Novel Single Nucleotide Polymorphism (9678G→A) for Linkage Analysis of Acute Intermittent Porphyria

To the Editor:
Acute intermittent porphyria (AIP) is an autosomal dominant inborn error of metabolism caused by a partial deficiency of the third enzyme of the heme biosynthetic pathway, hydroxymethylbilane synthase (EC 4.3.1.8.; HMBS). This enzyme catalyzes the condensation of four molecules of porphobilinogen to a tetrapyrrole hydroxymethylbilane. Clinically, AIP is characterized by acute attacks of neurological disorders manifesting as abdominal pain, hypertension, tachycardia, peripheral neuropathy, and mental dysfunction. Biochemical diagnosis of AIP relies on increased urinary porphobilinogen and fecal porphyrin excretion within the health-related reference interval. The definitive diagnosis requires the assay of red blood cell (RBC) HMBS activity (1).

AIP is a genetic disease characterized by poor penetrance; therefore, identification of presymptomatic AIP carriers in families with manifesting individuals is of utmost clinical importance because avoidance of precipitating agents, e.g., drugs and alcohol, can prevent the occurrence of the first porphyric attacks, which may be life-threatening. However, the identification of AIP carriers by assaying the RBC HMBS activity is problematic because there is substantial overlap between the enzyme activities of healthy individuals and patients with AIP (2). In addition, in one variant of AIP, the decreased enzyme activity is confined to the liver, and the RBC HMBS activity is within health-related values (3).

DNA-based diagnosis of presymptomatic AIP has proven to be more reliable than diagnosis by RBC HMBS activity. Direct detection of mutations in presymptomatic AIP is the definitive approach; however, this requires the prior identification of the mutations in the probands. To date, 117 mutations have been identified in the HMBS gene. Sixty-five are missense/nonsense mutations, 21 are splicing mutations, 18 are small deletions, 11 are small insertions, 1 is a gross deletion, and 1 is a gross insertion and duplication (4). Most of these mutations are found only in individual families, except those found in Dutch (R116W) (5) and Swedish (W198X) (6) AIP families. In most instances, the mutations can only be identified by DNA sequencing, an approach that is both labor-intensive and time-consuming. Although the exons containing the mutations can often be identified by one of the screening methods, such as single strand conformation polymorphism analysis (7), heteroduplex analysis (8), and denaturing gradient gel electrophoresis (9, 10), no methods at present can reliably detect all of the mutations.

In the course of mutation analysis of the HMBS gene in our patients with AIP, we have identified a single nucleotide polymorphism (SNP) by direct DNA sequencing (data not shown). The SNP is located 72 base pairs (bp) downstream of the last exon, flanking the 3’ end of the HMBS gene, at nucleotide position 9678 (numbered according to Genbank accession no. M95623), where the presence of G instead of A abolishes a BsrI restriction site. We developed a PCR-restriction analysis method to study this polymorphic marker in 48 unrelated Chinese individuals without symptoms or family histories of AIP.

Genomic DNA was extracted from whole blood samples by the salting out method as described (11). The study was performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects.

The sequence of the forward primer was 5’-TGCTGTCCAGTGCTCATTACATC-3’. The sequence of the reverse primer was 5’-GAACTCTGGCCAAAAGTCCC-3’. Genomic DNA (50 ng) was amplified in a 25-μL reaction mixture containing 1× Mg2+-free Taq DNA polymerase buffer, 200 μmol/L of each dNTP, 1.0 mmol/L MgCl2, 1.5 pmol of each primer, and 1.0 U of Taq DNA polymerase (Life Technologies). A Perkin-Elmer 480 thermal cycler was used, and the reactions were carried out with manual hot-start at 95 °C for 5 min, followed by 35 cycles of denaturation (30 s at 95 °C), annealing (60 s at 70 °C), and extension (40 s at 72 °C), and a final elongation step at 72 °C for 7 min.

