ONLINE MUTATION REPORT

Alkaptonuria in the Dominican Republic: identification of the founder AKU mutation and further evidence of mutation hot spots in the *HGO* gene

E Goicoechea de Jorge, I Lorda, M E Gallardo, B Pérez, C Peréz de Ferrán, H Mendoza, S Rodríguez de Córdoba

J Med Genet 2002;**39**:e40 (http://www.jmedgenet.com/cgi/content/full/39/7/e40)

Alkaptonuria (AKU, MIM 203500), the first human disease to be recognised as a recessive trait and Archibald Garrod's prototype "inborn error of metabolism",[1 2] is a rare disorder of the phenylalanine and tyrosine catabolic pathway caused by the deficiency of homogentisate dioxygenase (HGO, EC 1.13.11.5) activity.[3] AKU patients are homozygous, or compound heterozygous, for loss of function mutations in *HGO*.[4] As a consequence of this defect, AKU patients cannot convert homogentisate to maleylacetoacetate, which results in homogentisic aciduria, ochronosis, and arthritis.[5] AKU shows remarkable allelic heterogeneity. More than 40 different AKU mutations have been identified in a total of fewer than 100 unrelated patients from many different countries. In addition to the AKU mutations, 19 polymorphisms have been encountered within the human *HGO* gene (for a complete description of the *HGO* mutations and polymorphisms see the AKU database (http://www.cib.csic.es/~akudb/index.htm)). The analysis of the haplotype association of polymorphisms in the AKU chromosomes has been very useful for the identification of the different AKU alleles and for tracing their migration during recent human history. In this regard, it has been shown that the three most widespread AKU mutation in Europe, M368V, V300G, and P230S (representing 20%, 5%, and 5% of European AKU chromosomes, respectively) are not recurrent mutations. Instead they are probably old mutations that were introduced into Europe with the founder populations and have spread throughout western Europe with the different migrations.[6] Analysis of the *HGO* mutations and polymorphisms has also shown that the GGG sequence motif (or its reverse complement CCC) is a mutational hot spot in the *HGO* gene.[7]

AKU has a very low prevalence (1:100 000-250 000) in most populations. However, in certain areas, such as the Dominican Republic and Slovakia, the incidence of alkaptonuria is unusually high.[8 9] In Slovakia, AKU has an approximate incidence of 1:19 000 and shows unexpected allelic heterogeneity. As many as 10 different AKU mutations have been identified in AKU Slovak patients, suggesting that several independent founders have contributed to the AKU gene pool in this geographical location.[10–12] Recently, we provided evidence indicating that the most prevalent Slovak mutations (G152fs, G161R, G270R, and P370fs) most likely originated in Slovakia at a single and very small geographical location, the Kysuce region, in the northern part of the country.[11] Our data also suggested that increased mutation rates in *HGO*, associated with GGG triplets and other mutational hot spots, were probably involved in the origin of these Slovak mutations.

No molecular data are yet available from the Dominican AKU patients. However, early epidemiological data suggest that the high incidence of AKU in the Dominican Republic could be the consequence of a classical founder effect. In these early studies, as many as 47 AKU cases were reported occurring in eight highly inter-related kindreds in a mixed Spanish-Indian group living in rural Santo Domingo.[8]

To determine whether the high incidence of AKU in the Dominican Republic is the consequence of a founder effect (as suggested by the epidemiological data) or an increased mutation rate in the *HGO* gene (as seems to have been the case in Slovakia), we have performed *HGO* mutation/polymorphism analyses in eight unrelated Dominican AKU patients and their relatives. These patients are all from the same geographical area as the eight kindreds described by Milch[8] in 1960 and we have confirmed that at least one of the Dominican AKU patients included in our study belongs to one of those kindreds. This patient and the common geographical origin allow us to link the present molecular studies with Milch's epidemiological data.[8]

