Mutational analysis of the *HGO* gene in Finnish alkaptonuria patients

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Abstract

Alkaptonuria (AKU), the prototypic inborn error of metabolism, has recently been shown to be caused by loss of function mutations in the homogentisate-1,2-dioxygenase gene (*HGO*). So far 17 mutations have been characterised in AKU patients of different ethnic origin. We describe three novel mutations (R58fs, R330S, and H371R) and one common AKU mutation (M368V), detected by mutational and polymorphism analysis of the *HGO* gene in five Finnish AKU pedigrees. The three novel AKU mutations are most likely specific for the Finnish population and have originated recently.

(*J Med Genet* 1999;**36**:922–923)

Keywords: alkaptonuria; homogentisate-1,2-dioxygenase; Finland

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Revised version received
17 June 1999
Accepted for publication
5 August 1999

Alkaptonuria (AKU, OMIM No 203500), a metabolic defect in the Tyr/Phe catabolic pathway, was the first human disorder described as an autosomal recessive trait.[1] AKU is a rare disease with an incidence of approximately 1:250 000. The patients lack homogentisate-1,2-dioxygenase activity which causes accumulation of homogentisate (HGA) which is excreted into the urine. Upon oxidation at alkaline pH, the urine of AKU patients acquires a dark bluish brown coloration that is distinctively characteristic of the disease. The accumulation of an oxidation product of HGA, benzoquinone acetic acid (BAA), in the connective tissues and the accumulation of polymerised BAA causes pigmentation of the tissue (ochronosis) and leads to severe arthropathy during later life.[2]

Recently, it has been shown that a defect in the gene coding for homogentisate-1,2-dioxygenase (*HGO*) is responsible for alkaptonuria.[3] The *HGO* gene maps to chromosome 3q21-q23 and is expressed in liver, kidney, prostate, and, to a lesser extent, in small intestine and colon. *HGO* spans 54 363 bp of DNA and is composed of 14 exons. Upon transcription, it produces a single 1715 nt long transcript that encodes for a polypeptide of 445 amino acids.[4] To date, 17 mutations have been described in patients of different European origins.[3 5–7] Here, we present a mutational and polymorphism analysis of five Finnish AKU pedigrees and describe three novel AKU mutations.

Five unrelated Finnish AKU pedigrees with at least one affected member each were studied. All five AKU patients had parents who were either close relatives or originated from neighbouring villages or from the same region of the country. The clinical symptoms were similar in all affected members, with darkening of the urine in childhood, skin and scleral discoloring in youth, and ochronotic arthropathy in middle age. As control samples, genomic DNA from 38 random healthy Finnish subjects was used. Genomic DNA was purified from peripheral blood mononuclear cells according to standard protocols and all 14 *HGO* exons were amplified by PCR and studied by single strand conformational polymorphism assay (SSCP) and automated sequencing, as described previously.[7] For haplotyping, analysis of four single nucleotide polymorphisms (SNPs), IVS2+35T/A (intron 2), c407T/A (exon 4), IVS5+25T/C (intron 5), and IVS6+46C/A (intron 6), and three microsatellite polymorphisms *HGO*-1 (D3S4496, intron 4), *HGO*-2 (D3S4497, intron 13), and *HGO*-3 (D3S4556, intron 4) was performed as previously described.[7]

The mutational analysis of the *HGO* gene in the five Finnish AKU pedigrees resulted in identification of the mutations associated with each of the 10 chromosomes (table 1). All AKU patients in these pedigrees were homozygous for *HGO* mutations. In two pedigrees, the patients were homozygous for M368V, an AKU mutation in exon 13 that has been described previously in patients from other geographical regions.[7] In the other three Finnish pedigrees, the patients were homozygous for AKU mutations that are novel. One of them carries the frameshift mutation R58fs (c342delA) that leads to a truncated HGO polypeptide. R58fs is the third frameshift mutation described in AKU patients. The defective gene carrying the R58fs mutation

Table 1 HGO mutations identified in the Finnish AKU patients

Name	*Mutation*	*Exon*	*Nucleotide change*	*Amino acid change/predicted consequence*	*No of families*	*Ref*
R58fs	Frameshift	3	c342delA	Truncation after Arg58	1 (homozygous)	This study
R330S	Missense	12	c1157G→T	Arg330Ser	1 (homozygous)	This study
M368V	Missense	13	c1269A→G	Met368Val	2 (homozygous)	7
H371R	Missense	13	c1279A→G	His371Arg	1 (homozygous)	This study

Positions of nucleotide changes are from the transcription start site as previously described.[4]
The ATG initiation codon is located at nucleotide position c168.

