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SHORT REPORT

Localisation of the gene responsible for Fechtner syndrome in a region < 600 Kb on 22q11–q13

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Fechtner syndrome is an autosomal dominant disorder which has been thought to be a variant of Alport syndrome. It is characterised by nephritis, sensorineural hearing loss and eye abnormalities, as well as by macrothrombocytopenia and polymorphonuclear inclusion bodies. Recently, the Fechtner syndrome has been mapped in a 5.5 Mb region on the long arm of chromosome 22 by linkage analysis in an extended Israeli family. We describe here the genetic refinement of the Fechtner critical interval to a region less than 600 Kb by linkage analysis performed in a large Italian pedigree. The presence of several recombination events allowed the disease gene to be localised between markers D22S278 and D22S426, in a region containing only two non-recombinant markers, D22S1173 and D22S283. This interval, spanning < 600 Kb on genomic DNA, has been entirely sequenced and contains six known and three putative genes. *European Journal of Human Genetics* (2000) **8**, 895–899.

Keywords: Fechtner syndrome; May-Hegglin syndrome; chromosome 22; linkage analysis

Introduction

The Fechtner syndrome (FTNS; MIM 153640) is an autosomal dominant disorder first reported by Peterson *et al* in 1985.[1] The disorder represents a variant of the well characterised Alport syndrome,[2] since the affected individuals show nephritis, deafness and eye abnormalities together with leukocyte inclusions and macrothrombocytopenia. The inclusion bodies appear similar to toxic Dohle bodies and inclusions observed, under a light microscope, in the May-Hegglin anomaly (MHA, MIM 155100),[3,4] but their ultrastructural appearance is unique. Furthermore, a similar disorder is represented by Epstein syndrome (MIM 153650) which differs from FTNS by the lack of polymorphonuclear inclusion bodies.[5,6] Finally, Sebastian platelet syndrome is characterised by the same haematological changes present in FTNS but without the manifestations of Alport syndrome.[7,8] Other families with recurrence of FTNS have been reported.[9,10] In particular, Gershoni-Baruch *et al*[9] described a large family in which the affected members showed nephropathy, eye abnormalities, high-tone sensorineural hearing loss, impaired liver functions, hypercholesterolaemia, macrothrombocytopenia and polymorphonuclear inclusion bodies. By a genome-wide search carried out in this family, Toren *et al*[11] have recently mapped the locus responsible for FTNS syndrome on chromosome 22q11–q13, in a 5.5 Mb interval between markers D22S284 and D22S1167. Interestingly, Kunishima *et al*,[12] analysing a single Japanese family with MHA, have mapped this disease gene in a 13.6-cM region on chromosome 22q, between D22S280 and D22S272. The MHA locus is completely included in the critical interval defined for FTNS, suggesting the possibility that the two disorders could be due to mutations affecting the same gene.

We report here a large family originating from the south of Italy with occurrence of FTNS. In order to refine the critical region of FTNS, we performed linkage studies using a set of highly informative markers from the 22q11–q13 region, spanning the FTNS locus.

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Material and methods

Family

In this five-generation family (Figure 1) originating from the south of Italy, 10 affected and 15 unaffected individuals were available for the study. Among patients, the phenotype appeared quite variable and segregated in an autosomal

