Evidence that the RHDVI deletion genotype does not exist (letter)

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Congenital Haptoglobin Deficiency

To the Editor:

Haptoglobin is a dimeric glycoprotein comprising two β chains that bind to hemoglobin α dimers and two α chains.[1,2] In patients with active hemolysis, the hemoglobin escaping into the plasma is bound to the free haptoglobin and the haptoglobin-hemoglobin complex is cleared from the plasma with a T1/2 of 10 to 30 minutes.[3] Free haptoglobin, in contrast to the haptoglobin-hemoglobin complex, has a T1/2 of 5 days; hence, the depleted plasma levels in patients with active hemolysis and the clinical practice of measurement of plasma haptoglobin as a marker for hemolysis.[4]

This report describes two families with congenital deficiency of haptoglobin—an entity that has received little recognition in the hematology literature.

Report of Cases. A 47-year-old woman (Anglo-Saxon) presented in December 1990 with severe anemia and was found to have autoimmune hemolysis due to anti-e antibodies. Despite successful treatment with corticosteroids (normalization of hemoglobin and reticulocyte count and a negative antiglobulin test), her haptoglobin (measured by rate nephlometry [Beckman Instruments Inc]) remained very low, at less than 0.4 g/L (normal range, 1 to 3.8), for more than four years. The isolated nature of this abnormality prompted me to proceed with family studies. The family studies showed similar low haptoglobin levels in one of her brothers 51 years of age and her daughter 31 years of age; both were clinically well. Three other family members (a brother 55 years of age and 2 sons 28 and 20 years of age, respectively) had normal levels.

A 15-year-old (Greek) girl with Gilbert's syndrome (mild, hereditary glucuronyl transferase deficiency) was found to have very low serum haptoglobin level at less than 0.4 g/L (normal range, 1 to 3.8) without any other clinical or laboratory evidence of hemolysis. Family studies showed a similar low haptoglobin level in her brother 6 years of age, but the levels were normal in both parents and in her 17-year-old sister.

Reports of congenital deficiency of haptoglobin are rare.[5-7] Two of these reports[5,6] have highlighted the association with familial epilepsy, with the latter being attributed to encephalic inflammation secondary to oxidation of brain lipids by the free interstitial hemoglobin. One report[7] has documented a high incidence of haptoglobin deficiency amongst patients with respiratory allergies; this association was attributed to an increased prostaglandin synthesis resulting from the haptoglobin deficiency, with haptoglobin being a prostaglandin synthesis inhibitor. The haptoglobin-deficient subjects described in the present report did not manifest either of these associations.

Haptoglobin is genetically determined by two autosomal codominant allelic genes, Hp 1 and Hp 2, with three possible phenotypes Hp 1-1, Hp 2-1, and Hp 2-2.[6,7] The low levels of haptoglobin in three members from two generations in the first family suggests an autosomal dominant type of inheritance, but the negative/normal results in the parents of the second family do not support this. It is interesting to note the coexistence of Gilbert's disease—a congenital deficiency state of uncertain inheritance pattern—in the second family. Further studies are required to clarify the mode of inheritance and the incidence of congenital haptoglobin deficiency state.

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REFERENCES

1. Smithies O, Connell GE, Dixon GH: Gene action in the human haptoglobins. I. Dissociation into constituent polypeptide chains. J Mol Biol 21:213, 1966

2. Nigel RL, Gibson QH: The binding of hemoglobin to haptoglobin and its relation to subunit dissociation of hemoglobin. J Biol Chem 246:69, 1971

3. Garby L, Noyes WD: Studies on hemoglobin metabolism. I. The kinetic properties of the plasma hemoglobin pool in normal man. J Clin Invest 38:1479, 1959

4. Erslev AJ, Beutler E: Production and destruction of erythrocytes, in Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds): Williams Hematology, New York, NY, McGraw-Hill, 1995, p 438

5. Panter SS, Sadrzadeh SMH, Hallaway PE, Haines JL, Anderson VE, Eaton JW: Hypohaptoglobinemia associated with familial epilepsy. J Exp Med 161:748, 1985

6. Haines JL, Panter SS, Rich SS, Eaton JW, Tsai MY, Anderson VE: Reduced plasma haptoglobin and urinary taurine in familial seizures identified through the multisib strategy. Am J Med Gen 24:723, 1986

7. Piessens MF, Marien G, Stevens E: Decreased haptoglobin levels in respiratory allergy. Clin Allergy 14:287, 1984

Evidence That the *RHD*^{VI} Deletion Genotype Does Not Exist

To the Editor:

