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SHORT COMMUNICATION

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DNA polymorphisms in *ITPA* including basis of inosine triphosphatase deficiency

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Abstract Intracellular concentrations of the nucleotide inosine triphosphate (ITP) are regulated by ITP-ase (EC 3.6.1.19), which is encoded by *ITPA* on chromosome 20p. Subjects with complete deficiency of ITP-ase activity (MIM 147520) have elevated ITP concentrations in erythrocytes, but no obvious clinical abnormalities. Based on biochemical screening, complete ITP-ase deficiency has been postulated to result from homozygosity for a dysfunctional allele, with an estimated frequency of 0.05 in Caucasians. ITP-ase deficiency has not yet been characterized at the molecular genetic level. Sequencing of the genomic DNA from a Caucasian subject with complete ITP-ase deficiency revealed homozygosity for missense mutation 198C>A, which predicted a threonine for proline substitution at codon 32 (P32T), whereas among 125 normal Caucasians, there were no homozygotes for P32T ($P = 0.0079$). The P32T allele frequency of 0.07 in Caucasians was similar to the estimates derived from earlier biochemical studies. P32T was found to be present at varying frequency in other ethnic groups. Two common synonymous single-nucleotide polymorphisms were also identified. These *ITPA* markers, including P32T, provide tools for further study of association with clinical and biochemical phenotypes.

Key words Nucleotides · Erythrocytes · Genomic DNA · Sequencing · Monogenic disease

Introduction

Inosine triphosphate (ITP) is present in all cells, and its intracellular concentration is determined by the activity of cytosolic inosine triphosphatase (ITP-ase; EC 3.6.1.190), encoded by *ITPA* on chromosome 20p (Meera Khan et al. 1976; Hopkinson et al. 1976; Rudd et al. 1979). ITP-ase is a nucleoside triphosphate pyrophosphohydrolase that is specific for ITP (Vanderheiden 1975). Vanderheiden (1969) reported high levels of ITP in the erythrocytes of two siblings and suggested that this resulted from homozygous deficiency of ITP-ase (MIM 147520). Support was provided by subsequent larger scale biochemical studies, which determined an allele frequency for the putative dysfunctional variant of ~0.05 in Caucasian populations (Holmes et al. 1979; van Waeg et al. 1988; Duley et al. 1990). No clinical abnormalities have been associated with homozygous ITP-ase deficiency. To determine the molecular genetic basis of this condition, we sequenced *ITPA* in a subject with complete ITP-ase deficiency.

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Methods

Study subjects

DNA was obtained from the Coriell Cell Repositories (Camden, NJ, USA). Subject GM1617 was a 29-year-old woman with complete ITP-ase deficiency originally reported by Vanderheiden (1969). DNA from five normal Caucasian control subjects was also sequenced. In addition, samples from 125 clinically normal Caucasian subjects, 60 normal African subjects, 60 normal Chinese subjects, and 60 normal subjects of East Indian descent were screened to determine allele frequencies. The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *ITPA* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set using GenBank accession numbers NT_011387 and XM_045418. Primer sequences are shown in Table 1. Amplification conditions for all exons were 94°C for 5-min, followed by 30 cycles of 30 s each at 94°C, 60°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Table 1. Amplification primers for *ITPA*

Exon(s)	Primer sequence (5′ to 3′)	Product size (bp)
1	F- GCC GGA AGT TTT CTG TCA CT R- TGT TCT CTC TCC ATG CCT CC	240
2 and 3	F- CGT GCT CAC ATG GAG AAT CA R- CCT GGA AGC TAC CTG GAC AA	328
4	F- GTT CCA GGT GGG GAT GGT R- GGA CAA CGT GAA AGG CTG TT	255
5	F- CAG CCT GGA AAA GGT GGT AA R- GGT CCC TCC CCT AGA CAA AG	172
6	F- GTC TTG GGA GTG GGG GTG R- ACT GCA TCA AGG TGG GTA CG	200
7	F- AGA CTC ACC AAA CTC ATA CTG GC R- CAA ACC ATG GCT GTA CCA AC	220
8	F- GGT GCA CTT CCT TCC TGA AT R- CTA GCC CTC CCC AGA TCC	201