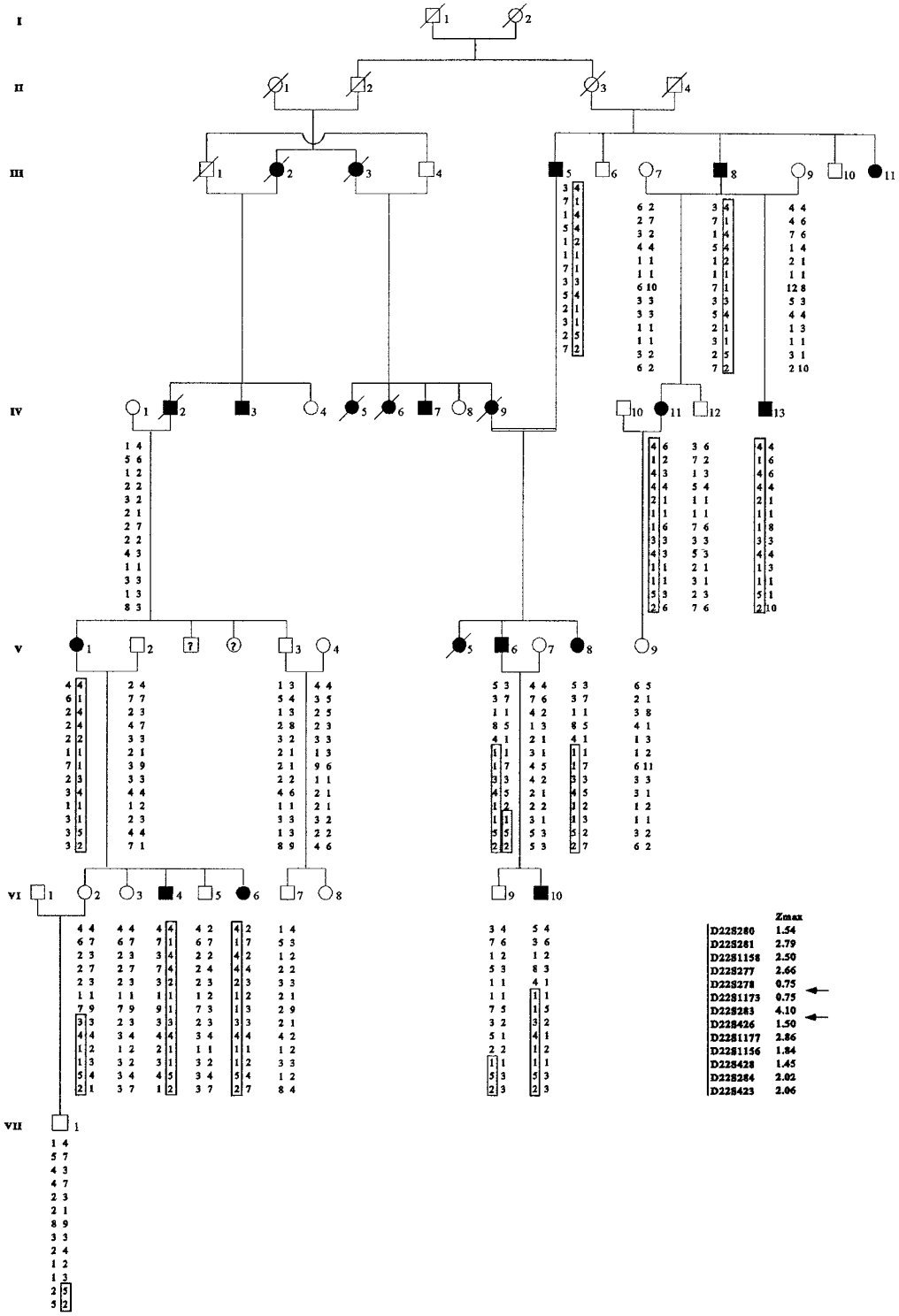

Figure 1 Pedigree and haplotype reconstruction of the large Italian FTNS family. Haplotype reconstruction for markers on chromosome 22q11–q13 as well as maximum lod score values are reported. The disease haplotype is boxed. The arrows indicate the recombination events and the boundaries of the FTNS critical interval.

Table 1 Clinical features of the family members affected with FTNS available for the study

Patient	*Age (years)*	*Macrothrom-bocytopenia*	*Inclusion bodies*	*Nephropathy*	*Eye abnormalities*	*Deafness*	*Additional features*
III-5	60	+	+	–	+	+	–
III-8	49	+	+	+	–	+	mildly abnormal liver function
IV-11	24	+	+	+	+	+	–
IV-13	10	+	+	–	–	–	–
V-1	46	+	+	–	+	+	hypercholesterolaemia
V-6	33	+	+	microscopic haematuria	–	–	mildly abnormal liver function hypercholesterolaemia
V-8	30	+	+	microscopic haematuria	+	–	mental retardation
VI-4	22	+	+	–	+	+	–
VI-6	6	+	+	–	+	+	–
VI-10	11	+	+	microscopic haematuria	–	–	–

dominant fashion. All the affected members showed polymorphonuclear inclusion bodies and macrothrombocytopenia. These features, in each patient, were associated in different combinations with high-tone neurosensorial deafness, cataract, abnormal renal function and other features shown in Table 1. Renal involvement varied from chronic renal failure (III-8 and IV-11 in Figure 1) to microscopic haematuria (V-6, V-8 and VI-10 in Figure 1). After informed consent obtained by participants, each subject underwent a full physical, ophthalmologic, hearing and haematological evaluation. Haematological studies included a complete blood count and Giemsa staining for the observation of platelet and polymorphonuclear inclusion bodies under a light microscope. Liver and renal function tests and lipid profile were also evaluated.

