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DNA Diagnosis for Hereditary Cerebral Hemorrhage with Amyloidosis (Dutch Type)

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Summary

Hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) is tightly linked to the Alzheimer amyloid precursor protein gene on chromosome 21, which codes for the amyloid β-protein. A point mutation detected at position 1852 of the amyloid precursor protein gene in four HCHWA-D patients was hypothesized to be the basic defect. This study proves that 22 HCHWA-D patients from three pedigrees all carry this point mutation, whereas the mutation is absent in escapees from the HCHWA-D families as well as in randomly selected Dutch individuals. A mutation-specific oligonucleotide is now available for the confirmation of the HCHWA-D diagnosis. Therefore, presymptomatic testing and prenatal evaluation of individuals at risk in the HCHWA-D families is now feasible.

Introduction

Hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) is a rare autosomal dominant disease occurring in four families living in two coastal villages in the Netherlands (Wattendorff et al. 1982; Luyendijk et al. 1988). About 50% of the patients die from the first massive cerebral hemorrhage at age 45–65 years. In the remaining patients recurrent hemorrhages occur, resulting in severe disablement, mental impairment, and death within 1–17 years after the first hemorrhagic accident (Haan et al. 1990). At present, the diagnosis of cerebral amyloid angiopathy can only be confirmed by histological examination. Extensive deposition of amyloid in the small leptomeningeal arteries and cortical arterioles, as well as plaques of the amorphous type, are the main characteristics. No neurofibrillary tangles or neuritic component is present. The vascular amyloid deposits in HCHWA-D brains are immunologically reactive with antibodies raised against a synthetic peptide homologous to Alzheimer disease (AD) amyloid β-protein (Aβ) (Van Duinen et al. 1987; Prelli et al. 1988). The deposits in HCHWA-D comprise 39 residues (Prelli et al. 1988) similar to the AβP (Glenner and Wong 1984). The AβP is encoded by a part of the amyloid precursor protein (APP) gene, located on chromosome 21 (Goldgaber et al. 1987; Kang et al. 1987; Tanzi et al. 1987*a*). Linkage studies in AD families proved that this disease is heterogeneous and that the majority of mutations are not linked to chromosome 21 (Tanzi et al. 1987*b;* Van Broeckhoven et al. 1987). In two HCHWA-D families, however, tight linkage (LOD score +7.59) with no recombinants of the APP gene was observed by analyzing 20 affected individuals from two large pedigrees (Van Broeckhoven et al. 1990). Sequence analysis of the two exons coding for the AβP revealed a single base mutation (G to C) at position 1852 of the APP_{695} sequence in four patients (Levy et al. 1990). The mutation leads to a GLN-for-GLU substitution, at position 22 in the AβP, that is expressed in the amyloid fibrils (Prelli et al. 1990). This region of the protein has recently been described as harboring a proteolytic cleavage site at positions 15–17 (Esch et al. 1990; Sisodia et al. 1990). Accord-

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ingly, the metabolism of APP is probably influenced by this GLN-for-GLU substitution at position 22 and contributes to the tissue-specific accumulation of AβP in HCHWA-D. Very recently, Goate et al. (1991) detected another missense mutation in the same exon of the APP gene that so segregates with some early-onset familial AD.

In order to establish the segregation of the 1852 (G-to-C) mutation, described by Levy et al. (1990), in four subjects with HCHWA-D, we examined 22 patients and two asymptomatic individuals (50%-at-risk family members who reached the age of 65 years without showing clinical signs of the disease). We also tested 50 randomly selected individuals to exclude the possibility that the point mutation, which abolishes an *Mbo*II restriction site, is a rare RFLP present in the Dutch population.

