Abstract  An intragenic deletion in the human PTPN6 gene is described. The PTPN6 gene maps to chromosome 12p12–13 and is shown to possess two alternative first exons. A 1.7-kb deletion occurring in the intron between the two alternatively used first exons is the result of an illegitimate recombination between two Alu-type repeats. The deletion increases the transcriptional activity of the distal promoter.

Source/description. The human PTPN6 gene has been localized to human chromosome 12p12–13 (Yi et al. 1992). Abnormalities of its murine homolog, hcpPh, have been described in mice carrying the motheaten (me) mutation (Tsui et al. 1993; Shultz et al. 1993), implicating this gene in the control of haematopoiesis. The genomic structure of the PTPN6 gene has not yet been described. The identification of cDNA clones with different 5'-ends (Shen et al. 1991; Yi et al. 1992) suggests a complex structure of this locus. Here, we describe the genomic structure of the PTPN6 gene and show that it is transcribed from two promoters (Fig. 1 A). In the RPMI8402 cell line, the PTPN6 gene exists in two forms; both alleles have been cloned and analysed here.

Polymorphism. The sequence of the more prevalent allele (A1) encompassing the two alternative first exons and exons 2–3 encoding the first SH2 domain (EMBL/Genbank/DDBJ databases accession number X82818) has been compared with the corresponding regions on the minor allele (A2) (EMBL/Genbank/DDBJ accession number X82817). The overall rate of sequence variation between the two alleles is low (less than 0.1%) and no differences occur in the coding and the transcribed 5'-untranslated regions. The major difference is the deletion of about 1.7 kb in allele 2. The deletion breakpoints lie within two Alu-type repeats (Fig. 1 A) in the first intron; the junction sequences are compared in Fig. 1 B, with the putative crossover point being indicated. The deletion can be detected by DNA filter hybridization (Fig. 1 C) with a probe encompassing exon 2 (Fig. 1 A) or, more conveniently, by the polymerase chain reaction (PCR) with primers 279 (5'-TTGTTCGCCTTCAGACTGC) and 280 (5'-GCTTTTCGCTACGCTGC) (Fig. 1 D) under the following conditions: denaturation at 94°C for 1 min; annealing at 66°C for 1 min; extension at 72°C for 1 min; 35 cycles using 25 ng DNA template in 20 μl final volume. In this assay, the size of the PCR fragment from allele A1 is about 2 kb, that from allele A2 is about 310 bp. However, in heterozygous subjects, the large fragment is usually very faint.

Frequency. The frequency was estimated from 70 chromosomes of Caucasian origin, using Southern blotting and/or PCR assay. Allele frequencies were: allele 1, 0.9; allele 2 (deletion), 0.1. The frequency of heterozygotes was 0.2 (calculated 0.18).

Chromosomal localization. The PTPN6 gene has been mapped to human chromosome 12p12–13 (Yi et al. 1992).

Mendelian inheritance. Co-dominant segregation was observed in one two-generation pedigree.

Comments. Four individuals were also analysed for a PvuII polymorphism occurring in the intron between exons 3 and 4 (Fig. 1 A). It can be detected by PCR (under the conditions described above) using primers 21 (5'-ACCCATATCGTCCAGACAGG) and 164 (5'-GTAGGGAACACACAGAATTT) with subsequent PvuII digestion (Fig. 1 E). In the cell line RPMI8402, the PvuII site is present on the chromosome with the deletion.