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The hereditary pancreatitis gene maps to long arm of chromosome 7

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Hereditary pancreatitis (HP) is an autosomal dominant disorder with incomplete penetrance characterized by recurring episodes of severe abdominal pain often presenting in childhood. Although this disorder has only been recently described, about 100 families have been documented worldwide. The pathophysiology of this disorder is unknown. Here, a large French family of 147 individuals (47 of whom were affected) from a four-generation kindred with HP has been examined and a genome segregation analysis of highly informative microsatellite markers has been performed. Linkage has been found between HP and six chromosome 7q markers. Maximal two point lod scores between HP and D7S 640, D7S 495, D7S 684, D7S 661, D7S 676 and D7S 688 were 4.00 (θ = 0.143), 5.85 (θ = 0.143), 4.91 (θ = 0.156), 8.58 (θ = 0.077), 8.28 (θ = 0.060), 4.40 (θ = 0.169), respectively. Multipoint linkage data combined with recombinant haplotype analysis indicated that the most likely order is : D7S 640 - D7S 495 - D7S 684 - D7S 661 - D7S 676 - D7S 688, with the HP gene situated in the underlined region. As in all families reported in the literature, the clinical presentation of the disease is identical to the presentation of sporadic cases, one could expect that the knowledge of the HP gene could be a clue to pancreatitis in general. Based on its map position, this is the first step towards the positional cloning of the Hereditary Pancreatitis Gene (HPG).

INTRODUCTION

Hereditary pancreatitis (HP) described by Comfort and Steinberg in 1952 (1), is a form of chronic pancreatitis often present in childhood. The first description of the disease, named by the authors as hereditary chronic relapsing pancreatitis, was in a family of which four members had a definite pancreatitis and two others were probably also affected. Since the initial description many other kindreds have been reported in different countries all over the world (2–5). Most of the families reported so far are of caucasian origin, although two families have been reported from Japan (6,7). Hereditary pancreatitis is indistinguishable from pancreatitis due to other causes. The disease is characterized by recurrent episodes of pancreatitis from childhood, equal sex distribution, a family history with at least two other affected members and the frequent presence of calcified stones in pancreatic ducts (8,9). The reports in the literature of about 100 kindreds and of at least three families with identical affected twins, strongly suggested the inheritance of the disease to be an autosomal dominant one with incomplete penetrance (10–13). The pathogenesis of hereditary pancreatitis is not well understood and the basic defect has yet to be identified. The first step to improve our understanding of the disease is to map and to clone the HP gene with the aim of identifying the gene as well as its protein product. We have studied for many years one of the largest kindreds reported so far with more than 220 individuals spanning five generations, and have recently launched a systematic whole genome search by linkage analysis (14). However as the major constituent of pancreatic stones in HP is the pancreatic stone protein, encoded by the '*reg*' gene on 2p2, this gene is a potential candidate for HP (15). Therefore as a first step, we looked for linkage on chromosome 2. However, the lod score results were consistently negative and this chromosome was excluded. The subsequent strategy to map this gene was a genome wide search by linkage analysis using sequence repeat polymorphisms. By studying a large four generation French family with 47 affected patients, we report in this paper the linkage data that map the gene responsible for the phenotype of hereditary pancreatitis to the long arm of chromosome 7 at 7q33-qter.

RESULTS

Description of the family

In 1962, Cornet *et al*. reported the first description of this family including a cluster of 17 related patients with HP from Vendée (western part of France) (16). Over the past 30 years different genealogical studies led us to establish the pedigree described in