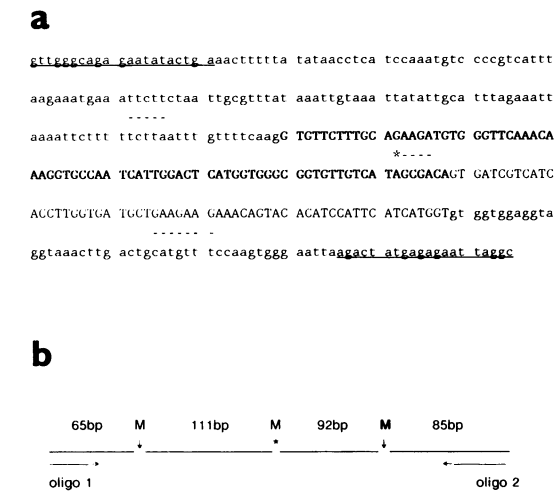

Figure 1 *a*, APP exon 15. The sequence of the genomic 355-bp fragment, which contains the exon 15 of the APP gene (Yoshikai et al. 1990), is shown. Indicated are the exonic sequence, in capital letters; the AβP part of the exon, in boldface; the primers used for amplification, underlined; the *Mbo*II restriction site, dashes; and the site of mutation (G→C) at position 1852, asterisk. *b*, *Mbo*II restriction-site map of 355-bp amplified fragment. The *Mbo*II sites are indicated by M's; the mutated *Mbo*II site is indicated by an asterisk.

Patients and Methods

Twenty patients belonging to two large HCHWA-D families living in Katwijk (Luyendijk et al. 1988) and two patients belonging to a family residing in Scheveningen (Wattendorff et al. 1982) were studied. The diagnosis of HCHWA-D was pathologically proved in seven patients and was based on clinical characteristics in the remaining 15. Two asymptomatic individuals from two different branches of the Katwijk pedigree, who had lived well past the mean age at onset (59.2 ± 7.9) in this family, one being 71 years old and the other being 77 years old, were studied as well. DNA from the HCHWA-D patients was isolated either from fresh blood or from postmortem brain tissue by using standard DNA isolation techniques (Sambrook et al. 1989).

For the PCR (Saiki et al. 1988), two oligonucleotides were prepared complementary to sequences which flank exon 15 (Lemaire et al. 1989; Yoshikai et al. 1990) of APP_{696} and which are 366 bp apart (fig. 1). *Thermus aquaticus* (*Taq*) heat-stable DNA polymerase was used for the amplification reaction. The reactions were performed in a total volume of 50 μl containing 0.5 μg genomic DNA, 50 pmol each of the two primers, 2 Units *Taq* DNA polymerase (Perkin Elmer–Cetus), 2.5 mM $MgCl_2$, 5 mM KCL, 10 mM Tris pH 8.4, 0.2 mM nucleotide triphosphates, and 10 μg BSA. Thirty-two cycles of 94°C for 1 min, 60°C for 1.5 min, and 70°C for 2 min were executed in a thermocycler (Perkin Elmer–Cetus), to, respectively, denature, anneal, and extend the DNA between the primer sequences. The PCR products were analyzed on 3% agarose gel (2.5% Nusieve and 0.5% Seakem; FMC) electrophoresed at 60 V in tris borate buffer pH 8.9 for 4 h. *Mbo*II-digested PCR products were analyzed on 3% agarose gels.

A second technique used was electrophoresis of undigested PCR products on a normal agarose gel (0.8%) in tris borate buffer for 1 h at 30 V. The DNA in the gel was alkaline transferred on nylon membrane Hybond N^+ (Amersham). The Southern blots were prehybridized for 2 h at 50°C in hybridization solution containing 2 × SSC, 10 × Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA), and 150 μg salmon sperm DNA/ml. The mutation-specific oligonucleotide (5′ ACC CAC ATC TGC TTG TGC AAA GAA CAC-3′) (Levy et al. 1990) was radiolabeled with ^{32}P by using T4 kinase according to the manufacturer's (Pharmacia) instructions and was added to the hybridization mixture (2×10^6 cpm/ml) for overnight hybridization at 50°C. Membranes were washed either (*a*) three times for 5 min in 2 × SSC and 0.1% SDS at room temperature and then once for 5 min at 48°C (low stringency) or (*b*) three or four times for 5 min at 68°C (high stringency), and this was followed by X-ray exposure for 2–4 h.

