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A *Pst*$^+$ Polymorphism in the HEXA Gene with an Unusual Geographic Distribution

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Key Words
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Abstract

A polymorphic variant in the human HEXA gene is described. This gene encodes the α-subunit of hexosaminidase A, the enzyme which is deficient in Tay-Sachs disease (TSD). In individuals carrying the polymorphism there is a T→C transition at position −6 in intron 13. The substitution creates a site for the restriction endonuclease *Pst*1. This variant has an unusual ethnogeographic distribution. It occurs on 1.4% of non-TSD carrier chromosomes in Ashkenazi Jews. All individuals ascertained carrying the *Pst*$^+$ allele have ancestry in Lithuania, Belarus and Ukraine. By contrast, no individuals carrying the *Pst*$^+$ allele have been detected among non-Jewish Lithuanians, Jews of Sephardic origin or in several other ethnic groups. Two unrelated non-Jewish families have been identified in which the *Pst*$^+$ variant occurs. In both cases the variant occurs on a chromosome carrying a novel TSD mutation (G772C) association with the B1 phenotype. The *Pst*$^+$ G772C chromosomes are of Scots-Irish descent.

Introduction

The HEXA gene, in which more than 30 mutations occur leading to variant forms of the neurodegenerative disorder Tay-Sachs disease (TSD), spans 35 kb on chromosome 15q22-24. TSD has elevated frequency in Ashkenazi Jews where 3 mutations predominate; a 4-bp insertion in exon 11 (73% of carrier chromosomes), a splice junction mutation in intron 12 (14%) and a G805A substitution in exon 7 (3%) [1–3]. The historical center of

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diffusion for the exon 11 mutation is believed to be in central Europe (corresponding to Austria, Hungary and Czeckoslovakia) [4].

We describe a polymorphism in the HEXA gene creating a new site for the restriction enzyme *Pst*1 with an allele frequency of 1.4% in Ashkenazi Jews. Individuals carrying this *Pst*$^+$ allele trace ancestry to Lithuania, Belarus and Ukraine, suggesting a center of diffusion different from that of the most common TSD gene. The *Pst*$^+$ allele was also found in two unrelated non-Jewish TSD patients in whom it occurred in association with a novel TSD mutation (G772C) with B1 phenotype [5, 6]. In both families the carriers of this chromosome were of Scots-Irish descent.

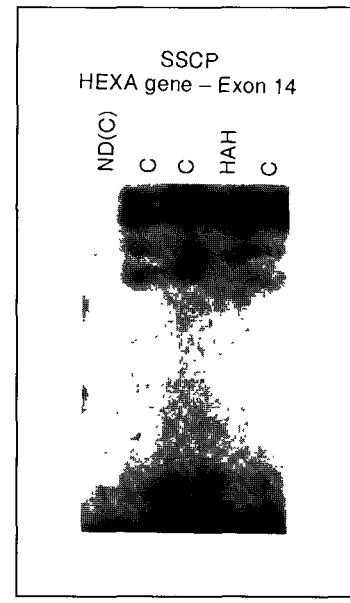

Fig. 1. Single stranded conformational polymorphism analysis of HEXA exon 14 and flanking sequences. ND(C) refers to a non-denatured control sample. The three lanes indentified as C are three different control individuals in whom the *Pst*$^+$ variant is *not* found. HAH is an individual in which the aberrant electrophoretic band is produced by the *Pst*$^+$ variant.

Materials and Methods

Subjects

DNA samples were obtained from Ashkenazi Jewish participants in the Montreal (n = 97) and California (n = 65) TSD screening programs, and from Israel (n = 39) and Lithuania (n = 2). Sephardic Jewish (n = 9) and Italian (n = 76) samples were obtained from the Montreal β-thalassemia screening program. French Canadians (n = 36) and French (n = 50) individuals were participants in a research program analyzing population origins. Samples from individuals of Scots-English-Irish descent were from Montreal (n = 154), California (n = 43) and from the United Kingdom (n = 6). Samples obtained from individuals of eastern European origin were provided either by the California TSD screening program or were from Lithuania (n = 12).

