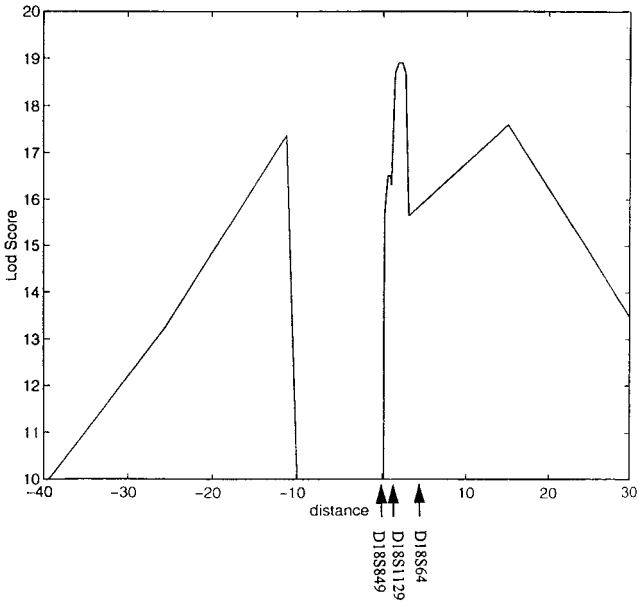


Figure 2 Multipoint analysis for markers D18S849, D18S1129, and D18S64. Linkage analysis was performed by use of the LINKMAP program. The distances used were 1 cM between D18S849 and D18S1129 and 2 cM between D18S1129 and D18S64.

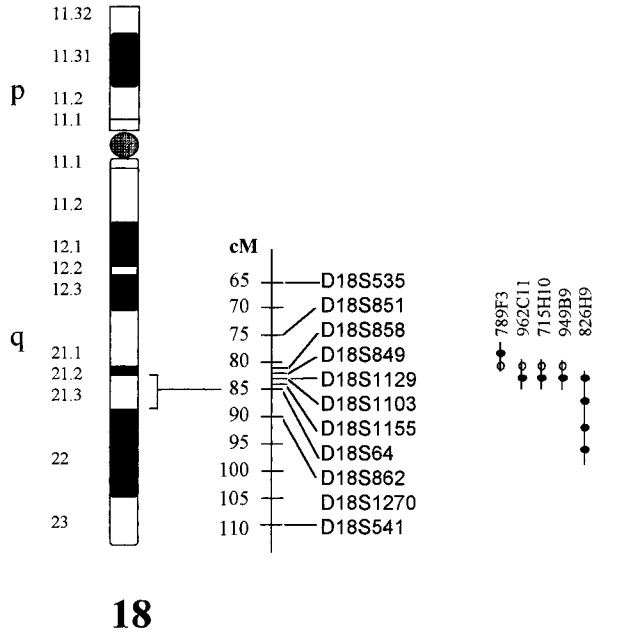


Figure 3 Schematic representation of a partial linkage map of 11 microsatellite markers located in 18q, around the mapping position of the F5F8D locus. The mapping order, genetic distances (in cM), and positions of CEPH YACs spanning the critical F5F8D locus were obtained from the MIT mapping project Webpage (http://www-genome.wi.mit.edu). Black dots in the YACs denote the presence of the corresponding markers; unblackened circles denote nonpolymorphic DNA segments linking the corresponding YACs.

nants—that is, recombinations generated in the past—thus explaining the presence of two differently marked but identical affected alleles in the first-cousin marriage. Indeed, when the consanguinity loop was removed from the pedigree of family A5, the two-point LOD score maximized at $\theta = 0$ for D18S1129 (data not shown). All LOD scores shown in this study were calculated with all consanguinity loops included, and therefore the LOD scores are quite probably underestimated. Another possibility is that there are two different mutations in the F5F8D locus in these families and that the consanguinity (which is common in these populations) is irrelevant.

In the families that we studied there is no apparent correlation between the haplotypes for polymorphic sites and the severity of the disease, suggesting that different independent mutations are responsible for similar F5F8D phenotypes. This is not unexpected and elsewhere has been found in numerous monogenic disorders, including hemophilia A (Antonarakis et al. 1995). In addition, comparison of haplotypes associated with "affected" F5F8D alleles vis-à-vis those of "nonaffected" chromosomes from the Iranian families examined did not reveal any differences, suggesting that different disease mutations had occurred in the common haplotypes of this population. In contrast, the results of the haplotype analysis from the study by Nichols et al. (1997), in eight unrelated but consanguineous Jewish families, strongly indicated a common founder effect.