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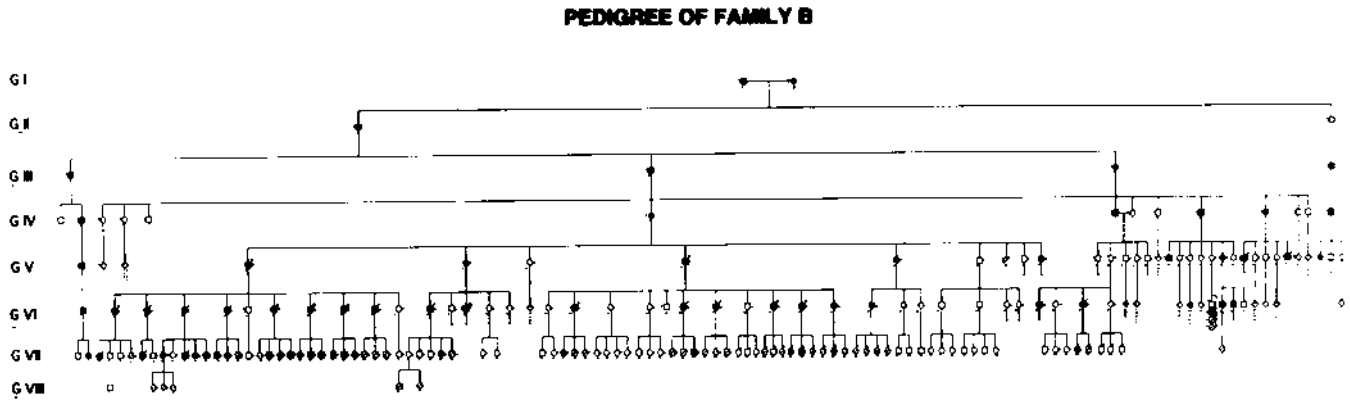

Figure 1. Pedigree of the family B.

Figure 1. Of the 227 individuals, 214 are alive, 147 were studied (47 affected) and these were only included after informed consent was obtained. All the patients were evaluated by a gastro-enterologist (LLB, TG, MS, FS) (see Fig. 1: pedigree of family B).

Systematic screening of the genome

Using 213 microsatellite markers spaced at intervals of about 20 cM, with heterozygosities greater than 0.7, linkage analysis allowed us to exclude half of the genome. Chromosomes 1, 2, 3, 9, 15, 16, 17, 19, 20 and 22 were scanned first as suggested by Antonorakis (17). Then we screened the distal part of other chromosomes. The first marker to demonstrate a lod score greater than 3 was D7S 640 (lod = 4.00 at θ = 0.143).

Refined map of the distal part of chromosome 7

To determine the position of the HP gene relative to the microsatellite markers in this region, 14 other markers were examined (data not shown). Among these, lod scores greater than 3 were obtained with six markers. The greatest values were obtained at D7S 661: lod = 8.58 at θ = 0.077; and at D7S 676: lod 8.28 at θ = 0.060. Two point lod scores obtained between eight chromosome 7 markers and HP are shown in Table 1. Markers are ordered from centromere (top) to telomere (bottom), inter-marker distances (cM) are shown between them.

In order to determine the position of HP relative to the markers used for the two point analysis, the location score was determined by testing the locus of the disease and the markers.

Multipoint linkage analysis

As shown on Table 1, six chromosome 7 polymorphic markers gave lod scores above the threshold value of +3, thus clearly demonstrating linkage to HP.

Table 1. Results of two-point linkage analysis of family B

Locus		Lod score (θ)								Z_{max}	θ Z_{max}
		0.00	0.001	0.01	0.05	0.1	0.2	0.3	0.4		
D7S 640		–3.92	–1.36	0.64	3.02	3.85	3.83	2.92	1.48	4.00	0.143
	7										
D7S 495		0.78	0.96	2.47	4.63	5.66	5.61	4.35	2.29	5.85	0.143
	4										
D7S 684		–8.69	–1.20	0.80	3.50	4.63	4.78	3.80	2.09	4.91	0.156
	8										
D7S 661		6.00	6.01	6.97	8.46	8.52	7.37	5.39	2.77	8.58	0.077
	1										
D7S 676		3.33	5.56	7.40	8.27	8.10	6.84	4.93	2.49	8.28	0.060
	3										
D7S 688		–6.24	–3.31	–0.38	2.50	3.92	4.33	3.36	1.61	4.40	0.169
	2										
D7S 505		–13.96	–11.55	–7.35	–3.01	–0.59	1.29	1.61	1.04	1.63	0.280
	2										
D7S 642		–1.98	–1.96	–1.37	–0.07	0.53	0.96	0.94	0.60	0.99	0.240

