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Combined factors V and VIII deficiency — the solution

D. GINSBURG*†‡, W. C. NICHOLS*, A. ZIVELIN‡‡, R. J. KAUFMAN**‡ and U. SELIGSOHN ‡‡

**Departments of Internal Medicine, †Human Genetics and **Biological Chemistry and ‡Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI, USA; ‡‡Institute of Thrombosis and Hemostasis, The Chaim Sheba Medical Center, Tel Hashomer and Sackler Faculty of Medicine, Tel Aviv, Israel*

Summary. Combined deficiency of coagulation factor V and factor VIII is an autosomal recessive disorder which has been observed in a number of populations around the world. However, this disease appears to be most common in the Mediterranean basin, particularly in Jews of Sephardic and Middle Eastern origin living in Israel. We have taken a positional cloning approach toward identifying the gene responsible for this disorder. We initially studied 14 affected individuals from nine unrelated Jewish families using a panel of polymorphic genetic markers spaced throughout the human genome. The combined factors V and VIII deficiency gene was mapped to a locus on the long arm of chromosome 18 with a maximal LOD score of 13.22. A detailed genetic analysis identified two distinct haplotypes among these families, suggesting two independent founders or, alternatively, a single ancient founder with a more recent split of these subpopulations. Further work to identify and characterize the gene responsible for combined factors V and VIII deficiency should provide important insights into the biosynthesis of these homologous proteins.

Keywords: Factor V deficiency, FVIII deficiency, haemophilia, chromsome mapping, linkage, haplotypes.

Combined deficiency of factors V and VIII was first reported by Oeri and co-workers in 1954 [1]. Since that time, a number of additional families have been identified around the world (reviewed by Seligsohn *et al.* [2]). As of the time of the latter review, a total of 89 patients in 58 families had been reported. Twenty-four of these families are from the Mediterranean region, including nine from Italy and nine from Israel. Additional families have been reported from Japan, North America, and Europe.

Patients with this disease generally exhibit plasma coagulation factor V and factor VIII antigens and activities in the range of 5–30% of normal. Bleeding is similar to that observed in other coagulation factor deficiencies and includes post-surgical bleeding, menorrhagia, and post-partum haemorrhage. Epistaxis and easy bruising are frequently noted. From the published reports, it appears that the bleeding severity observed in these patients is somewhat greater than would be expected in patients with single factor deficiency with similar residual levels of activity [2, 3].

The levels of all other plasma proteins that have been measured in these patients appear to be normal, including those of the homologous copper-binding protein, ceruloplasmin. Thus, this defect appears to be restricted solely to these two clotting proteins. Haemophilia A (factor VIII deficiency) has a prevalence in the population of approximately 1:10,000 whereas factor V deficiency (parahaemophilia) is estimated to occur in approximately 1:1,000,000 individuals [4]. Though there have been several case reports of individuals who have simultaneously inherited these two independent genetic diseases [5–7], this would be expected to be quite rare, (approximately $1:10^{10}$). In addition, the inheritance pattern observed in the reported cases of combined factors V and VIII deficiency are clearly consistent with a single gene, autosomal recessive disorder.

In 1980, Marlar and Griffin reported an apparent deficiency of protein C inhibitor as the underlying mechanism for combined factors V and VIII deficiency [8]. This explanation was logically compelling, given the function of activated protein C to proteolytically degrade factors V and VIII. Deficiency of protein C inhibitor would be expected to result in unopposed activity of protein C, explaining the simultaneous decrease in activity of factor V and factor VIII. Despite the attractiveness of this hypothesis, subsequent studies failed to confirm the deficiency of protein C inhibitor in these patients [9–12]. Since that time, no other explanation for this puzzling disorder has been identified. Zivelin and co-workers [13] recently used a linkage approach to exclude the protein C, protein S, factor V and prothrombin genes as potential candidates for this disease.

Correspondence: David Ginsburg, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109-0650, USA.

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With the advent of powerful new tools for genetic analysis through the efforts of the human genome project, it has now become possible to clone a disease-causing gene by a purely genetic approach, without prior knowledge of the corresponding protein or its function. This approach is referred to as positional cloning [14]. We sought to identify the combined factors V and VIII deficiency gene by positional cloning, using a panel of nine families that our group had previously characterized [3]. The first step in this process is to map the gene to a specific region within the human genome.