The Rhesus (RH) blood group system, one of the most complex polymorphic systems in humans, encompasses at least 45 antigens. These antigens are carried by at least two red blood cell membrane proteins that are encoded by two homologous genes, *RHD* and *RHCE*.[1] The RHD antigen is a mosaic structure of at least 37 epitopes. Rearrangements of the *RHD* gene with the *RHCE* gene or point mutations in the *RHD* gene result in the loss of one or more D epitopes. Individuals with partial D phenotypes can produce antibodies to the missing epitopes in response to transfusion with D-positive blood or by pregnancy with a D-positive fetus. Until now, the following partial D phenotypes have been described: D^{II}, D^{IIIa}, D^{IIIb}, D^{IIIc}, D^{IVa}, D^{IVb}, D^{Va}, D^{VI}, D^{VII}, D^{DFR}, D^{DBT}, D^{DNU}, D^{HMi}, D^{HMii}, and R_o^{Har}.[2]

Of the partial D^{VI} phenotype, the partial D category that is most frequently leading to alloimmunization, two genotypes have been described[3]: the conversion type and the deletion type. In the conversion type, exons 4, 5, and 6 of the *RHD* gene are replaced by *RHCE* equivalents occurring in individuals of the D^{VI} Ccee phenotype. Individuals of the D^{VI}ccEe phenotype were originally described as belonging to the deletion type in which exons 4, 5, and 6 of the *RHD* gene are lost. Recent evidence suggests that these two types can also be distinguished at the serologic level using anti-BARC serum[4] or several monoclonal antibodies (IgG MoAbs NOI, SAL17-

4E8, BRAD-3 and IgM MoAb CA27-4C5, all distributed via the Third International Workshop on Monoclonal Antibodies[5]).

In contrast to our previous results,[3] we show now that exon replacement is involved in both D^{VI} genotypes. In the present study, 12 individuals of the D^{VI} phenotype were analyzed: 9 individuals of the D^{VI}Ccee phenotype and 3 individuals of the D^{VI}ccEe phenotype, including the 2 individuals (307 and DEL) who were described as having the deletion type of D^{VI}.[3]

Standard *RHD* genotyping[6] suggested the absence of *RHD* exon 4 and *RHD* intron 4 and the presence of *RHD* exon 7 and *RHD* 3′-noncoding region in all D^{VI} individuals. In addition, six exon-specific primer sets amplifying *RHD* exons 3, 4, 5, 6, 7, and 9 were developed. From DNA of 3 individuals of the $D^{VI}Ce$ haplotype, *RHD* exons 3, 7, and 9 could be amplified, whereas exons 4, 5, and 6 could not. This is in agreement with the D^{VI} exon 4/5/6 conversion genotype. From the DNA of 2 individuals of the D^{VI}cE haplotype, *RHD* exons 3, 6, 7, and 9 could be amplified, whereas exons 4 and 5 could not. Furthermore, in polymerase chain reaction (PCR) experiments that were performed on the reticulocyte transcripts from the D^{VI} variants previously described,[3] we amplified hybrid D(exon 3)-CE(exon 4) fragments from both D^{VI}ccEe and D^{VI}Ccee samples and a hybrid CE(exon 5)-D(exon 6) fragment only from the D^{VI}ccEe sample (Fig 1). From genomic DNA, a hybrid CE(exon 6)-D(exon 7) fragment could only be amplified from D^{VI}Ccee samples.[7] As expected, no amplification product was obtained with control dccee and DccEE samples.

Together with the sequence analysis of full-length *RHD* cDNA of individual 307, previously described as having the D^{VI} deletion genotype,[3] these results showed replacement of *RHD* exons 4 and 5 by their equivalent exons from the *RHCE* gene and not the deletion of exons 4, 5, and 6 in variants with the $D^{VI}cE$ haplotype.

The different conclusions from our previous published results could be explained by (1) the comigration of the rearranged CE(exon 4-5)-D(exon 6) *Bam*HI genomic fragment with the restriction fragment of 5.3 kb carrying exons 4, 5, and 6 of the *RHCE* gene, which resulted in a higher intensity of this band in the D^{VI}ccEe (DEL and 307) as compared with the other D^{VI}Ccee samples and D controls; and (2) the fact that the original sequence analysis of the D^{VI} DEL transcripts was most likely performed on a splice variant of the Rh transcripts lacking exon 4-5-6.