Results

All samples showed the expected fragment of 355 bp (fig. 2, *left panel,* lanes 1 and 2) comprising the APP_{695} exon 15. Two approaches showed the presence or absence of the 1852 point mutation. First, *Mbo*II digestion of the PCR material, followed by agarose gel electrophoresis, yielded fragments of 111, 92, 86, and 65 bp in patients and normal individuals, but an additional fragment of 203 bp was only present in the HCHWA-D patients, whereas none of the 50 randomly selected Dutch individuals and neither of the two asymptomatic individuals showed this 203-bp band. Agarose gel detection of the mutation after *Mbo*II digestion was difficult to control. Partial digestion and diffuse bands urged us to confirm the results by using a second technique. Southern blotting of the PCR products, followed by hybridization with specific oligonucleotide, identified the mutation in all 22 patients, whereas the two asymptomatic family members and the 50 normals did not demonstrate the mutation. In figure 3, parts of two sibships are shown, each of which contains one person, who, on basis of age, is judged to have escaped the disease. When the amplified DNA from members of these two sibships was hybridized with the mutation-specific oligonucleotide, under high stringent washing conditions, the nonaffected family members were shown to lack the mutation. The finding of the same mutation confirms that three pedigrees analyzed may have originated from a common ancestor who carried the mutation. The data prove that the G-to-C mutation at position 22 in the AβP segregates with the disease in the HCHWA-D patients examined.

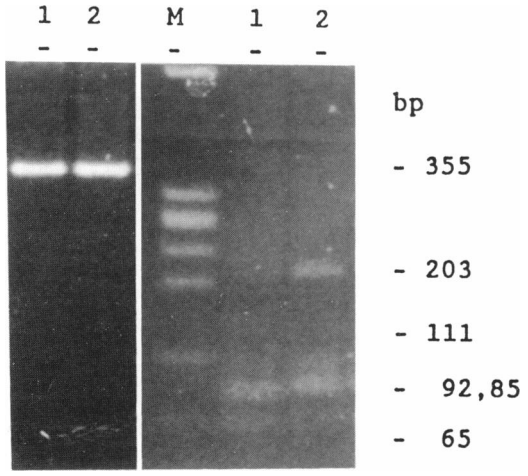

Figure 2 Mutation detection by *Mbo*II digestion. Lanes 1 and 2, PCR-amplified DNA of respectively normal individual and HCHWA-D patient, shown before (*left panel*) and after (*right panel*) both digestion with *Mbo*II and agarose gel electrophoresis. Lane M, Size marker X174 digested with *Hae*III.

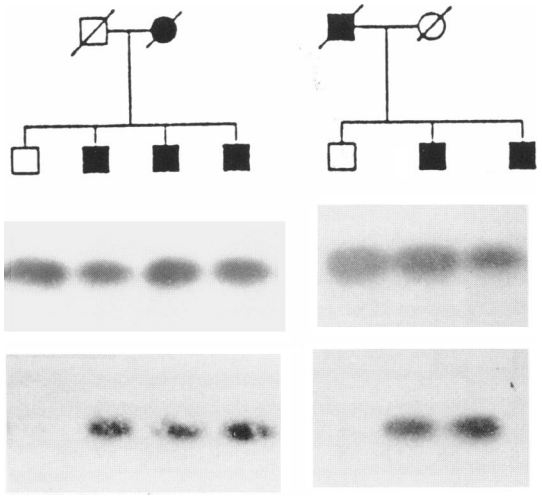

Figure 3 Mutation detection by use of mutation-specific oligonucleotide. Two sibships of the Katwijk pedigree are shown, both of which contain one asymptomatic individual (indicated as an unblackened square) who has lived well past the average age at onset. HCHWA-D patients are indicated as blackened squares. Southern-blotted undigested PCR products were hybridized with the mutation-specific oligonucleotide. The top row shows bands which are visible after low-stringency washing; the bottom row shows the same bands after further washings at a higher stringency.

Discussion

We confirmed (*a*) the presence of a mutation at position 1852 of the APP gene in DNA of 22 HCHWA-D patients belonging to three HCHWA-D pedigrees and (*b*) that cosegregates with the disease in every case. No mutation was detected among 50 randomly selected Dutch persons and two HCHWA-D family members who escaped the disease. Therefore, the point mutation can be considered diagnostic for HCHWA-D.

Bands obtained by RFLP analysis indicated the importance of confirming the results by a second technique, to optimize reproducibility. The hybridization with the mutation-specific oligonucleotide appeared, in our hands, to be a reliable method for diagnosis of HCHWA-D.

Previously the disease could only be confirmed either by postmortem examination of the brain or by brain biopsy. The mutation-specific oligonucleotide

hybridization used in the present study can be used not only to confirm that a patient with a cerebral hemorrhage has HCHWA-D but to offer the possibility of presymptomatic and prenatal evaluation of at-risk people in the HCHWA-D families. However, application for presymptomatic DNA analysis should follow a counseling routine.

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