MATERIAL AND METHODS

DNA from patients and their relatives was extracted from buccal mucosa cells collected with MasterAmp buccal brushes (Epicentre Technologies, USA) following informed consent. Mutation and polymorphism analyses were performed as previously described.[6] Briefly, the 14 exons of the *HGO* gene were amplified from genomic DNA using specific primers derived from the 5′ and 3′ intronic sequences and the corresponding PCR products were purified using Wizard PCR Preps DNA Purification System (Promega, USA). Direct sequencing of the PCR products was performed with a dye terminator cycle sequencing kit (Applied Biosystems, USA) and resolved in an ABI PRISM 3700 automatic DNA sequencer. Analysis of polymorphisms at the *HGO-1* (D3S4496), *HGO-2* (D3S4497), and *HGO-3* (D3S4556) microsatellites was performed by PCR using total human genomic DNA. The *HGO* intragenic dimorphic markers IVS3-112T/C, c407T/A, IVS4+31A/G, IVS5+25T/C, IVS6+46C/A, and IVS11+18A/G were characterised in the same PCR fragments that were sequenced during the search for AKU mutations.

RESULTS

AKU mutations were identified in all 16 Dominican AKU chromosomes. Seven of the eight AKU patients are homozygous for a c.527T→G substitution in exon 6 of the *HGO* gene, resulting in a missense mutation (C120W), which replaces a cysteine in position 120 with a tryptophan. The remaining Dominican AKU patient is homozygous for the G270R (c.975G→A) missense mutation. G270R has been described previously as a prevalent AKU mutation in Slovakia.[11 13]

We used the recently discovered HGO crystal structure[14] to determine the consequences of the C120W and G270R mutations. C120W is expected to have a strong disruptive effect on

Abbreviations: AKU, alkaptonuria; HGO, homogentisate dioxygenase; SNP, single nucleotide polymorphism

Mutation	5′ ← *HGO* polymorphisms → 3′									Origin	Chromosome Code	Reference
	IVS3-112T/C	c.407T/A (H80G)	IVS4 + 31A/G	*HGO-3*	*HGO-1*	IVS5 + 25T/C	IVS6 + 46C/A	IVS11 + 18A/G	*HGO-2*			
C120W												
	T	A	A	191	161	T	A	A	197	Dom Rep	73a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	73b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	74a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	74b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	75a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	75b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	76a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	76b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	78a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	78b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	79a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	79b	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	80a	PR
	T	A	A	191	161	T	A	A	197	Dom Rep	80b	PR
G270R												
	T	A	A	191	163	C	A	A	189	Dom Rep	77a	PR
	T	A	A	191	163	C	A	A	189	Dom Rep	77b	PR
	C	T	A	195	161	T	C	A	187	Italy	29a	15
	C	T	A	195	161	T	C	A	187	Italy	29b	15
	T	T	A	195	161	T	C	A	181	Slovakia	52a	13
	T	T	A	195	161	T	C	A	181	Slovakia	53a	13
	T	T	A	195	161	T	C	A	181	Slovakia	59b	13
	T	T	A	195	161	T	C	A	181	Slovakia	61c	13
	T	T	A	195	161	T	C	A	181	Slovakia	66b	13
	T	T	A	195	161	T	C	A	181	Slovakia	67b	13

Figure 1 *HGO* haplotypes associated with the Dominican AKU mutations. The figure shows the allelic associations of nine *HGO* intragenic polymorphisms for each of the 16 Dominican AKU chromosomes included in this study. The *HGO* polymorphic loci ordered from 5′ to 3′ are indicated at the top. *HGO-1*, *HGO-2*, and *HGO-3* are (CA)n or (CT)n dinucleotide repeats. All other polymorphisms are diallelic SNPs. AKU chromosomes are grouped by mutation. In the G270R mutation group, we have also included the chromosomes described in Italy and Slovakia that carry the same *AKU* mutation. The position of the *AKU* mutations within the HGO haplotype is indicated with a vertical bar. The chromosomes are identified by the pedigree code number. PR, present report.

the HGO active site, whereas G270R interferes with the formation of the HGO hexamer. Therefore, both mutations have structural consequences that should impair HGO enzyme activity.

The haplotype associations between the AKU mutations and nine polymorphic sites within the *HGO* gene were established for the 16 Dominican AKU chromosomes. Fig 1 shows that in all 14 C120W chromosomes the AKU mutation is associated with identical polymorphisms and that, therefore, these seven Dominican AKU patients should share a common ancestor from whom they have inherited the AKU mutation. Moreover, since one of the seven AKU patients carrying the C120W mutation belongs to the very large AKU pedigree described in 1960,[8] it is reasonable to expect that C120W is the founding mutation common to most AKU patients in the Dominican Republic. It is interesting in this regard that the only other AKU patient previously identified carrying this mutation was a 75 year old Dominican male living in the USA.[16] However, no HGO haplotype data are available from this patient. Whether the C120W mutation originated in the Dominican Republic or was introduced there by immigrants from other geographical regions is at present unknown.