Homozygosity mapping

To map the combined factors V and VIII deficiency gene, we took advantage of an efficient strategy to approach autosomal recessive diseases first proposed by Lander and Botstein in 1987 [15]. The idea behind this approach is shown schematically in Fig. 1. Successful application of this strategy requires consanguineous families in which the patient can be assumed to have inherited the same mutant gene from both parents. The affected patients in seven of the nine families we studied were the products of first cousin marriages (see Fig. 2). As illustrated in Fig. 1, a single mutant allele present in one of the common grandparents could be passed through both parents, resulting in a homozygous state in the affected child. Several hypothetical polymorphic DNA markers are shown flanking the disease gene. Markers close to the gene should be inherited along with it. Since the affected patient is homozygous for the mutation, homozygosity for nearby genetic markers can also be expected. Indeed, in this way, the child of a first cousin marriage would be expected to be homozygous for 1/16 of its genome. By testing a large number of such individuals, a DNA marker near the disease gene would be expected to be homozygous in many or nearly all of the patients, depending on how close the marker is to the gene. In contrast, an unlinked, highly polymorphic, random marker should be homozygous by chance in about 1/16 of affected patients. By this approach, it is possible to obtain significant evidence for linkage with as few as three affected patients.

Genetic linkage analysis to map the combined factors V and VIII deficiency gene

The pedigrees for the families analyzed in this study are shown schematically in Fig. 2. A total of 54 individuals from nine families, including 14 affected individuals, were studied. DNA prepared from these individuals was genotyped for a total of 241 polymorphic markers spaced throughout the genome [16]. Analysis of a genetic marker on chromosome 7, which is unlinked to the combined factors V and VIII deficiency gene, is shown in Fig. 3.

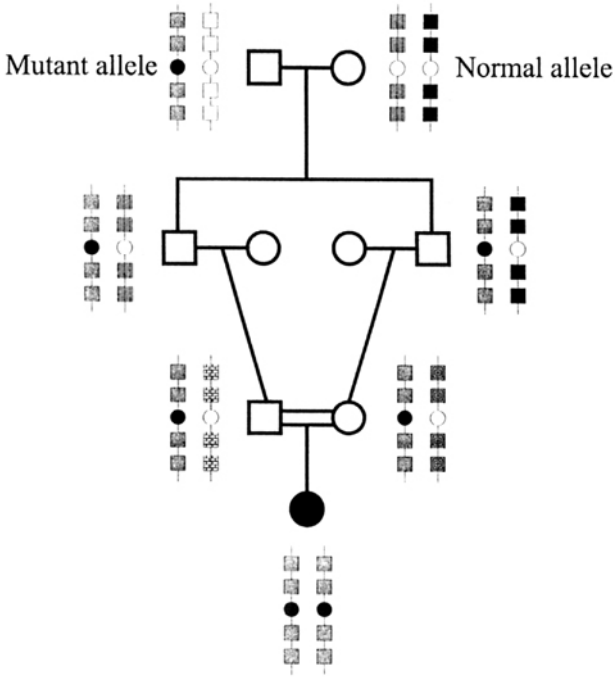

Fig. 1. Homozygosity mapping. A hypothetical disease gene locus is represented by the circles, with a filled circle indicating the mutant allele and an open circle the normal allele. The square boxes represent nearby polymorphic genetic markers. The patient's parents have each inherited the same copy of the mutant allele from their common great-grandfather and the patient is homozygous for this mutation. The patient has also inherited the same polymorphic marker alleles flanking this mutation on both chromosomes (shown in solid gray boxes) and thus is homozygous for these markers, as well as the mutation.

Most of the affected patients are heterozygous for this marker. In contrast, the marker used for the analysis in Fig. 4 shows a homozygous genotype in all but one of the affected patients. Such an outcome is very unlikely to occur by chance. Fig. 5 shows the results of this analysis on three representative chromosomes. As is evident, a region on the long arm of chromosome 18 shows the most suggestive evidence for linkage to the combined factors V and VIII deficiency gene [16].

