Letters to the Editor

Am. J. Hum. Genet. 69:235–237, 2001

Variant 1859G→A (Arg620Gln) of the "Hairless" Gene: Absence of Association with Papular Atrichia or Androgenetic Alopecia

To the Editor:

Recent studies have shown that mutations in the human "hairless" gene (*HR*) are responsible for complete, congenital absence of hair in families with autosomal recessive universal congenital alopecia (ALUNC [MIM 203655]) and autosomal recessive papular atrichia (APL [MIM 209500]) (for review, see Kruse et al. 1999). In the October 1998 issue of the Journal, Ahmad et al. (1998) reported that a missense mutation (1859G→A, which results in Arg620Gln) in the zinc-finger domain caused papular atrichia among members of a family of Irish descent. Evidence that Gln620 represents a disease-causing mutation was based on the following four observations (Ahmad et al. 1998): (1) Gln620 was found in a homozygous state only in affected family members, (2) Gln620 was not observed in 50 unaffected control individuals, (3) Arg620 is located in the zinc-finger domain of *HR*, and (4) Arg620 is conserved among human, mouse, and rat genes.

We recently conducted a mutation screen of *HR* in 46 individuals with androgenetic alopecia (AGA) (A.M. Hillmer, R. Kruse, F. Macciardi, U. Heyn, R.C. Betz, T. Ruzicka, P. Propping, M.M. Nöthen, S. Cichon, unpublished data). Unexpectedly, we detected one individual who was heterozygous for Arg620Gln. Because this observation raised the possibility that Arg620Gln may be a polymorphism and not a disease-causing mutation, we tested for the presence of Arg620Gln in a further 820 individuals of various ethnic origins: 601 from Germany (comprising 288 individuals recruited among laboratory staff and students, 198 anonymous blood donors, 57 individuals with AGA, and 58 control individuals without AGA), 79 from Japan, 65 from Oman, 49 from Mexico, and 26 from Pakistan. In all individuals, exon 6 of *HR* was amplified from genomic DNA, using a nested PCR approach. In a first PCR, primers Ex6MutF1 (5′-GGA GGT GGA GGA AAG AAT GTG C-3′) and Ex6MutR1 (5′-CTG CAG AGA GGG GAA GTC TGC T-3′) were used to generate a 686-bp PCR product comprising exon 6 and flanking intronic sequences. To enhance the specificity and yield of the reaction, 1 μl of the PCR product was used for a second PCR, with primers Ex6MutF2 (5′-ATG GAA GCT GCT CCT TGC TTC-3′) and Ex6MutR2 (5′-GTA GGG GGC TTT TTG GGG AG-3′), which gave a 380-bp PCR product. Tests for the presence of the mutation were conducted after digestion with restriction endonuclease *Pvu*II, as described by Ahmad et al. (1998).

Among 820 individuals, we identified 44 who were heterozygous for Arg620Gln and 1 who was homozygous for Gln620. These alleles were distributed among the ethnic groups as follows: among Germans, allele Gln620 was found in 33 individuals in a heterozygous state and in 1 individual (ANA 87-01) in a homozygous state, resulting in a Gln620 allele frequency of 2.7%; heterozygosity for Gln620 was detected in 5 individuals from Oman, 3 from Pakistan, and 3 from Mexico, resulting in allele frequencies of 3.8%, 5.8%, and 3.1%, respectively. Gln620 was not detected among the 79 individuals from Japan.

Homozygosity for Gln620 in individual ANA 87-01 was verified by sequence analysis (fig. 1*A*) and *Pvu*II digestion (fig. 1*B*) of a 119-bp PCR-fragment directly amplified from genomic DNA with primers HREx6internF (5′-CCA CCA TGG ACT CTT CAA CA-3′) and HREx6internR (5′-CCC CTC TCC TAC CTG CTT T-3′). In addition, to check for Mendelian inheritance, we investigated DNA samples of the parents and brother of ANA 87-01. Both parents (ANA 87-02 and ANA 87-03) are heterozygous (Arg620/Gln620), and the brother (87-04) is homozygous for allele Arg620 (fig. 1*B*). To exclude allele-specific amplification of the exon 6–bearing allele Gln620, the binding sites of primers HREx6internF, Ex6MutF1, Ex6MutF2, Ex6MutR1, Ex6MutR2, and HREx6internR were sequenced in ANA 87-01. We observed no mutation in the primer binding sites. Furthermore, we genotyped seven microsatellite markers (D8S550, LPL, D8S258, D8S280, D8S282, D8S283, and D8S285) flanking *HR* in family ANA 87. The parents had four distinguishable haplotypes. ANA 87-01 showed two different haplotypes that were distinct from the two haplotypes of his brother ANA 87-04. These results confirm the transmission of two parental Gln620 alleles to ANA 87-01 and two Arg620 alleles to ANA 87-04.