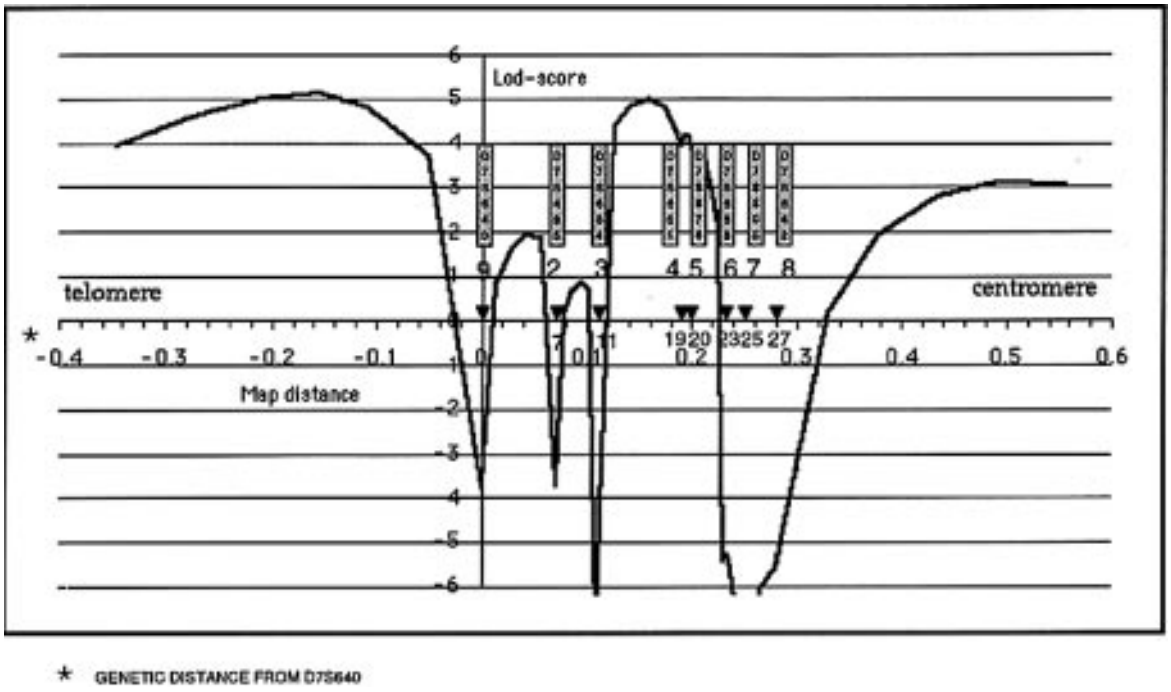

Figure 2. Multipoint linkage analysis between HPG locus and markers D7S 640, D7S 495, D7S 684, D7S 661, D7S 676, D7S 688, D7S 505, D7S 642. Lod score (base 10) for HP versus marker map (cM). D7S640 is at distance 0, marker positions are shown by a little triangle. This composite map was generated with overlapping five point linkmap analyses, due to computation limitations. Kosambi mapping function was used.

In order to localize more accurately the disease locus, a linkmap analysis between the published map of these markers and HP was performed. A composite map was generated by moving a four marker window along the map and by determining the lod scores (five points) for HP, for five equally spaced positions in the central interval. This approach was adopted as it was impossible to run a linkmap analysis with more than five points. Results were compatible with those obtained with the various combinations of four markers (from the eight used: not shown). We also checked that the order of markers determined in our family by the various five point ilink analysis was the same as the published order with only slight differences in some distances (18).