Once this initial evidence for linkage was obtained, the DNA samples from these patients and their families were typed with a larger panel of markers localized to this region of chromosome 18q [16]. The results of this genotype analysis are shown in Fig. 6. All of the affected patients are homozygous for a number of markers in this region. A segment of overlap in all of these individuals is identified, including markers D18S1129, D18S1103, and D18S1155. These data indicate that the combined factor V and VIII deficiency gene is located within this region. As shown schematically in Fig. 7, the size of this region is estimated at approximately 2.5 cm. Statistical analysis

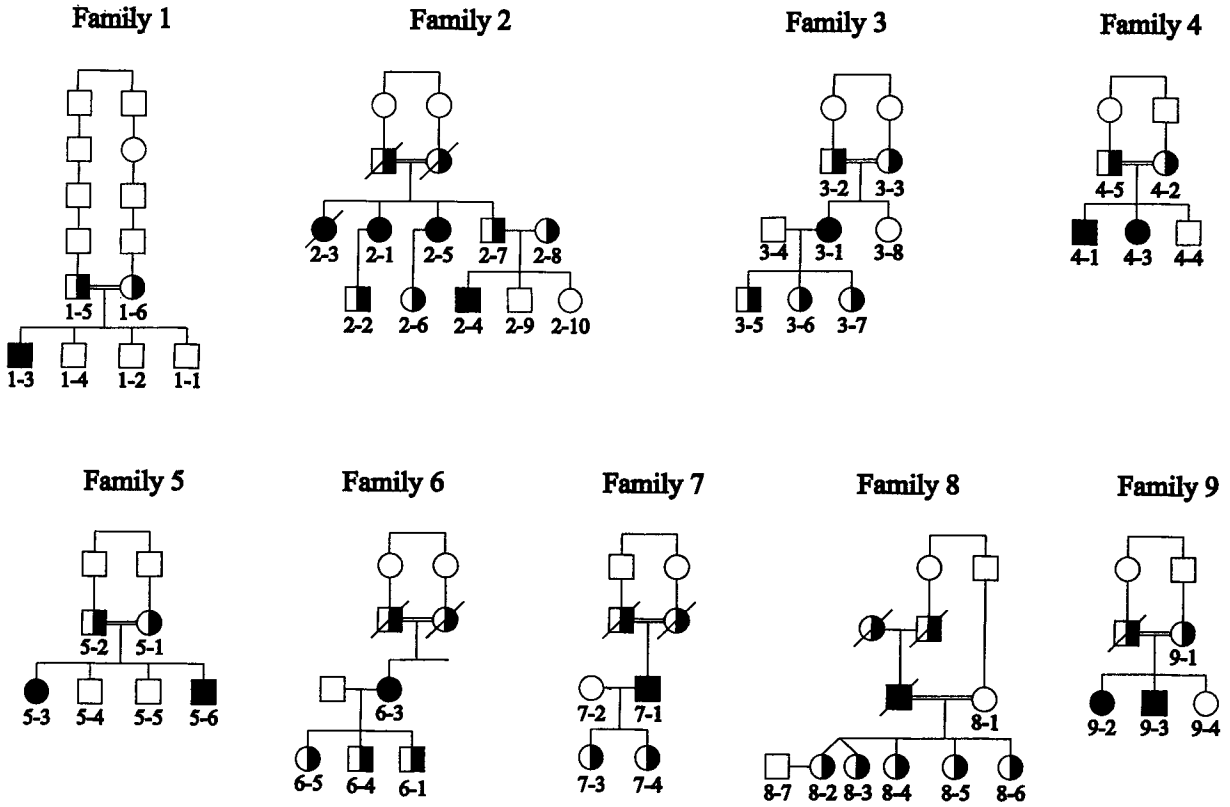

Fig. 2. Combined factors V and VIII deficiency families. The pedigrees used for linkage analysis to localize the combined factors V and VIII deficiency gene are shown here. Solid symbols indicate homozygously affected individuals and half-filled symbols, obligate heterozygotes.

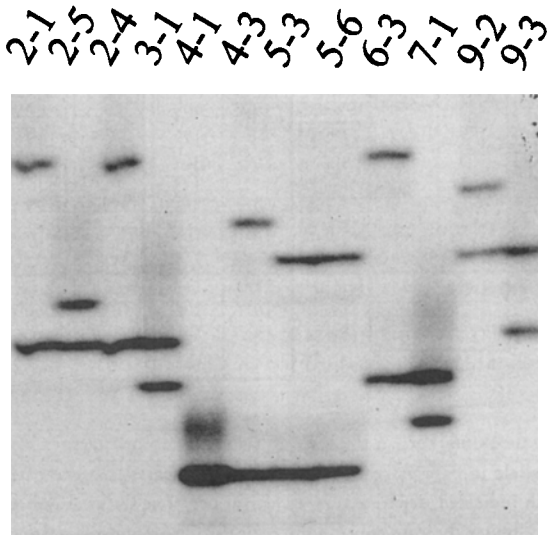

Fig. 3. DNA analysis of combined factors V and VIII deficiency patients with an unlinked polymorphic marker on chromosome 7q. Patients identifiers above each lane, corresponding to the numbers shown on the pedigrees in Fig. 2. Individual 4-1 is seen to be homozygous for a single, small band, whereas all other individuals are heterozygous for this marker.