A single-gene defect causing deficiency of proteins encoded by two other loci raises a number of intriguing mechanistic explanations. Since factor V and factor VIII are highly similar in structure and function, having clearly diverged from a common ancestor (Gitschier et al. 1984; Cripe et al. 1992), one may propose several types of candidate genes, including the following: a highly specific transcription regulator, a specific protein-processing factor (both factor V and factor VIII have highly glycosylated B-domains), a secretion-pathway factor, a chaperone, or a protein involved in the extracellular life of these factors.

We have localized the F5F8D critical region to an ~1-cM region of 18q, between markers D18S849 and D18S1103. This region is entirely spanned by YAC contig WC18.4 (MIT at http://www-genome.wi.mit.edu/), with YACs 789F3, 962C11, 715H10, 949B6, and 826H9 containing, between them, all of the critical region as defined by our data. Positional cloning efforts (exon trapping, cDNA selection, and sequence sampling) are now in progress to clone the F5F8D gene.

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References

Antonarakis SE, Kazazian HH Jr, Tuddenham EGD (1995) Molecular etiology of factor viii deficiency in haemophilia A. Hum Mut 5:1–22

Canfield WM, Kisiel W (1982) Evidence of normal functional levels of activated protein C inhibitor in combined factor V/VIII deficiency disease. J Clin Invest 70:1260–1272

Cohen D, Chumakov I, Weissenbach J (1993) A first-generation physical map of the human genome. Nature 366:698–701

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Cripe LD, Moore KD, Kane WH (1992) Structure of the gene for human coagulation factor V. Biochemistry 31:3777–3785

Dacie JV, Lewis SM (eds) (1995) Practical haematology, 8th ed. Churchill Livingstone, London

Gardiner JE, Griffin JH (1984) Studies on human protein C inhibitor and factor V/VIII deficient plasmas. Thromb Res 36:197–203

Gitschier J, Wood WI, Goralka TM, Wion KL, Chen EY, Eaton DH, Vehar GA, et al (1984) Characterization of the human factor VIII gene. Nature 312:326–330

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Breg WR, et al (1977) Analysis of human Y-chromosome specific reiterated DNA in chromosome variants. Proc Natl Acad Sci USA 4:1245–1249

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Marlar RA, Griffin JH (1980) Deficiency of protein C inhibitor in combined factor V/VIII deficiency disease. J Clin Invest 66:1186–1189

Mazzone D, Fichera A, Pratico G, Sciacca F (1982) Combined congenital deficiency of factor V and factor VIII. Acta Haematol 68:337–338

Murray J, Buetow K, Weber J, Lugwidsen S, Scherpbier-Heddema T, Manion F, Quillen J, et al (1994) A comprehensive human linkage map with centimorgan density. Science 265: 2049–2054

Nichols WC, Seligsohn U, Zivelin A, Terry VH, Arnold ND, Siemieniak DR, Ginsburg D (1996) The gene for combined factors V and VIII deficiency maps to a 2.4 cM region on chromosome 18. Blood Suppl 88:656a

Nichols WC, Seligsohn U, Zivelin A, Terry VH, Arnold ND, Siemieniak DR, Kaufman RJ, et al (1997) Linkage of combined factors v and viii deficiency to chromosome 18q by homozygosity mapping. J Clin Invest 99:596–601

Ozsoylu S (1983) Combined congenital deficiency of factor V and factor VIII. Acta Haematol 70:207–208

Rahim Adam KA, El Seed A, Karrar Z A, Gader AMA (1985) Combined factor V and factor VIII deficiency with normal protein C and protein C inhibitor: a family study. Scand J Haematol 34:401–405

Seligsohn U (1989) Combined factor V and factor VIII deficiency. In: Seghatchian J, Savidge GT (esds) Factor VIII-von Willebrand factor. CRC Press, New York, pp 89–100

Seligsohn U, Ramot B (1969) Combined factor V and factor VIII deficiency: report of four cases. Br J Haematol 16:475–486

Seligsohn U, Zivelin A, Zwang E (1982) Combined factor V and factor VIII deficiency among non-Ashkenazi Jews. N Engl J Med 307:1191–1195

——— (1984) Decreased factor VIII clotting antigen levels in the combined factor V and factor VIII deficiency. Thromb Res 33:95–98

Sheffield VC, Weber JL, Buetow KH, Murray JC, Even DA, Wiles K, Gastier JM, et al (1995) A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution genome-wide linkage maps. Hum Mol Genet 4:1837–1844

Suzuki K, Nishioka J, Hashimoto S, Kamiya T, Saito H (1983) Normal titer of functional and immunoreactive protein C inhibitor in plasma of patients with congenital combined factor V and factor VIII deficiency. Blood 62: 1266–1270

Zivelin A, Yatuv R, Seligsohn U (1995) Combined factor V and factor VIII deficiency is probably not related to genetic disorders of components of the protein C system. Thromb Haemost 73:1439