Patients carrying a novel TSD gene (G772C; B1 phenotype) were previously reported [5, 6].

Determination of TSD Carrier Status

Samples from Montreal, California and England were ascertained for carrier status as previously reported [7, 8]. Samples from Israel were screened for the 3 common Ashkenazi TSD alleles.

Identification of Pst$^+$ *Polymorphism.* DNA was isolated from blood leukocytes or fibroblasts by standard procedures. Primer pairs used to amplify exon 14 and flanking sequences of the HEXA gene, including intron 13, were as reported [9]. Single-stranded conformational polymorphism (SSCP) analysis was performed according to Orita et al. [10] as modified by Triggs-Raine [9]. Direct sequencing of the DNA fragment was carried out using a sequenase kit according to Wong et al. [11]. Following *Pst*1 digestion of the amplified DNA, samples were electrophoresed on 1.4% agarose.

Results and Discussion

SSCP analysis of the HEXA gene fragment containing the Pst$^+$ allele yielded an altered electrophoretic pattern (fig. 1). Direct sequencing of the PCR-amplified fragment identified a T→C transition at position −6, in intron 13 (fig. 2). This mutation creates a *Pst*1 site (fig. 3). A screen of 203 Ashkenazi Jewish

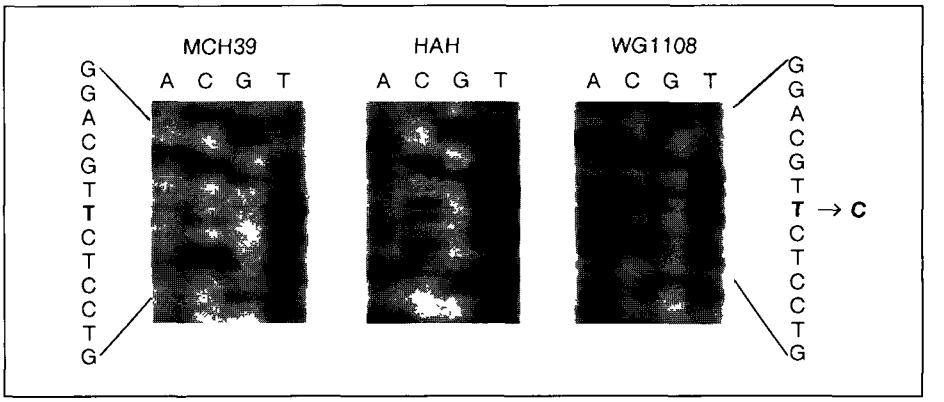

Fig. 2. Direct DNA sequencing of amplified HEXA exon 14 products. Templates are prepared from control fibroblasts (MCH39), a *Pst*+ individual (HAH), and a TSD patient who is a compound heterozygote for the G772C mutation (WG1108).

individuals (160 normal, 43 TSD heterozygotes) identified 5 *Pst*+ alleles (frequency = 1.4% on normal chromosomes). No *Pst*+ alleles were observed on TSD chromsomes (table 1). Three of the 5 *Pst*+ individuals came from the Montreal sample, 1 from the Israel group and one from the California sample. A survey of individuals with normal HEXA genotypes of varied ethnic background did not identify any more *Pst*+ alleles (table 1).

The *Pst*+ variant was also found in 2 patients on chromosomes carrying a novel G772C TSD allele. The *Pst*+ allele was confirmed in one of these patients and in his father by direct sequencing (fig. 2).

