Letters to the Editor

Am. J. Hum. Genet. 66:1721–1722, 2000

Complement Factor H Gene Mutation Associated with Autosomal Recessive Atypical Hemolytic Uremic Syndrome

To the Editor:

We congratulate Ying et al. (1999) on their study demonstrating failure of secretion of factor H in a family with autosomal recessive hemolytic uremic syndrome (HUS) (MIM 235400). Their study indicates factor H (HF) involvement in the recessive form of the disease as well as the autosomal dominant form (Warwicker et al. 1998). HF is a member of the regulators of complement activity gene cluster that includes four factor H–related proteins (FHR1-4) (Zipfel and Skerka 1994). All secreted proteins encoded by this gene family are organized into repetitive elements known as short consensus repeats (SCRs) or complement control protein modules, and, generally, each SCR is encoded by one exon. We have found it difficult to design HF exon 20 specific primers because of the sequence similarity between HF exons 18–20 and FHR1 exons 3–5. We have now designed an intronic forward primer and a reverse primer in the 3′ untranslated region and have confirmed their specificity by sequencing PCR products.

Ying et al. state that they have identified a point mutation in HF exon 20, the last coding exon of the gene. We were also sent DNA samples from two affected individuals from this Bedouin pedigree and found them to be homozygous for microsatellites spanning HF. We amplified exon 20 by means of the primers shown in figure 1. Electrophoresis of the product through a 2% agarose gel indicated a single smaller product, and sequencing revealed a T substitution and a 24-bp deletion. The effect of this is to replace the last seven amino acids of the protein with three different amino acids, as shown in figure 2. Crucially, this deletes the final cysteine residue. Each SCR has four cysteine residues at conserved positions that form two disulphide bridges essential for protein folding (Janatova et al. 1989). Mutations that disrupted these disulphide bridges led to a profound secretion block in a child with HF deficiency and membranoproliferative glomerulonephritis (Ault et al. 1997; Schmidt et al. 1999.

The change reported by Ying et al. is present in FHR1 exon 5, although these investigators did not observe the second nucleotide substitution in FHR1 exon 5. The forward and reverse primers used by Ying et al. are complementary to both HF and FHR1 (fig. 1). The reverse primer overlaps the deletion in the Bedouin family (fig. 1) and, hence, these primers will amplify FHR1 exon 5 but not HF exon 20 in the affected individuals.

In the discussion, the authors suggest that the recessive form of HUS results from HF deficiency and the dominant form results from the presence of abnormal HF protein in serum. However, in the isolated case that we reported, with an exon 1 mutation, the HF levels were half the normal level. Why did our patient develop HUS whereas the heterozygotes in the Bedouin family did not, and why did the child with abnormal HF secretion described by Ault develop membranoproliferative glomer-

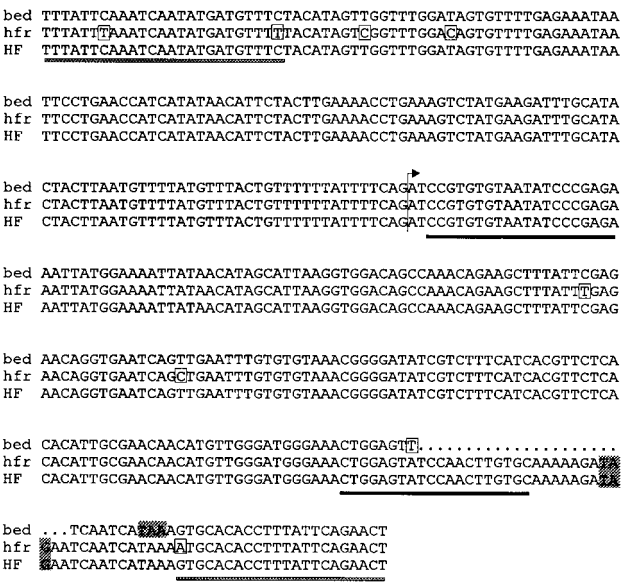

Figure 1 Alignment of genomic nucleotide sequence in the Bedouin family, FHR1 and HF. The start of the exon is indicated by an arrow and the termination codons are shaded in gray. All nucleotide differences are in the unshaded boxes. The primer sequence used by Ying et al. is shown as a blackened bar under the nucleotide sequence, and the primer sequence that we used is shown by the gray-shaded bar. (Abbreviations: bed, Bedouin family; hfr, factor H-related protein 1; HF, Factor H)

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bed PCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLEFQS.....
hfr PCVISREIMENYNIALRWTAKQKLYLRTGESAEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR.
HF  PCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR.
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Figure 2 Alignment of the amino acid sequence of exon 20 in the Bedouin family, FHR1 exon 5 and HF exon 20. The two amino acid differences between FHR1 exon 5 and HF exon 20 are in the boxes. (Abbreviations: bed, Bedouin family; hfr, factor H-related protein 1; HF, Factor H)

ulonephritis rather than HUS? These unanswered questions emphasize the need for further investigation of the genotype-phenotype correlations in FH abnormalities.

Acknowledgments

This work has been supported by the Northern Counties Kidney Research Fund, the Foundation for Nephrology, the Newcastle upon Tyne University Hospitals Special Trustees, and the Medical Research Council and Action Research.

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Electronic-Database Information

The URL for data in this article is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for HUS [MIM 235400])

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0002-9297/2000/6605-0030$02.00

Am. J. Hum. Genet. 66:1722, 2000

Reply to Buddles et al.

To the Editor:

We congratulate Buddles et al. on their thorough evaluation of the factor H gene and on the identification of a mutation that we failed to detect in our study. The primary conclusions of our study were that (1) on the basis of segregation in a large Bedouin kindred with flanking microsatellite markers, hemolytic uremic syndrome can be inherited as an autosomal recessive disease tightly linked to the factor H locus, and (2) the affected Bedouin patients have abnormal cellular transport of factor H. These conclusions remain unchanged and are, in fact, strengthened by the excellent work of Buddles et al. We agree with their conclusion that further investigation of genotype-phenotype correlations in factor H abnormalities are needed to answer a number of interesting questions.

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0002-9297/2000/6605-0031$02.00