The results in Figure 2 show that the most likely HP gene localisations are proximal to D7S 640 at $\theta = 0.15$ or between D7S 684 and D7S 661 at $\theta = 0.028$ from D7S 684. Positions between D7S 661 and D7S 688 cannot be excluded because the odds against this were around 10.

Haplotype analysis

We performed haplotype analysis with the markers listed in Table 1. This analysis revealed several recombination events. Two recombination events in part 1 of family B (Fig. 3) were observed between markers D7S 684 and D7S 661 both in subjects GVI_8 and GVI_{10} and one event was found in part 2 of family B (Fig. 4) between markers D7S 676 and D7S 688 (individual GVI_{23}). The two recombination events in part 1 of family B place the locus distal to D7S 684 and the recombination event in family 2 places the HP gene proximal to D7S 688. Individual GVI_{25} is carrying the haplotype 1122221 linked to the disease but is still not affected. We have observed two other individuals, VI_{28} and his daughter (VII_{57}) who are carrying the 'disease haplotype' but are also still asymptomatic, this could be due to incomplete penetrance of the disease. We have assumed the penetrance to be 80% for people over 20 and 65% for those under 20.

DISCUSSION

Over the past few years a very high quality short tandem repeat polymorphism map has been generated by Weissenbach and colleagues (19,20). These dinucleotide markers, which are highly polymorphic have increased the efficiency of linkage mapping in recent years, as further illustrated in this paper. Using this strategy we have mapped the gene responsible for hereditary pancreatitis to 7q. The markers were of $(CA)_n$ repeats and were chosen because of their high heterozygosity (over 70%) and spacing of about 20 cM. To facilitate the microsatellite genotyping, we used a non radio-active multiplex procedure (21).

During our systematic screening effort to map the HP gene, we followed the recommendation of Antonarakis who proposed the use of an 'intelligent linkage scanning' technique (17). This approach is based on the fact that the locations of genes already cloned in the genome are non random; chromosomes which are G+C rich have the highest densities of coding sequences, which correspond to the CpG islands which are present at the 5′ end of the majority of housekeeping genes. Chromosomes 1, 9, 15, 16, 17, 19, 20, 22 contain the highest densities of CpG islands, as do the majority of telomeric bands. Following this strategy, we have successively excluded chromosomes 1, 9, 15, 16, 17, 19, 20, 22; following this we systematically screened the distal part of other chromosomes (4, 6 and 7).

