Alkaptonuria: such a long journey

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Alkaptonuria holds an honoured place in the edifice of human genetics. Two important perceptions emerged from early studies of the disorder: first, realization that the laws of mendelian inheritance apply to *Homo sapiens*; second, that a variant chemical trait can be a manifestation of human biological individuality (Table 1). The enormous significance of this marginal disorder was first recognized by Archibald Garrod when he was laying an intellectual cornerstone in human genetics (the one for biochemical genetics); now, almost a century later, as described on page 19 in this issue, Fernandes-Cañón and colleagues have put in place the keystone to the alkaptonuria story[1].

In 1898, when he was 41, Archibald Garrod (Fig. 1) identified homogentisic acid (HGA) as the substance responsible for blackening of urine (or nappies) on prolonged exposure to air in "black urine disease". The disease was alkaptonuria, a very rare disorder, first reported in 1822, known to appear in infancy and associated in later life with discolouration of skin, sclera and cartilage (ochronosis) and with disabling arthritis[2]. Garrod showed that alkaptonuria was a congenital condition by demonstrating its occurrence in the newborn sibling of a known case. Awareness of the increased frequency of parental consanguinity in his alkaptonuria families provoked brisk correspondence with William Bateson and led the correspondents to surmise that the disorder was inherited, by rediscovered Mendel's rules, as an autosomal recessive; it was one of the first human traits to illustrate mendelian inheritance. In 1902, Garrod published his famous paper under the subdued title: "The incidence of Alkaptonuria: A study in chemical individuality"[3]. In it, the reader encounters the source of two concepts now playing important roles in the Human Genome Project and its applications: (i) inborn errors of metabolism and (ii) inborn factors in disease; both have been incorporated into our thinking as mendelian and complex (multifactorial) traits respectively.

Science is an attack on ignorance and medicine is the art of restoring and preserving health. Garrod, the model physician–scientist, used the tools of science (hypothesis, measurement, observation and experiment) to better understand why his patients had their particular disorders. Various pressures on contemporary geneticists and physicians may explain why many may not have read, or even know of, Garrod's paper despite its secure place in the history of genetics and medicine. An excellent biography is available for those interested in learning more of this extraordinary man[4].

Progress in 'forward genetics' — the classical history — of alkaptonuria was slow (Table 1). Although the step from clinical to metabolic phenotype occurred during a single decade, emerging as the concept of the "inborn error of metabolism"[5], half a century elapsed before reduced activity of the putative enzyme (homogentisate 1, 2 dioxygenase, HGO, EC 1.13.11.5) was demonstrated[6]; almost four more

Table 1 Classical and contemporary histories of alkaptonuria

	Clinical	Metabolic	Enzymic	Locus
"CLASSICAL"	(1902) Garrod observes[3]:	(1908) Trait used to develop concept of the:	(1958)	(1993/94)
	Mendelian inheritance →	"Inborn error of metabolism" (Homogentisic aciduria)[5] →	Homogentisate 1,2 dioxygenase deficiency demonstrated[6] →	Trait mapped to chromosome 3q2; Symbol, *AKU*[7,8]
	Biochemical individuality			OMIM 203500
"CONTEMPORARY"				
Fernandes-Cañón *et al.*[1]	Autosomal recessive inheritance confirmed (DNA & clinical levels) ←	Co-segregation of phenotype (HGA) with *HGO* genotype [2 pedigrees] ←	Tissue-specific expression (mRNA) ←	Gene-cloned (Symbol, *HGO*)
				Mapped to *AKU* locus (by FISH)
	Implications for treatment		*In vitro* expression of P230S shows it is a loss-of-function *HGO* mutation	*AKU* = *HGO*
				Mutations found c817C→T (P230S) c1028T→G (V300G)

decades passed before the locus (symbol *AKU*) was assigned to chromosome 3q2 by homozygosity mapping[7] (capitalizing on the consanguinity noted by Garrod) and by comparative mapping[8] (capitalizing on homology of synteny between mouse chromosome 16 and human chromosome 3q). There, the forward journey halted in 1994.

Garrod was familiar with evolutionary conservation of biological properties; Fernandes-Cañón and colleagues used that theme anew to clone the human gene mutated in alkaptonuria[1]. They started with an ascomycete fungus (*Aspergillus nidulans*), cloned and characterized a fungal gene (*hmgA*) that encodes an *HGO* enzyme, and used that information to search a human EST (expressed sequence tag) library; EST clone 77725 (1,715 bp) proved to be a human *HGO* cDNA homologue[9]. The latter was confirmed to encode the entire HGO enzyme by expressing it as a fusion protein; it was then used for the remainder of the work (Table 1).

With probes derived from the *HGO* cDNA clone, the Spanish group screened human genome libraries and found 60 kb of DNA containing the 14 exons of the *HGO* gene (details to be published elsewhere). *HGO* is a single-copy gene; in somatic cell hybrids, and with flourescence *in situ* hybridization (FISH), it mapped to human chromosome 3q21–q23 at the *AKU* locus.

Northern blot hybridization with EST-77725 revealed strong tissue-specific expression of *HGO* (mRNA, 1.8 kb) in liver and kidney as expected; but also in prostate where deposits of HGA can occur in affected patients[2]. Loss-of-function mutations are a key to finding candidate genes; the human *HGO* gene contains at least one, probably many more. One of them, in exon 10, Pro230Ser, involves a residue conserved in human and fungal HGO proteins; another, Val300Gly in exon 12, also involves a conserved residue. Alkaptonuric individuals in the Spanish pedigrees all carried two copies of a mutant allele; asymptomatic obligate carriers had only one copy. A survey of over 100 normal chromosomes revealed neither mutation. Accordingly, Garrod's hypothesis that alkaptonuria is a rare autosomal recessive trait has been confirmed at the molecular level.

Association of a mutant allele with the variant phenotype is not direct proof that the former causes the latter. The Spanish team performed replicate expression analyses of the mutation in *E. coli*, as a Pro230Ser fusion protein; relative to wild-type expres-sion, the mutant protein had no HGO enzyme activity. *In vitro* unit-protein analysis of mutant HGO is thus concordant with the *in vivo* variant phenotype associated with a mutant *AKU* genotype. Wherever it is, the spirit of Garrod is probably smiling.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Fig. 1 Archibald E. Garrod in 1899, the year he reported a method to detect homogentisic acid in "black urine disease" (alkaptonuria). (Taken from ref. 4, with permission.)

Why such a long journey? Perhaps because Garrod's insights were premature[4] and, for lack of tools, they could not have been articulated, as has been done by the Spanish team. History is all about questions put to the past that resonate in the present. From the past, the resonance of alkaptonuria is instructive because it helps us to remember that not everything in human genetics is happening today even though our tools are potent. In the present, alkaptonuria validates the Human Genome Project; and while noting that the mutant allele (symbol a) is almost always plural ($a_1 + a_2 + a_3 \ldots a_n$), at any locus, it also validates the Human Genetic Diversity Project, where locus-specific rare disease-producing alleles can be powerful markers of affinities and origins of human populations. Even better when knowledge of the allelic variation improves counselling and treatment of the disease; Fernandez-Cañón and colleagues have something to say about this heretofore unresolved aspect of alkaptonuria.

The Spanish team write fluent, lucid English — the language of science today. Garrod also wrote in English, although in his day German was the popular language of science. *Sic transit gloria mundi* — So passes away earthly glory. □

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