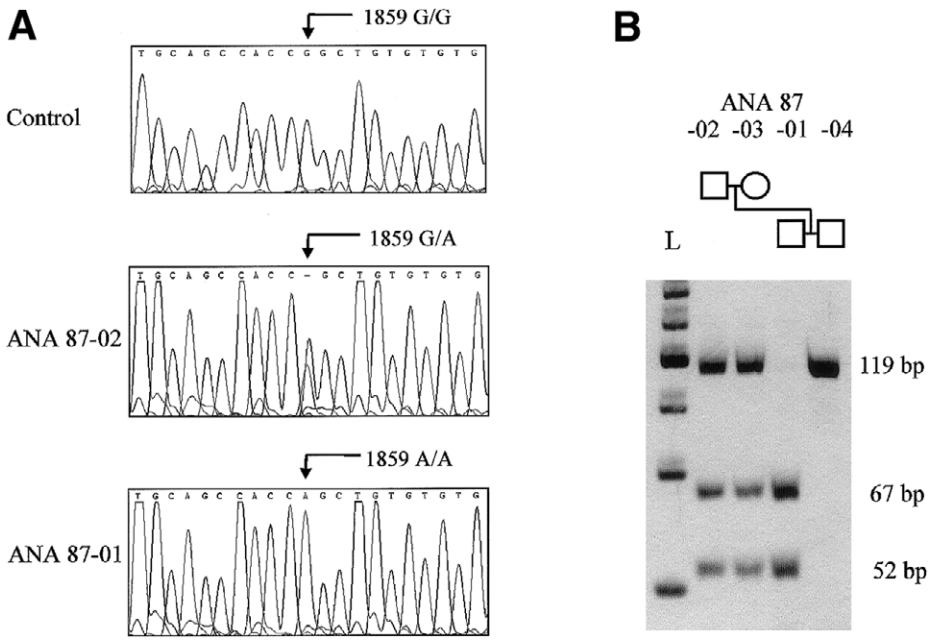

Figure 1 Genotyping of the 1859G→A (Arg620Gln) variant in exon 6 of *HR*. *A,* Sequence analysis of exon 6 of a control individual (*top*) with the genotype 1859G/G, individual ANA 87-02 (*middle*) with the genotype 1859G/A, and individual ANA 87-01 (*bottom*) with the genotype 1859A/A. *B, Pvu*II RFLP analysis of a 119-bp PCR fragment that contains exon 6 of *HR* (including nucleotide position 1859) in four members of family ANA 87. Fragments were separated on a 10% polyacrylamide gel and visualized by silver staining. Allele 1859G remains uncut (119-bp fragment), and allele 1859A produces a 67-bp and a 52-bp fragment. ANA 87-01 is homozygous for allele 1859A, and the brother, ANA 87-04, is homozygous for allele 1859G. The parents, ANA 87-02 and ANA 87-03, are heterozygous for 1859G/A. *Lane L,* 25-bp ladder (Gibco).

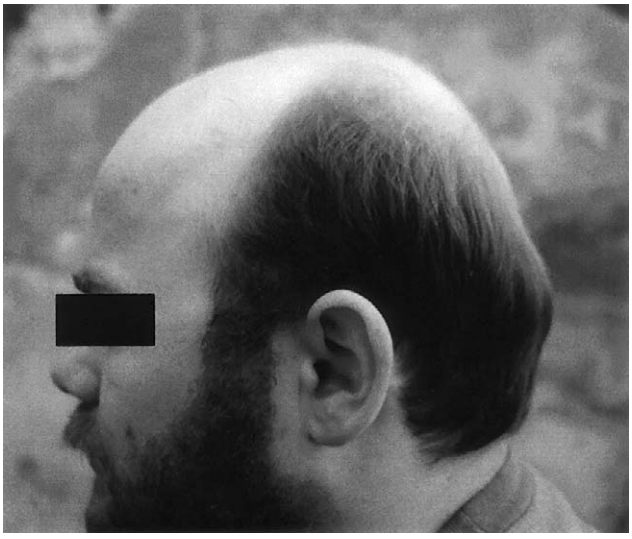

Figure 2 Phenotypic appearance of individual ANA 87-01, who is homozygous for 1859A (Gln620) of *HR*. He displays androgenetic alopecia but is clearly not affected with papular atrichia. The hair of the scalp and beard (as well as the rest of the body hair) is normally developed, and there are no signs of papular rash.