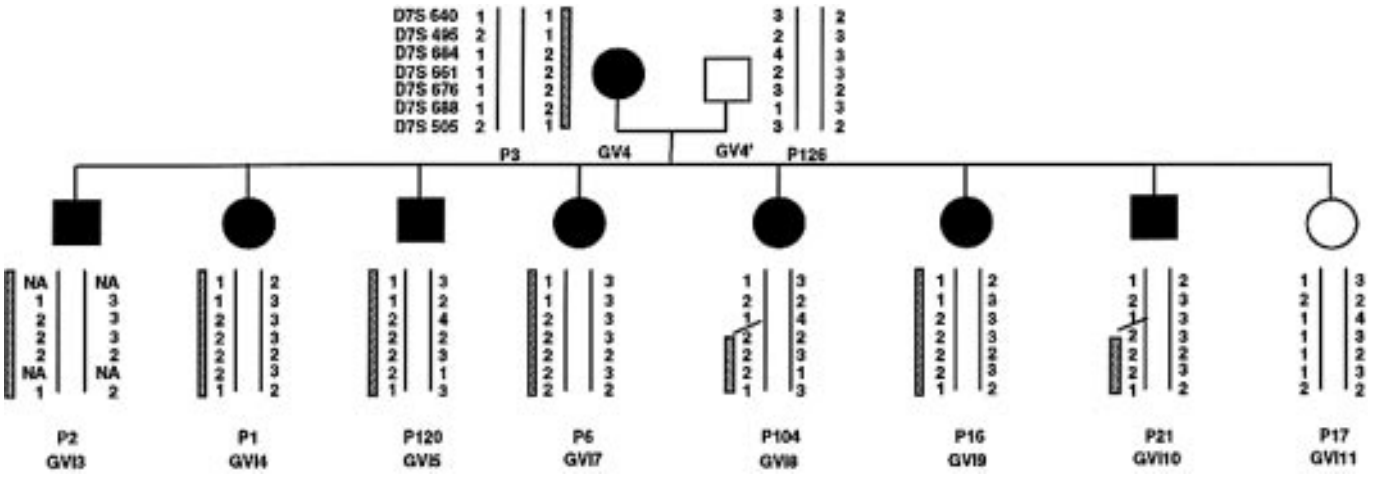

Figure 3. Pedigree and haplotype of parts 1 of Family B. The markers used for haplotype construction are shown, the cross-hatched bars represent haplotypes which co-segregate with the disease, NA = not amplified fragment.

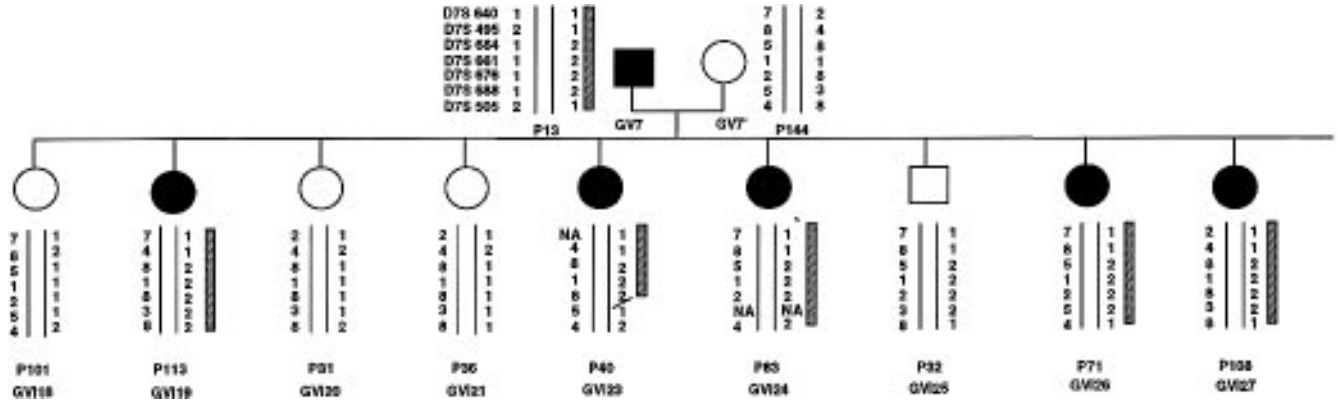

Figure 4. Pedigree and haplotype of parts 2 of Family B. The markers used for haplotype construction are shown, the cross-hatched bars represent haplotypes which co-segregate with the disease, NA = not amplified fragment.

The first linkage obtained was to marker D7S 640, on chromosome 7, subsequently, the marker D7S 661 also showed very strong linkage with a lod score of 8.58 at $\theta = 0.077$.

To refine the localization of the HP gene, polymorphic markers proximal and distal to D7S 661 were studied and a multi-point analysis was performed. Multipoint linkage data indicate that the most likely order is: D7S 640 - D7S 495 - D7S 684 - D7S 661 -D7S 676 - D7S 688, with the HP gene situated in the underlined region.

Haplotype analysis and studies of crossovers show that the HP gene is located between DS7684 and DS7688. Multipoint linkage data indicate that the most likely position is distal to DS7684 or between DS7661 and DS7676 as between these two markers no recombination events were observed.