PCR products (15 μL) were digested with 10 units of BsrI (New England Biolabs) at 65 °C overnight as recommended by the manufacturer. The digested products were then loaded on 6% acrylamide:bisacrylamide (19:1, by weight) gels and electrophoresed in Tris-borate-EDTA buffer. Electrophoresis was carried at 40 mA in 1× Tris-borate-EDTA buffer. When the primers listed above were used, the PCR product size was 331 bp. Digestion of wild-type DNA with BsrI generated fragments of 213, 104, and 14 bp for 9678G. In the presence of 9678A, fragments of 213, 86, 14, and 18 bp were observed (Fig. 1).

When we analyzed 48 apparently healthy Chinese individuals, we found that 4 were 9678A/A, 27 were 9678A/G, and 17 were 9678G/G. The allele frequency of 9678A was 0.36, and that of 9678G was 0.64. The unbiased estimation of heterozygosity was 0.47, and the polymorphism information content was 0.36. Thus, this SNP is informative in Chinese; however, it would be interesting to know whether this dimorphism will also be informative in other ethnic groups.

Linkage analysis using polymorphic DNA markers is suitable for the identification of presymptomatic AIP carriers in families with manifesting individuals. AIP is an autosomal dominant disease; hence, more than one family member is usually affected, facilitating the phasing of the polymorphic markers. In addition, the entire genomic sequence encoding the HMBS gene is only 10 kilobases in size (12). Thus, recombination between the mutation and the polymorphic marker is negligible, increasing the reliability of this approach. Because highly polymorphic microsatellite markers have not been
Fig. 1. Identification of 9678G→A by BsrI restriction analysis.

The 9678G allele produced fragments 213, 104, and 14 bp in size, whereas the 9678A allele produced fragments 213, 86, 14, and 18 bp in size. The 14- and 18-bp bands were not seen on the gel.

identified in this gene, SNPs (12, 13) remain the only available genetic markers for linkage analysis to identify presymptomatic carriers in AIP families.

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References


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Reference Values of Serum IgA Subclasses in Caucasian Adults by Immunonephelometry

To the Editor:

Immunoglobulin A (IgA) is a glycoprotein present in plasma (as part of the systemic compartment) and in body secretions (as part of the secretory compartment). Two different subclasses, IgA1 and IgA2, have been described. The IgA1/IgA2 ratio is much higher in the systemic compartment than in the secretory compartment. Serum IgA2 concentrations have been used as an index for mucosal pathology (1, 2). Changes in serum IgA subclass concentrations and changes of the IgA1/IgA2 ratio have been associated with specific diseases and conditions, e.g., subclass deficiency leading to anaphylactic transfusion reactions, chronic alcohol abuse (increased IgA2 concentration and IgA2/IgA1 ratio), and primary IgA nephropathy (increased IgA1 in Caucasians) (4).

Various methods (e.g., immunoradiometry, immunodiffusion, and ELISA) that use subclass-specific antisera (2) or monoclonal antibodies (4–6) have been proposed for the determination of IgA subclass concentrations in various human body fluids. Recently, subclass-specific antisera have been introduced for application in a nephelometric assay.

The aim of our study was to establish reference values for serum concentrations of the two IgA subclasses in Caucasian adults, using this immunonephelometric assay.

Total IgA and IgA subclasses were assayed using commercial reagents (Hu IgA Subclass BNA Kit; The Binding Site, Birmingham, UK) on a Behring nephelometer (Behringwerke AG). The coefficient of variation, calculated from the results of 10 consecutive determinations of a single sample, was 2.6% for total IgA (at a mean concentration of 1.02 g/L), 4.3% for IgA1 (at a mean concentration of 1.12 g/L), and 3.6% for IgA2 (at a mean concentration of 0.11 g/L). The total serum IgA concentration was standardized against the IFCC/BCR/CAP Reference Material (CRM 470) (7).