Since the original description of the HEXA gene structure [13], several reports of rare silent mutations have appeared [14–17]. To date, however no neutral (benign) polymorphisms (population allele frequency >1%) have been reported. Although the elaboration of haplotypes at the HEXA locus would facilitate study of the spread of TSD genes and the migration of human populations, HEXA (unlike HEXB) has thus far proved to be quite 'barren' of polymorphisms. Such find-

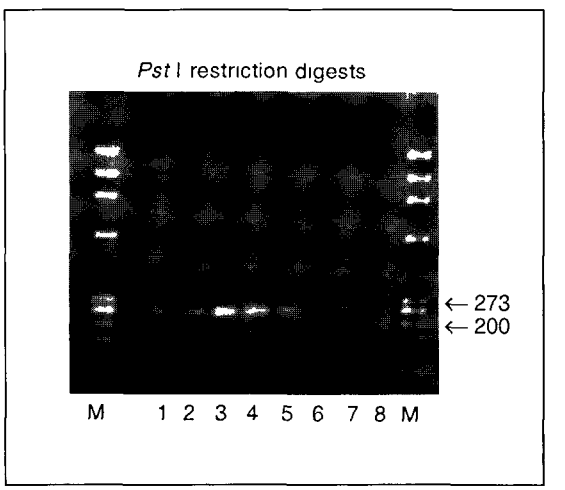

Fig. 3. *Pst*+ digestion of amplified (HEXA) exon 14 products. Genomic DNA was amplified using exon 14 primers (5′TGACTGGTGTGAAAAGTGTTGC3′, 5′CCTTTCTCTCCAAGCACAGG3′ [9] and digested with *Pst*1 according to supplier. ∅X174 HaeIII digest markers are shown. Lanes 1, 3, 5, 7; amplified DNA. Lanes 2, 4, 6, 8 corresponding *Pst*1 digests illustrating *Pst*+ polymorphisms in 2 Ashkenazi individuals (lanes 4 and 6) and the individual carrying the G772C mutation (lane 8).

Table 1

Chromosomes	*Pst*$^+$	Ethnicity
363	5	Ashkenazi Jewish
43	0	Ashkenazi Jewish TSD
18	0	Sephardic Jewish
384	0	Scots-Irish, English
22	2	Scots-Irish, English TSD
152	0	Italian
100	0	French
72	0	French Canadian
56	0	mixed (unknown)
24	0	Eastern Europe non-Jewish

ings are generally rare but have been noted at some other loci of medical interest (e.g. factor IX) [18].

The *Pst*$^+$ mutation occurs in the pyrimidine tract of intron 13 which is believed to have functional significance. Intronic mutations in the −6 to −10 position are associated with splicing abnormalities leading to disease phenotypes in the β-globin and the HEXA genes, respectively [9, 19]. We have no evidence for reduced splicing or altered enzyme activity in *Pst*$^+$ individuals, but believe it to be unlikely since this polymorphism was ascertained predominantly in individuals with normal levels of serum hexosaminidase A.

The Jewish individuals harboring the *Pst*$^+$ allele each have ancestry in Lithuania and its surrounding regions. The identification of *Pst*$^+$ Ashkenazi Jewish individuals in samples from three centers (Montreal, California, Israel) makes it unlikely that this variant is 'private' in the sense of being confined to a single family. While the *Pst*$^+$ individuals all shared ancestry in and around Lithuania, the Ashkenazi cohort (n = 203) represented individuals with ancestry spread over the European continent (Russia, Poland, Germany, etc.) and included relatively few of Lithuanian origin.

The ethnic origin of the *Pst*$^+$ chromosome associated with the G772C (TSD mutation) does not appear to be the same as that of the normal chromosome carrying the *Pst*$^+$ variant. The unrelated carriers of *Pst*$^+$ and G772C were both Americans of Scots-Irish descent. We therefore surveyed individuals of Celtic (Scottish and Irish) origins (n = 203) currently living in England, Ireland and Montreal including 20 who tested positively for the most common 'Irish' TSD gene (IVS 9 splice junction mutation) [12]. No *'Pst*$^+$*'* individuals were identified in this group (table 1). It is therefore likely that *'Pst*$^+$*'* is in linkage disequilibrium with G772C in this population. The *Pst*$^+$ allele, occuring in two unrelated populations and not involving the highly mutagenic CpG dinucleotide, may be more ancient than the common Ashkenazi TSD 4-bp insertion mutation.

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