Table 2 AKU alleles associated with HGO haplotypes in Finnish patients

		Associated polymorphisms						
Mutation	*Frequency*	*IVS2+35A/T*	*c407T/A*	*HGO-3*	*HGO-1*	*IVS5+25T/C*	*IVS6+46A/C*	*HGO-2*
R58fs	2/10	A	T	197	161	T	C	181
		A	T	197	161	T	C	181
R330S	2/10	A	T	191	161	T	A	177
		A	T	191	161	T	A	177
M368V	4/10	A	T	195	161	T	C	183
		A	T	195	161	T	C	183
		A	T	195	161	T	C	183
		A	T	195	161	T	C	183
H371R	2/10	A	T	199	161	T	C	189
		A	T	199	161	T	C	189

codes for the first 58 amino acids of the normal HGO protein followed by 31 additional unrelated amino acids. The R58fs mutation was also detected in heterozygosity in an unaffected brother and in one of the patient's three children. Two missense mutations, R330S and H371R, were found in homozygosity in the AKU patients of the two remaining AKU pedigrees. R330S in exon 12 is caused by transversion of G to T at position c1157 that eliminates a *Stu*I restriction enzyme site. Similarly, H371R in exon 13 is caused by an A to G transition at position c1279 and destroys a *Nco*I restriction enzyme site. None of the mutations was observed in unaffected controls. The three Finnish missense mutations change amino acids in positions that are highly conserved in corresponding HGO proteins from mouse (Genbank Acc U58988), fungi (*Aspergillus nidulans*, AJ001836), and nematode (*Caenorhabtitis elegans*, Z93778).

Four SNPs (IVS2+35T/A, c407T/A, IVS5+25T/C, and IVS6+46C/A) and a dinucleotide (CT)n repeat (*HGO*-1) were characterised in the five AKU pedigrees and in 34 normal Finns. The *HGO*-2 and *HGO*-3 polymorphisms were also characterised in the AKU pedigrees. Three Finnish mutations, R58fs, R330S and H371R, cosegregated with *HGO* haplotypes that have not previously been associated with AKU mutations (table 2). In contrast, the M368V mutation in the four Finnish AKU chromosomes was associated with an *HGO* haplotype found earlier in a German and a French AKU patient carrying the same mutation. Previous data on M368V associated *HGO* haplotypes suggest that M368V is probably an old AKU mutation that has spread throughout Europe with the different population migrations.[7] Thus, these AKU chromosomes might have been introduced into Finland by immigrants from other European populations.

Three of the AKU mutations found in the Finnish patients are missense mutations, which is in agreement with the observation that the majority of the AKU mutations found so far are missense mutations.[7] The AKU mutations are distributed throughout the whole length of the *HGO* sequence, with a slightly higher occurrence in exons 7 to 10. Within this region, a cluster of three mutations (R225H, F227S, and P230S) has been described to occur within a short region of six amino acids, suggesting that this region is important for enzyme activity.[7] Similarly, two mutations (M368V and H371R) found in the Finnish patients are separated from each other by two amino acids, suggesting that the region close to amino acid 370 might also be critical for protein function.

Most of the *AKU* mutations encountered have been found in single families and are considered to be relatively new mutations. Finland has a high prevalence of several rare hereditary diseases, which is thought to be a consequence of its isolated population history, in which founder effects played an important role.[8] All Finnish AKU patients, according to their family histories, had parents or grandparents who originated from very close geographical regions. Considering the low prevalence of AKU in the Finnish population, the finding of three patients homozygous for the novel R58fs, R330S, and H371R mutations suggests that these mutations may have originated recently and are most likely specific for the Finnish population.

We thank the families with AKU for their collaboration and donation of DNA samples. This research was supported from Medical Research Fund of Tampere University (PP, KK, AR), the Fundación Jose Antonio de Castro, the Spanish Comisión Interministerial de Ciencia y Tecnología (SAF99/0013), and the Comunidad de Madrid (08.6/0015/1997). In addition, this study is based on work supported by the Fundación Conchita Rábago de Jiménez Díaz under a grant awarded to DB-VdeB.

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