DNA extraction

Blood samples were obtained from members of the family under informed consent and DNA was extracted using standard protocols.

Genotyping

To refine the critical region for FTNS, the following dinucleotide repeats on 22q11–q13 were typed in the family members: D22S280, D22S281, D22S1158, D22S277, D22S278, D22S1173, D22S283, D22S426, D22S1177, D22S1156, D22S428, D22S284, D22S423. The order of these markers has been physically demonstrated by the sequence of the entire chromosome 22.[13] Primers for markers D22S280, D22S283 and D22S423 were present in the ABI-Prism Linkage Mapping Set Version 2 (ABI, Perkin-Elmer, Norwalk, CT, USA), whilst primers for the remaining markers were synthesised, labelling the forward primer with FAM, TET or HEX fluorescent dyes. PCR was carried out under standard conditions. Amplification products were loaded in an automated sequencer ABI mod 377 (ABI, Perkin-Elmer, Foster City, CA, USA) and allele size defined using the Genescan software.

Linkage analysis

Classic pairwise lod score analysis was carried out in the pedigree using the programs included in the LINKAGE package.[14] The analysis was performed modelling the FTNS as a fully penetrant dominant disorder with an estimated disease allele frequency of 0.001. All the individuals showing at least polymorphonuclear inclusion bodies and macrothrombocytopenia were considered to be affected, since no evidence for a reduced penetrance came out of the analysis of pedigrees already published. Haplotypes reconstruction was performed in order better to characterise the meiotic recombinants and define the critical region for the disease gene.

Results

Figure 1 reports the haplotype reconstruction for the chromosome 22q11–q13 markers tested in the family. The maximum lod score values (Z_{max}) are also shown. In particular, a lod score of 4.10 was obtained for marker D22S283.

Several recombination events were detected in the family. In particular, the presence of one recombination event in the healthy individual VI-2 places the FTNS locus proximal to the D22S426 locus. This recombinant defines the telomeric boundary of the FTNS locus between markers D22S426 and D22S283. Furthermore, the affected individuals V-6 and V-8 inherited from the affected mother a recombinant haplotype which shares only the distal part, from marker D22S1173 to D22S423, with the disease haplotype present in the other patients. This recombination event defines the centromeric boundary of the FTNS critical interval between markers D22S278 and D22S1173. Thus, the region containing the *FTNS* gene is located between markers D22S278 and D22S426 and contains two non-recombinant markers, D22S1173 and D22S283.

Discussion

The results of the present work confirm linkage of FTNS to loci on chromosome 22q11–q13 in a large family originating from the south of Italy. In particular, a lod score of 4.10 at $\theta = 0.00$ was obtained with marker D22S283. The presence of