For the two reasons, our results lead to the conclusion that Arg620Gln is indeed not a disease-causing mutation but a polymorphism without consequences for hair development. (1) Given a Gln620 allele frequency of 2.7%, one would expect almost 60,000 individuals affected with APL in Germany; however, no German has yet been reported to be affected with this disease. (2) We identified an individual who is homozygous for Gln620 but, unambiguously, is not affected with papular atrichia. Male-pattern loss of scalp hair started at the age of 18–20 years. He developed severe androgenetic alopecia (Norwood-Hamilton grade VI) by the age of 26 years. Figure 2 shows the individual at the age of 33 years. The rest of the scalp hair, eyebrows, and beard hair, as well as pubic, axillary, and body hair developed normally and remained completely normal. There are no signs of a papular rash.

In the family of Irish Travellers reported by Ahmad et al. (1998), Arg620Gln cosegregates with papular atrichia. In the light of our findings, however, it must be concluded that it is a polymorphism in close linkage with a yet unidentified disease-causing mutation at the *HR* locus in the reported family. An alternative explanation

of the discrepancy between results of our study and those of the report by Ahmad et al. (1998) would be the existence of modifier genes. However, this is very unlikely, given the fact that none of the reported molecular studies of families with APL has observed a case of incomplete penetrance.

Interestingly, although the individual who is homozygous for Gln620 is not affected with papular atrichia, he displays AGA. We therefore examined the frequency of Gln620 in a sample of 103 males with severe AGA and in 58 males (age > 60 years) without AGA. Allele frequencies were similar in both groups (2.91% and 3.45%, respectively; $P = .79$), which suggests that Arg620Gln does not play a role in the development of AGA.

Acknowledgments

We thank all individuals who consented to the study and gave blood samples. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Forschergruppe "Keratinozyten-Proliferation und differenzierte Leistung in der Epidermis" and Forschergruppe "Genetische Epidemiologie und Medizinische Genetik komplexer Erkrankungen"). R.C.B. is a research fellow from the Deutsche Forschungsgemeinschaft.

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ALUNC [MIM 203655] and APL [MIM 209500])

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0002-9297/2001/6901-0026$02.00

Am. J. Hum. Genet. 69:237–241, 2001

The Presence of Mitochondrial Haplogroup X in Altaians from South Siberia

To the Editor:

For American Indians, extensive RFLP and HVSI sequence analysis has unambiguously identified four major founding mtDNA haplogroups (A, B, C, D), which account together for ~97% of modern American Indian mtDNAs (Wallace 1995). Examination of the distribution of the four founding lineage haplotypes (A, B, C, and D) in American Indian populations (both contemporary and ancient) shows that all four lineages were present in the New World prior to European contact (Wallace 1995; Lalueza et al. 1997; Stone and Stoneking 1998), thus indicating that all American Indian mtDNAs are apparently descended from these four founding lineages. mtDNAs apparently not from haplogroups A–D may result from recent admixture with non–American Indians or may represent additional American Indian founding mtDNA lineages.

A striking example of the presence in American Indians of genotypes not from haplogroups A–D is haplogroup X. This haplogroup represents a minor founding lineage that is restricted in distribution to northern Amerindian groups, including the Ojibwa, the Nuu-Chah-Nulth, the Sioux, and the Yakima, as well as the Na Dene–speaking Navajo (Brown et al. 1998). Unlike haplogroups A–D, haplogroup X is also found at low frequencies of ~4% in western Eurasian populations. Despite a shared consensus RFLP haplotype, substantial genetic differences exist between the American Indian and European haplogroup X mtDNAs. Phylogenetic analysis and coalescence estimates for American Indian and European haplogroup X mtDNAs exclude the possibility that the occurrence of haplogroup X in American Indians is due to recent European admixture. They also clearly indicate that the two branches/subgroups are distantly related to each other and that considerable genetic substructure exists within both groups (Brown et al. 1998).

Haplogroup X is remarkable in that it has not been found in Asians, including Siberians, suggesting that it