Our study maps the gene responsible for HP to 7q33qter. It also illustrates the validity of the approach proposed by Antonarakis, as HP gene was in fact mapped to a CpG island at the distal telomeric part of chromosome 7. Definition of the haplotype linked to the HP gene would allow presymptomatic diagnosis to be performed in this family and in other families found to be linked to chromosome 7q. This could be important for genetic counselling in these families.

At the present time, it is difficult to speculate whether HP is a homogeneous genetic disease or whether other families have markers linked to the 7q locus. However, the general description of families in the literature seems to be quite similar and stereotyped. Linkage analysis performed in other families will yield information about the genetic homogeneity or heterogeneity of the disease. Clinically there are no clear definite criteria to distinguish hereditary pancreatitis from idiopathic relapsing pancreatitis. Both show similar symptoms. We are currently collecting other pedigrees with HP to try to determine if other loci for this disorder exist. The rapid development of improved genetic maps as well as localization of expressed sequence tags (22) would facilitate the search for candidate genes. Today it is interesting to note that, among other genes, the carboxypeptidase A has been mapped to 7q32-q ter which is close to our critical region. Carboxypeptidase A is a pancreatic exopeptidase, assigned by Honey (23) to 7q32-q ter. As soon as mutations in the gene causing HP have been identified, the next logical step will

be to study patients with sporadic pancreatitis. This may lead to a greater understanding of pancreatitis in general (24,25).

Our locus assignment represents the first step towards the identification of the gene responsible for hereditary pancreatitis; identification of gene defects will contribute to, and improve our understanding of the molecular basis of this disease.

MATERIALS AND METHODS

Linkage analysis

The Fastlink package of programs, version 2,3P (26,27) was used for linkage analysis. This was derived from the Linkage package version 5.1 (28–30) but it was rewritten in the C programming language and many of the routines were optimized. These modifications resulted in an important increase in the speed of computation. Computations were performed at Infobiogen on a Sparc Server 2000 with 6 CPU and 1 gigabyte memory.

The penetrance for HP was assumed to be 80%, for people aged over 20, and 65% for those under 20. For ilink and linkmap five point analyses, genotypes were downcoded to four equally frequent alleles. Various two or three point runs were checked with the three programs to ensure that downcoding did not illicit noticeable changes in results.

Family studies

Each family member was examined by one of the clinicians (LLB, TG, MS or FS) to confirm the affected or unaffected status, and living participants gave informed consent. The diagnosis criteria were those proposed by Gross *et al.* (4). An acute episode of pancreatitis is the classical clinical presentation that is indistinguishable from pancreatitis due to any other cause; that is severe epigastring pain radiating to the back, which is alleviated in the knee-chest foetal position. Symptoms in this family start as early as 2 or 3 years of age for some children, and recurrences are frequent. Calcifications of the pancreatic gland can be demonstrated by plains films of the abdomen and ultrasonography which demonstrate enlargement of the gland. Computed tomography is often added for better delineation of certain features suggested by ultrasonography. In the family studied other metabolic defects have been conclusively excluded, especially disorders of calcium and lipid metabolism.

DNA analysis

EDTA blood samples (20 ml) were collected and DNA prepared according to standard procedures. Amplification of each dinucleotide repeat was performed using a Perkin Elmer 9600 Thermocycler in a final volume of 50 µl containing 40 ng of genomic DNA, 50 pmol of each primer, 1.25 mM dNTPs and 0.4 U *Taq* polymerase. Amplification conditions were 94°C for 5 min followed by 40 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 30 s, then 94° for 120 s and 68°C for 15 min. Three amplification products obtained with separate primer sets on identical DNA samples were coprecipitated and comigrated in a single lane of a 6% polyacrylamide denaturing gel containing 8 M urea. Separated products were then transferred to Hybond N^+ Nylon membranes which were successively hybridized with probes labelled using a non radioactive labelling procedure and detected using the ECL system (Amersham, UK) (21).

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