

## To the editor:

## Molecular characterization of a third case of human atransferrinemia

Atransferrinemia/hypotransferrinemia is an unusual disorder generally inherited in an autosomal recessive pattern.<sup>1,2</sup> The hypochromic, microcytic anemia and hemochromatotic siderosis seen in atransferrinemia/hypotransferrinemia are of interest, as they provide clues to mechanisms and tissue distributions of iron-handling pathways that do not involve transferrin.<sup>1</sup> A spontaneously arisen form of atransferrinemia in the mouse has been traced to a splice-site mutation in *Tf*,<sup>3</sup> the orthologue of *TF*, which encodes transferrin in humans. The molecular-genetic basis of atransferrinemia/hypotransferrinemia has been previously determined in only 2 human instances. One proband, a woman, was found to be a compound heterozygote for mutations in *TF*. These were 1429G>C (A477P) and 562\_571del 572\_580dup, resulting in a stop codon 27 amino acids downstream.<sup>1</sup> (Several different nomenclatures have been used to designate transferrin mutations and polymorphisms. We use the recommended nomenclature,<sup>4,5</sup> in which the A of the upstream ATG is counted as nucleotide number 1 and the initiator methionine is amino acid number 1.) Another, a boy,<sup>6-8</sup> was a compound heterozygote for 1180G>A (E394K) and an inferred null allele not traced to mutation in either coding region or intron-exon junctions of *TF*.<sup>8</sup>

The second instance of atransferrinemia identified, described in 1968 and now analyzed at the molecular level, occurred in a Slovak girl,<sup>9-12</sup> at present 34 years old. She came to medical attention at age 2 months with severe hypochromic, microcytic anemia; her healthy parents denied consanguinity. Atransferrinemia was diagnosed using serum electrophoresis. Serum transferrin concentrations in her parents, a brother, and a grandfather were approximately half normal values.<sup>9,11</sup> Periodic infusions of purified transferrin led to improved erythropoiesis, although complications ascribed to siderosis developed.<sup>10-12</sup> Blood from the girl, her parents, and her healthy brothers was used to establish lymphoblastoid lines (National Institute of General Medical Sciences [NIGMS] Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ; GM10911-14 and GM10929).

All 17 exons of *TF*, with intron-exon junctions, were sequenced from cell-line genomic DNA extracted by standard techniques. Primers and sequencing conditions are given elsewhere.<sup>1</sup> A missense mutation (229G>A) in exon 3 predicted to result in a nonconservative amino acid change (D77N) was found in homozygous form (229A/A) in the proband (GM10914). The mutation segregated with disease status in parents (both heterozygous [229G/A]) and brothers (one heterozygous, one [GM10911] wild-type [229G/G]). The mutation was not found in 48 chromosomes from other subjects. The known polymorphisms -84G>G, -2G>G, IVS4 + 110T>T, IVS4-60T>T, 739T>T (L247L), IVS8 + 61C>C, and IVS10-23C>C also were identified in the proband.

The proband came from a town of 7000 inhabitants in a relatively isolated region of West Slovakia. Given homozygosity in the proband for the 229G>A (D77N) mutation and for all

markers (data not shown), it may safely be presumed that parental consanguinity was present, albeit unknown, rather than that the mutation arose independently twice.

These findings establish the molecular-genetic etiology of a clinically severe form of atransferrinemia. The previously reported female patient<sup>1</sup> came to medical attention, for anemia, at the age of only 20 years. The previously reported boy<sup>6-8</sup> was first evaluated at the age of only 7 years, for pallor of new onset. Substitution of a polar asparagine residue for a negatively charged aspartate residue in the region of the human transferrin molecule encoded by exon 3 of *TF* appears largely to have abrogated synthesis or secretion of functional transferrin in our patient, whose disease was severe in early infancy. Molecular characterization of other instances of atransferrinemia may permit further correlations between mutation and clinical manifestations.

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## References

1. Beutler E, Gelbart T, Lee P, Trevino R, Fernandez MA, Fairbanks VF. Molecular characterization of a case of atransferrinemia. *Blood*. 2000;96:4071-4074.
2. Stavem P, Saltvedt E, Eljjo K, Rootwelt K. Congenital hypochromic microcytic anaemia with iron overload of the liver and hyperferritaemia. *Scand J Haematol*. 1973;10:153-160.
3. Trenor CC, Campagna DR, Sellers VM, Andrews NC, Fleming MD. The molecular defect in hypotransferrinemic mice. *Blood*. 2000;96:1113-1118.
4. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations: Nomenclature Working Group. *Hum Mutat*. 1998;11:1-3.
5. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat*. 2000;15:7-12.
6. Goya N, Miyazaki S, Kodate S, Ushio B. A family of congenital atransferrinemia. *Blood*. 1972;40:239-245.
7. Hayashi A, Wada Y, Suzuki T, Shimizu A. Studies on familial hypotransferrinemia: unique clinical course and molecular pathology. *Am J Hum Genet*. 1993;53:201-213.
8. Asada-Senju M, Maeda T, Sakata T, Hayashi A, Suzuki T. Molecular analysis of the transferrin gene in a patient with hereditary hypotransferrinemia. *J Hum Genet*. 2002;47:355-359.
9. Čáp J, Lehotská V, Mayerová A. Kongenitálna atransferrinémia u 11-mesačného dieťaťa. *Cesk Pediatr*. 1968;23:1020-1025.
10. Schwick HG, Čáp J, Goya N. Therapy of atransferrinemia with transferrin [abstract]. *J Clin Chem Clin Biochem*. 1977;16:73.
11. Hromec A, Čáp J, Schwick HG. Kongenitale Atransferrinämie: Klinische Beobachtungen während einer 18jährigen Substitution mit Humantransferrin. *Pädiatr Praxis*. 1989;38:633-637.
12. Hromec A, Payer J Jr, Killinger Z, Rybár I, Rovensky J. Kongenitale Atransferrinämie. *Dtsch Med Wochenschr*. 1994;119:663-666.