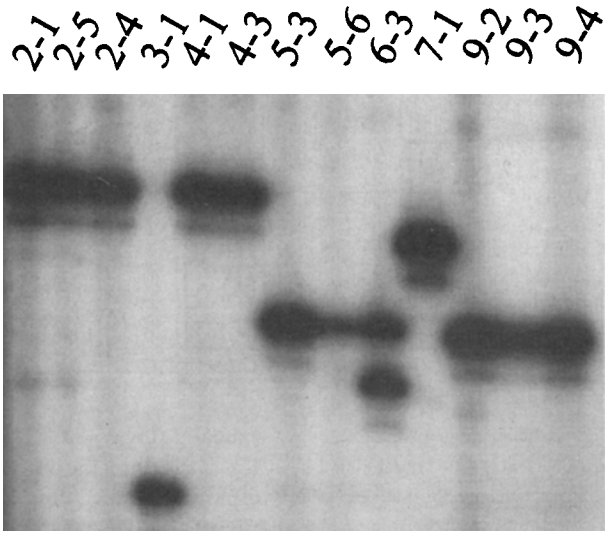

Fig. 4. DNA analysis with marker D18S849. The same DNA samples shown in Fig. 3 are analyzed here with a polymorphic marker from the long arm of chromosome 18. Though individual 6-3 is heterozygous with two distinct bands evident, all other individuals are homozygous for this market.

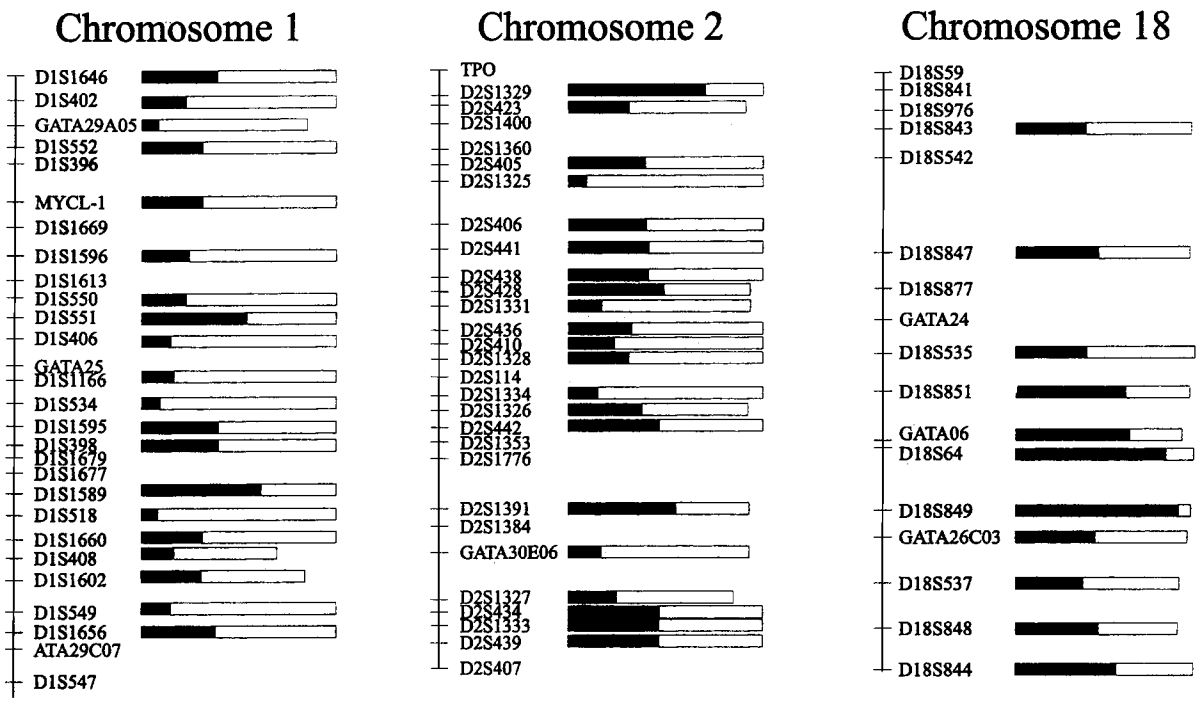

Fig. 5. Genetic linkage analysis for markers on chromosomes 1, 2 and 18. The results of DNA analysis for polymorphic markers on three representative chromosomes are shown here. For each marker, the filled section of the bar shows the fraction of patients who are homozygous, whereas the open area indicates the fraction who are heterozygous. Only markers in a restricted region of chromosome 18 (D18S64 and D18S49) show a markedly increased number of homozygous individuals. The actual genotype results for marker D18S49 are shown in Fig. 4.