One of the eight Dominican AKU patients is homozygous for the G270R mutation. As indicated above, the G270R mutation is one of the most prevalent *AKU* mutations in Slovakia. We have previously indicated that the G270R mutation originated in Slovakia associated with a GGG mutation hot spot.[11 13] Interestingly, the Slovak and the Dominican G270R mutations are associated with two completely different *HGO* haplotypes (fig 1), illustrating that they have originated through independent mutational events.

Key points

- The aim of this study was to determine whether the high incidence of AKU in the Dominican Republic is the consequence of a founder effect or an increased mutation rate in the *HGO* gene, as seems to have been the case in Slovakia, the only other known location with a high incidence of AKU.
- We performed *HGO* mutation analysis in eight unrelated Dominican AKU patients and their relatives and characterised the 16 *HGO* haplotypes by analysing nine intragenic polymorphisms.
- AKU mutations were identified in all 16 Dominican AKU chromosomes. Seven of the eight AKU patients are homozygous for a c.527T→G substitution in exon 6 of the *HGO* gene, resulting in a missense mutation (C120W), which replaces a cysteine in position 120 with a tryptophan. The remaining Dominican AKU patient is homozygous for the G270R (c.975G→A) missense mutation.
- This study provides molecular evidence that the increased incidence of AKU in the Dominican Republic is the consequence of a classical founder effect and that C120W is the founder mutation. We have also identified a Dominican AKU patient homozygous for the G270R mutation and have shown that G270R, one of the prototype Slovak *AKU* mutations, is a recurrent mutation in the Dominican Republic. This finding reinforces our previous conclusion that the GGG sequence motif, or its reverse complement CCC, is an important mutational hot spot in the *HGO* gene.

In conclusion, *HGO* mutation analysis in 16 AKU chromosomes from eight unrelated patients from the Dominican Republic has shown two AKU mutations, C120W and G270R. C120W is the most prevalent mutation (87% of the AKU chromosomes tested) and most likely the mutation responsible for AKU in the 47 patients described in Milch's epidemiological report published in 1960.[8] These data provide molecular evidence that the increased incidence of AKU in the Dominican Republic is the consequence of a classical founder effect and that C120W is the founder mutation. The identification of a patient carrying a homozygous G270R mutation suggests the existence of additional AKU founders at specific locations within the Dominican Republic. We have shown here that G270R, one of the prototype Slovak AKU mutations, is a recurrent mutation in the Dominican Republic. This finding reinforces our previous conclusion[7] that the GGG sequence motif, or its reverse complement CCC, is an important mutational hot spot in the *HGO* gene.

ACKNOWLEDGEMENTS

We thank the families with AKU for their collaboration and donation of blood (DNA) samples. We would also like to thank L Gulliksen for her contribution to this work. This research was supported by the Fundación José Antonio de Castro, the Spanish Comisión Interministerial de Ciencia y Tecnología (SAF99/0013), and the Comunidad de Madrid (08.6/0015/1997).

Authors' affiliations

E Goicoechea de Jorge, M E Gallardo, B Pérez, S Rodríguez de Córdoba, Unidad de Patología Molecular, Fundación Jiménez Díaz, Av Reyes Católicos 2, 28040 Madrid, Spain
I Lorda, Servicio de Genética, Fundación Jiménez Díaz, Av Reyes Católicos 2, 28040 Madrid, Spain
E Goicoechea de Jorge, M A Gallardo, B Pérez, S Rodríguez de Córdoba, Departamento de Inmunología, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Velázquez 144, 28006 Madrid, Spain
C Pérez de Ferrán, H Mendoza, Centro Nacional de Investigación en Salud Materno Infantil, Hospital de Niños Robert Reid Cabral, Abraham Lincoln 2, Santo Domingo, Dominican Republic

Correspondence to: Dr S Rodríguez de Córdoba, Departamento de Inmunología. Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Velázquez 144, 28006-Madrid, Spain; SRdeCordoba@cib.csic.es