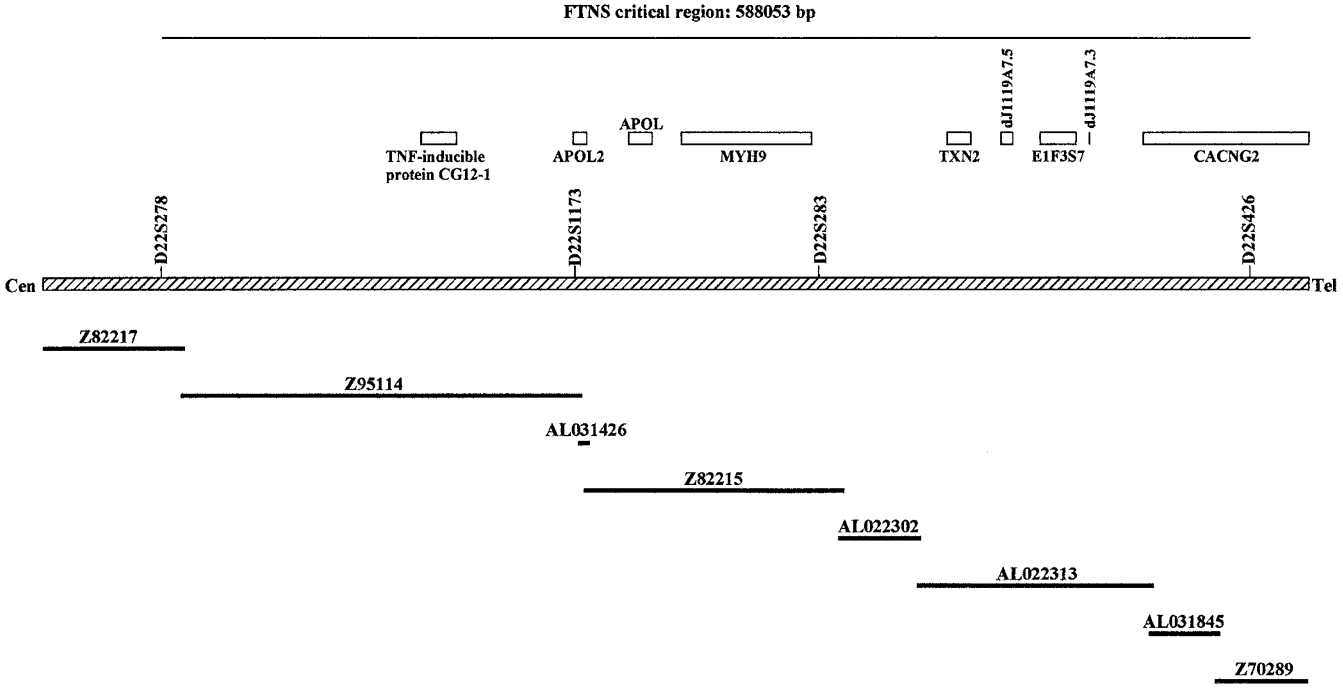

Figure 2 Schematic representation of the FTNS critical region. All these data are resumed from the sequence of chromosome 22.[13] The white boxes indicate the genomic regions covered by the different genes present in the FTNS locus. The *APOL* gene is transcribed by the '+' strand (cen-tel), whereas *MYH9*, the TNF-inducible protein *CG12-1* mRNA, *EIF3S7*, *TXN2* and *CACNG2* by the '–' strand (tel–cen). dJ1119A7.5 and dJ1119A7.3 are putative genes. The *CACNG2* gene is only partially located in the FTNS critical region.

several recombination events allowed the *FTNS* gene to be localised between markers D22S278 and D22S426, greatly reducing the critical region of 18 cM between markers D22S1167 and D22S284, corresponding to 5.5 Mb, recently reported by Toren *et al.*[11] From a genetic point of view, this region spans about 5.6 cM on genomic DNA[15] and includes a minimum of two non-recombinant loci: D22S1173 and D22S283. Interestingly, the MHA has been mapped in the same area by Kunishima *et al,*[12] in an interval of 13.6 cM located between markers D22S280 and D22S272. These data suggest that the two syndromes, FTNS and MHA, could be allelic and the small region defined by us could contain the gene responsible for these two disorders. These observations are strongly supported by the recent refinement of the MHA locus by Martignetti *et al*[16] to a 6.6 cM interval between markers D22S683 and D22S1177. Nevertheless, a contiguous gene syndrome could not be excluded.

The chromosome 22 is the only human chromosome to has been entirely sequenced. The critical region defined by the recombinants identified in the FTNS family studied is covered by eight overlapping genomic sequences (http://www.sanger.ac.uk/HGP/Chr22) with accession numbers Z82217, Z95114, AL031426, Z82215, AL022302, AL022313, AL031845 and Z70289 respectively,[13] (Figure 2). Based on these data, the size of the FTNS locus is <600 Kb. In particular, since the proximal recombinant marker D22S278 is localised from bp 67993 to bp 68280 in sequence Z82217 and the distal recombinant marker D22S426 is localised from bp 26234 to bp 26594 in sequence Z70289, we can calculate that the entire critical region for the FTNS locus is 588.053 bp in size, excluding the presence of some small gaps.

Several genes have been localised in this interval but have yet to be associated with specific genetic disorders. The apolipoprotein L gene (*APOL*), the gene encoding for the myosin heavy polypeptide 9 non-muscle (*MYH9*), the TNF-inducible protein *CG12-1* mRNA, the eukaryotic translation initiation factor 3 subunit 7 gene (*EIF3S7*) and the thioredoxin gene (*TXN2*) are completely included in the FTNS locus, while the calcium channel voltage-dependent gamma subunit 2 gene (*CACNG2*) is only partially located in the FTNS critical region. In addition, the FNTS locus included a putative *APOL2* gene, a sequence matching an EST cluster and a sequence encoding a putative protein similar to human Hermansky-Pudlak syndrome protein as well as several pseudogenes. All the above genes are strong positional candidate for FTNS based on their location within the small critical region identified. Mutational analysis of all these genes is in progress in order to identify the one responsible.

Acknowledgements
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