F, Forward primer; R, reverse primer

Genotyping of *ITPA* gene polymorphisms

The 198C>A (P32T) and 242G>A single-nucleotide polymorphism (SNP) were genotyped by amplifying genomic DNA using the primers for exons 2 and 3 shown in Table 1, and the amplification program described earlier. For the 198C>A SNP, the 328-bp product was digested with endonuclease *Nsp*I. The 198C allele yielded a single 328-bp fragment after digestion with *Nsp*I. The 198A allele yielded two fragments with sizes 238 and 90 bp after digestion with *Nsp*I. The fragments were resolved on 2% agarose gels. For the 242G>A SNP, the 328-bp product was digested with endonuclease *Bst*NI. The 242G allele yielded five fragments with sizes 220, 51, 43, 11, and 3 bp after *Bst*NI digestion. The 242A allele yielded four fragments with sizes 271, 43, 11, and 3 bp after *Bst*NI digestion. The fragments were resolved on 2% agarose gels.

The *ITPA* exon 8 665G>A SNP was genotyped by amplifying genomic DNA using the primers in Table 1, and the amplification program described earlier. This was followed by digestion of the 201-bp product with endonuclease *Rsa*I. The 665G allele yielded three fragments with sizes 73, 72, and 56 bp after *Rsa*I digestion. The 665A allele yielded two fragments with sizes 145 and 56 bp after *Rsa*I digestion. The fragments were resolved on 2% agarose gels.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Chi-square analysis tested differences in proportions and the deviation of genotype frequencies from Hardy-Weinberg predictions, with the nominal $P < 0.05$. Fisher's exact test was used to compare the single ITP-ase deficient subject with normal controls.

Results

Identification of polymorphisms

Genomic DNA sequencing experiments uncovered three polymorphisms. These were a missense SNP 198C>A in exon 2 (which changed the amino acid sequence P32T), a silent 242G>A SNP in exon 3, and a silent 665G>A SNP in exon 8. Homozygosity for the missense P32T SNP was seen only in the *ITPA*-deficient subject, whereas, among 125 normal Caucasians, there were no homozygotes for P32T ($P = 0.0079$, Fisher's exact test). The exon 8 SNP already has an entry in the National Center for Biotechnology Information SNP database (ID rs9101). All three SNPs are shown in Table 2.

Polymorphism frequencies in normal samples

The observed genotype frequencies of all polymorphisms did not deviate from the predictions of the Hardy-Weinberg equation. The allele frequency in four ethnic groups for each polymorphism is shown in Table 2. Using Caucasian allele frequencies as the referent for each comparison, we found that (1) the frequency of the P32T allele was significantly higher in Chinese subjects only ($P = 0.03$), (2) the frequency of the 242A allele was significantly higher in African and Chinese subjects (both $P < 0.0001$), and (3) the frequency of the 665A allele was significantly lower in African subjects ($P < 0.0001$) and significantly higher in Chinese subjects ($P = 0.002$).

Table 2. *ITPA* SNP allele frequencies

Polymorphism name	Ethnic group (number)	Allele frequency
198C>A (P32T)	Caucasian (125)	0.07
	African (60)	0.05
	Chinese (60)	0.15
	East Indian (60)	0.11
242G>A	Caucasian (125)	0.32
	African (60)	0.52
	Chinese (60)	0.57
	East Indian (60)	0.42
665G>A	Caucasian (125)	0.32
	African (60)	0.08
	Chinese (60)	0.50
	East Indian (60)	0.38

SNP, Single-nucleotide polymorphism

Discussion

We report (1) primer sets to amplify coding regions of the *ITPA* gene and (2) use of these primers to sequence genomic DNA. This has resulted in the identification of three polymorphisms in *ITPA*, of which one, P32T, is the likely molecular genetic basis of homozygous ITP-ase deficiency. Of 125 normal Caucasians, none was homozygous for P32T. Furthermore, the observed allele frequency of 0.07 in the Caucasian sample was consistent with previous estimates that were derived from biochemical measurements. The proline at residue 32 is evolutionarily conserved in species ranging from human to mouse (Lin et al. 2001), supporting the functionality of this residue. The P32T allele appears to be more prevalent in subjects of Chinese and East Indian origin. The higher frequency of the P32T allele in Chinese and East Indian subjects suggests that homozygous ITP-ase deficiency may be higher in these populations than in Caucasians, although this has not been previously assessed biochemically. The current opinion that ITP-ase deficiency has no clinical or biochemical consequences can now be assessed more systematically in various ethnic groups at the molecular level in other experiments using the reagents described in this report.

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