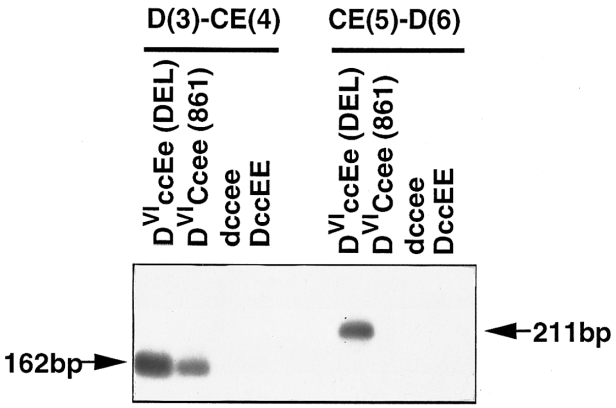

Fig 1. Hybrid D-CE and CE-D PCR of reticulocyte transcripts from D^{VI} phenotype individuals. cDNAs derived from reticulocytes of donors with the indicated phenotypes were amplified between two sets of primers in which one oligonucleotide is specific of the *RHD* gene and the other one of the *RHCE* gene. Set 1: 5′TTTGTCGGTGCTGATCTCAGTGGA3′ (*RHD,* exon 3); 5′GAACACGTAGAAGTGCCTCAG3′ (*RHCE,* exon 4). Set 2: 5′GGATGTTCTGGCCAAGTG3′ (*RHCE,* exon 5); 5′AGGTACTTGGCTCCCCCGGAC3′ (*RHD,* exon 6). PCR products were resolved by electrophoresis on 2.5% agarose gel and characterized by hybridization with the Rh cDNA probe.

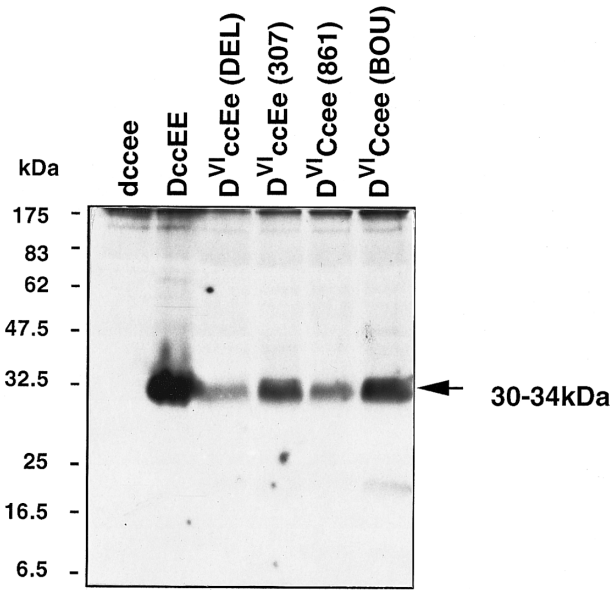

Fig 2. Western blot analysis of RhD proteins from red blood cells of different D^{VI} and common Rh phenotypes. Total membrane proteins from red blood cells of different D^{VI} (D^{VI} ccEe and D^{VI} Ccee) and common (dccee and DccEE) Rh phenotypes were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5%) in nonreducing conditions, transferred to a nitrocellulose membrane (0.45 mm; Schleicher & Schuell, Dassel, Germany), and immunostained with LOR-15C9 monoclonal antibody.[8] Immunoblots were finally stained with antihuman IgG peroxidase-tagged antibodies (Biosys, Compiegne, France) and the peroxidase activity was shown by the ECL chemoluminescence system from Amersham (Bucks, UK).

Western blot analysis performed with a monoclonal antibody recognizing a nonconformation epitope of the RhD antigen[8] showed a 30- to 34-kD RhD polypeptide in the red blood cell membrane of D^{VI}ccEe (DEL and 307) and D^{VI}Ccee (861 and BOU) variants, as in control DccEE samples (Fig 2). These results provided the definitive proof that the variant phenotypes of the D^{VI}ccEe samples previously investigated[3] did not result from the expression of a deleted isoform of the RhD polypeptide.

In conclusion, our results show that the D^{VI} deletion genotype does not exist. In the conversion type described before, exons 4, 5, and 6 of the *RHD* gene are replaced by *RHCE* equivalents occurring in individuals of the D^{VI}Ccee phenotype. Individuals of the D^{VI}ccEe phenotype have the newly described conversion type in which *RHD* exons 4 and 5 are replaced by *RHCE* equivalents. Our results confirm the recent analysis of unrelated D^{VI}ccEe samples performed by Avent et al[9] and Huang.[10] Based on the serologic heterogeneity among D^{VI} variants, it may be possible that, in the future, more rare D^{VI} genotypes will be described.