	Tunisian	Tunisian		Tunisian	Tunisian	Tunisian			Iraqi				Iraqi		Egyptian	Iranian
	1-3	5-3	5-6	6-3	8	9-2	9-3	2-1	2-3	2-5	2-4	4-1	4-3	7-1	3-1
centromere															
AFM191XCP	193|181	187	187	195|193	195	185|181	185|181	195	195	195	197|195	197	197	195	185
D18S1152	114|118	106	106	106	108	102|108	102|108	106		106	108|106	108	108	106	114
D18S858	199|205	205	205	205|193	199	196|205	196|205	205	205	205	199|205	199	199	208	205
D18S41	199|203	203	203	203|193	203	197|199	197|199	199	199	199	199	199	199	207	199
D18S849	302	288	288	288|282	286	288	288	302	302	302	302	302	302	298	270
D18S1144	173	159	159	159|165	177	159	159	177	177	177	177	177	177	163	163
D18S1129	91	107	107	107	109	107	107	97	97	97	97	97	97	95	97
D18S1103	94	94	94	94	94	94	94	106	106	106	106	106	106	106	106
D18S1155	198	198	198	198	198	198	198	208	208	208	208	208	208	208	208
D18S1109	136	136	136	136	136	136	136		124	124	118|124	118	118	124	120|118
D18S64	200	200	200	200	200	200	200	192	192	192	192	192	192	192	206|192
GATAP32103	180	180	180	180	180	180	180|192	188|184	184	184	188|184	188	188	184	180
D18S862	141	141	141	141	141	141	141		141|135	141|135	144|135	144	144	141	126|129
telomere															

Fig. 6. Haplotype data for chromosome 18 markers. Genotype data for chromosome 18 markers in the vicinity of the combined factors V and VIII deficiency gene are tabulated here. The numbers indicate the size of each allele in base pairs. A single number indicates that the patient is homozygous for this marker. When two alleles are present, their sizes are both indicated, separated by a vertical bar. The dark, gray-boxed area indicates the region for which all patients are homozygous. The light gray boxes indicate continuous regions of homozygosity in each individual patient (taken from Nichols *et al.* [16], with permission).

for the significance of this linkage result was performed using several standard linkage analysis computer programs (MULTIMAP and MAPMAKER/HOMOZ). The result of this LOD score analysis is shown in Fig. 8. The peak LOD is obtained at D18S1129 with a maximum LOD score of 13.22. This result means that the odds ratio for the combined factors V and VIII deficiency gene being located in this region over the odds of these results occurring purely by chance are in excess of 10^{13}!

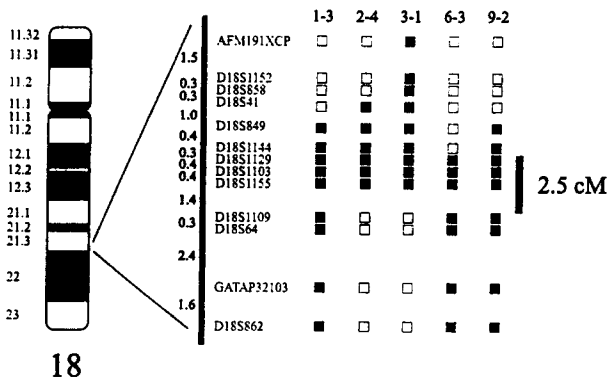

Fig. 7. Location of the candidate genetic interval for the combined factors V and VIII deficiency gene on chromosome 18. The relative positions for each marker along chromosome 18 are shown here. The numbers to the left of the chromosome 18 ideogram indicate cytogenetic bands. The names for each polymorphic marker studied are shown to the left of the vertical black bar, with the distance between them (in centimorgans) indicated to the left of the bar. The genotype results are shown for five critical patients, with open boxes indicating heterozygous and closed boxes homozygous marker genotypes. The common region of homozygosity, indicated by the vertical bar at the right, is approximately 2.5 cM and defines the candidate region for the location of the combined factors V and VIII deficiency gene.