REFERENCES

1 **Garrod AE**. The incidence of alkaptonuria: a study in clinical individuality. *Lancet* 1902;**ii**:1616-20.
2 **Garrod AE**. The Croonian lectures on inborn errors of metabolism. Lecture II. Alkaptonuria. *Lancet* 1908;***ii***:73-9.
3 **La Du BN**, Zannoni VG, Laster L, Seegmiller JE. The nature of the defect in tyrosine metabolism in alkaptonuria. *J Biol Chem* 1958;**230**:251-60.
4 **Fernández-Cañón JM**, Granadino B, Beltrán-Valero de Bernabé D, Renedo M, Fernández-Ruiz E, Peñalva MA, Rodríguez de Córdoba S. The molecular basis of alkaptonuria. *Nat Genet* 1996;**14**:19-24.
5 **La Du BN**. Alkaptonuria. In: Scriver CR, Beaudet AL, Sly W, Valle D, eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill, 1995:1371-86.
6 **Beltrán-Valero de Bernabé D**, Granadino B, Chiarelli I, Porfirio B, Mayatepek E, Aquaron R, Moore MM, Festen JJ, Sanmarti R, Peñalva MA, Rodríguez de Córdoba S. Mutation and polymorphism analysis of the human homogentisate 1,2-dioxygenase gene in alkaptonuria patients. *Am J Hum Genet* 1998;**62**:776-84.
7 **Beltrán-Valero de Bernabé D**, Jiménez FJ, Aquaron R, Rodríguez de Córdoba S. Analysis of alkaptonuria (AKU) mutations and polymorphisms reveals that the CCC sequence motif is a mutational hot spot in the homogentisate 1,2 dioxygenase gene (*HGO*). *Am J Hum Genet* 1999;**64**:1316-22.
8 **Milch RA**. Studies of alkaptonuria. Inheritance of 47 cases in eight highly inter-related Dominican kindreds. *Am J Hum Genet* 1960;**12**:76-85
9 **Srsen S**, Cisarik F, Pasztor L, Harmecko L. Alkaptonuria in the Trencin district of Czechoslovakia. *Am J Med Genet* 1978;**12**:159-66
10 **Muller CR**, Fregin A, Srsen S, Srsnova K, Halliger-Keller B, Felbor U, Seemanova E, Kress W. Allelic heterogeneity of alkaptonuria in central Europe. *Eur J Hum Genet* 1999;**7**:645-51.
11 **Zatkova A**, Beltrán Valero de Bernabé D, Poláková H, Zvarík M, Feráková E, Bošák V, Ferák V, Kádasi D, Rodríguez de Córdoba S. High frequency of alkaptonuria in Slovakia. Evidence for the appearance of multiple mutations in *HGO* involving different mutational hot spots. *Am J Hum Genet* 2000;**67**:1333-9.
12 **Gehrig A**, Schmidt SR, Muller CR, Srsen S, Srsnova K, Kress W. Molecular defects in alkaptonuria. *Cytogenet Cell Genet* 1997;**76**:14-16.
13 **Zatkova A**, Polakova H, Micutkova L, Zvarik M, Bosak V, Ferakova E, Matusek J, Ferak V, Kadasi L. Novel mutations in homogentisate-1,2-dioxygenase gene identified in Slovak patients with alkaptonuria. *J Med Genet* 2000;**37**:539-42.
14 **Titus GP**, Mueller HA, Rodríguez de Córdoba S, Peñalva MA, Timm DA. Crystal structure of human homogentisate dioxygenase. *Nat Struct Biol* 2000;**7**:542-6.
15 **Porfirio B**, Chiarelli I, Graziano C, Mannoni A, Morrone A, Zammarchi E, Beltrán-Valero de Bernabé D, Rodríguez de Córdoba S. Alkaptonuria in Italy: polymorphic haplotype background, mutational profile, and description of four novel mutations in the homogentisate 1,2-dioxygenase gene. *J Med Genet* 2000;**37**:309-11
16 **Introne WJ**, Rausche M, Anikster Y, Gilbert F, Gahl WA. Alkaptonuria: new studies of an old disease. Poster presented at the 50th Annual Meeting of The American Society of Human Genetics. Philadelphia. *Am J Med Genet* 2000;**67**(S2):289.