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REFERENCES

1. Smythe JS, Avent ND, Judson PA, Parsons SF, Martin PG, Anstee DJ: Expression of *RHD* and *RHCE* gene products using retroviral transduction of K562 cells establishes the molecular basis of Rh blood group antigens. Blood 87:2968, 1996

2. Cartron JP, Rouillac C, Le Van Kim C, Mouro I, Colin Y: Tentative model for the mapping of D epitopes on the RhD polypeptide. Transfus Clin Biol 6:497, 1996

3. Mouro I, Le Van Kim C, van Rhenen DJ, Le Pennec PY, Bailly P, Cartron JP, Colin Y: Rearrangements of the blood group RhD gene associated with the D^{VI} category phenotype. Blood 83:1129, 1994

4. Daniels G: Human Blood Groups. Oxford, UK, Blackwell Science, 1995

5. van Rhenen DJ, Vermeij J, Ligthart P, Overbeeke MAM: Serological differences between partial D VI of the deletion type and partial D VI of the conversion type. Transfus Clin Biol 3:17s, 1996

6. Simsek S, Faas BHW, Bleeker PMM, Overbeeke MAM, Cuijpers HThM, van der Schoot CE, von dem Borne AEGKr: Rapid Rh *D* genotyping by polymerase chain reaction-based amplification of DNA. Blood 85:2975, 1996

7. Maaskant-van Wijk PA, Faas BHW, Beckers EAM, Wildoer P, Ligthart PC, Overbeeke MAM, von dem Borne AEGKr, van Rhenen DJ, van der Schoot CE: PCR-based genotyping of partial D category VI conversion type. Transfus Clin Biol 3:32s, 1996

8. Apoil PA, Reid ME, Halverson G, Mouro I, Colin Y, Roubinet F, Cartron JP, Blancher A: A human monoclonal anti-D antibody detecting by immunoblotting a non conformation dependent epitope on the RhD protein. Br J Haematol (submitted)

9. Avent ND, Liu W, Jones JW, Scott ML, Voak D, Pisacka M, Watt J, Fletcher A: Molecular analysis of Rh transcripts and polypeptides from individuals expressing the D^{VI} variant phenotype: An *RHD* gene deletion event does not generate all D^{VI}ccEe phenotypes. Blood 89:1779, 1997

10. Huang CH: Human D^{VI} category erythrocytes: Correlation of the phenotype with a novel hybrid RhD-CE-D gene but not an internally deleted RhD gene. Blood 89:1834, 1997

The A20210 Allele of the Prothrombin Gene Is Not Frequently Associated With the Factor V Arg 506 to Gln Mutation in Thrombophilic Families

To the Editor:

A genetic variation in the 3′-untranslated region of the prothrombin gene was recently linked to an increased risk for venous thrombosis. In 28 families selected for hereditary thrombophilia, 5 (18%) of the probands carried the G to A transition at nucleotide 20210 of the factor II gene, whereas the A20210 allele was found in 1% of 100 healthy subjects.[1] Two (40%) of the A20210 carriers also had the factor V (FV) Arg 506 to Gln mutation. This prompted us to look for an association of the two risk alleles in 26 families carrying the FV mutation in two French centers. As recently underlined,[2] the selection of such families is based on the severity of clinical expression that motivates the laboratory background. It is thus concevable that several genetic risk factors might account for the expression of the thrombotic phenotype.

We screened 288 subjects belonging to the 26 families; 151 carried the FV Arg 506 to Gln mutation and 66 had had thromboses. The G to A transition at position 20210 was identified after amplification with primer A (5′-TTACAAGCCTGATGAAGGGA-3′) and primer B (5′-CCATGAATAGCACTGGGAGCATTGAAGC-3′). The latter was designed with a C to A substitution at position 20214 to create a restriction site for *Hin*dIII when the G to A transition is present. None of the probands or family members had the prothrombin gene mutation. We also screened 400 apparently healthy subjects and found the mutation in 2.8% of them. The frequency in the normal population was therefore comparable to that found in the Dutch population (1% in healthy subjects and 2.3% in a population-based case/control study). We conclude that the newly identified prothrombin gene mutation does not frequently contribute to thrombosis in individuals with the FV Arg 506 to Gln mutation.

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REFERENCES

1. Poort RS, Rosendaal FR, Reitsma PH, Bertina RM: A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3699, 1996

2. Lensen RPM, Rosendaal FR, Koster T, Allaart CF, de Ronde H, Vandenbroucke JP, Reitsma PH, Bertina RM: Apparent different thrombotic tendency in patients with factor V Leiden and protein C deficiency due to selection of patients. Blood 88:4205, 1996