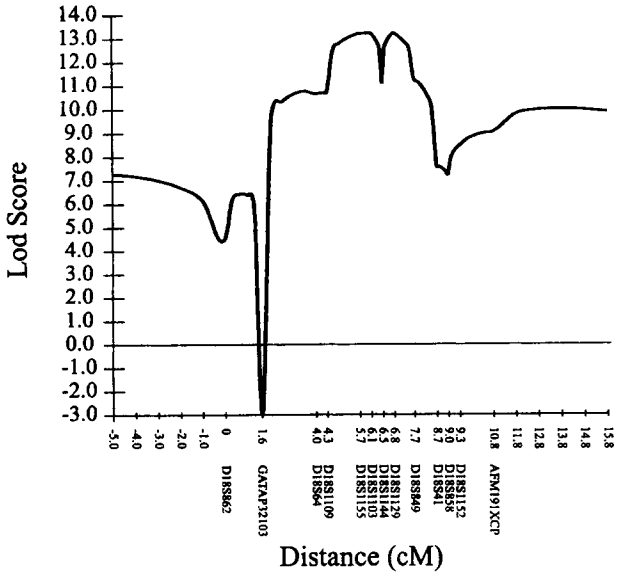

Fig. 8. Multi-point linkage analysis of combined factors V and VIII deficiency. Multi-point linkage analysis was performed using the 13 markers shown on the abscissa with MAPMAKER/HOMOZ. The best distanced and order between the markers was determined using MULTIMAP. The markers are shown centromere (left) to telomere (right). The dotted horizontal line within the plot indicates a LOD score of 0. MAPMAKER/HOMOZ analysis using all 13 markers in the region yielded a maximum LOD score of 13.22 at D18S1139 (taken from Nichols *et al.* [16], with permission).

Haplotype analysis

Analysis of the genotype data shown in Fig. 6 identifies two distinct shared haplotypes among these affected families. Focusing our attention on markers D18S1103 and D18S1155, there are only two patterns present in all of these families. All of the Tunisian families have a 94 base pair (bp) allele for D18S1103 and a 198 bp allele for D18S1155. The corresponding alleles in the Iraqi, Egyptian, and Iranian families are 106 and 208, respectively. Analysis of the adjacent markers reveals extension of this common haplotype all the way to the most telomeric marker D18S862 in many of the Tunisian patients and centromeric as far as D18S849 in some patients. Similarly, there are larger regions of sharing among the Iraqi, Egyptian, and Iranian families.

These results suggest two independent founder mutations responsible for the disease in these families. The founder mutation in the Tunisian population would appear to have arisen on the 94-198 haplotype and the mutation in the remaining families on the 106-208 haplotype. There are no common marker alleles shared between these two haplotypes, suggesting that they are completely independent. However, from this analysis, we cannot exclude the possibility of an ancient common founder whose offspring then split into these two populations. This division would have occurred sufficiently far in the past to allow multiple chromosomal recombination events to generate apparently unrelated haplotypes. These possibilities will be resolved by characterization of the actual mutation in the combined factors V and VIII deficiency gene. These two haplotypes may also be consistent with the histories of these populations. The Tunisian families appear to share a common ancestor distinct from the other middle eastern Jewish families in Egypt, Iraq and Iran. The latter group of families has presumably remained in this area since the destruction of Israel in ancient times.

Further studies to identify the gene for combined factors V and VIII deficiency

Since our initial report mapping the gene for combined factors V and VIII deficiency to chromosome 18q [16], these data have been confirmed in an independent study by Neerman-Arbez and co-workers [17]. This group studied unrelated non-Jewish families, primarily from Iran. There is no clear relationship between the haplotypes identified in their study and those we have reported. These data suggest multiple unrelated founders.

The results reviewed here provide the first critical step toward identifying the gene for combined factors V and VIII deficiency. However, no additional information can be deduced from these data about the biological function

of the responsible genes. Progress in this area continues to be rapid and advances toward identifying and characterizing the combined factors V and VIII deficiency gene will be discussed in the presentation corresponding to this manuscript at the May 1998 World Federation of Hemophilia Meeting.

Addendum

Recent progress in the laboratory has led to the identification of the gene responsible for combined factors V and VIII deficiency as a previously described resident protein of the endoplasmic reticulum-Golgi intermediate compartment termed ERGIC-53. Independent mutations responsible for each of the chromosome haplotypes described above in our patient population have been identified [18]. In addition to identifying the gene responsible for this intriguing human inherited bleeding disorder, these findings have important implications for our understanding of protein biosynthesis and intracellular transport between the endoplasmic reticulum and Golgi apparatus.

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