Rapid Detection of a Pentanucleotide Deletion Polymorphism in the Human α_2-Macroglobulin Gene

To the Editor:

Alpha-2 macroglobulin (α_2M) is a serum glycoprotein and a panproteinase inhibitor found in various tissues, including plasma and cerebrospinal fluid. α_2M is thought to inactivate proteinases by a specific trapping mechanism in the so-called "bait" region of the protein *(1)*. α_2M is also a ligand for the LDL receptor-related protein, and both are upregulated after brain injury and in regions of the brain affected by Alzheimer disease. Additionally, α_2M binds to the amyloid β peptide *(2, 3)*, which leads to attenuation of both fibrillogenesis and neurotoxicity *(4)* and which is cleared by the LDL receptor-related protein. Recently, a pentanucleotide deletion in the 5′ splice site of exon 18, which encodes a portion of the α_2M bait region, has been suggested to be genetically associated with an increased risk for developing Alzheimer disease *(5)*. α_2M and the ϵ4 allele of the apolipoprotein E gene seem to confer a similar degree of risk for developing late-onset Alzheimer disease. The conventional methods for measuring α_2M are ELISA, immunoblotting, or enzymatic assays, but these methods can not be applied to the detection of α_2M pentanucleotide polymorphism. The DNA-based method for detecting this polymorphism deletion is not amenable to large-scale screening *(5, 6)*.

The method described below is based on the observation that the α_2M pentanucleotide deletion polymorphism *(6)* leads to the loss of the *Hph*I restriction site at the intronic sequence in the 5′ splice site of exon 18 (Fig. 1A). The primers amplify a 196-bp region in individuals without the pentanucleotide deletion (Fig. 1B). Genomic DNA was extracted from leukocytes, using HQIAamp (Qiagen), and was amplified by PCR using oligonucleotide primers α_2MF (5′-GGT GGC AAC TAT TAC ATT CTC TCA-3′) and α_2MR (5-ACT TAC TTT ACC ACC ACC AAA TCC-3′). In addition to the buffer and nucleotide components, each amplification reaction contained ~200 ng of genomic DNA, 20 pmol of each primer, and 2 U of Taq polymerase (Life Technologies) in a final volume of 50 μL. The reaction mixture was first denatured at 94 °C for 2 min and then subjected to 35 cycles of PCR (94 °C for 1 min, 59 °C for 40 s, 72 °C for 40 s), after which it was incubated at 72 °C for 10 min. A 20-μL aliquot of the amplification product was then digested in the presence of 2.2 μL of 10× buffer and 20 U of *Hph*I for at least 2 h at 37 °C. Restriction digest products were size fractionated by electrophoresis on a 2% agarose gel with 1 mg/L ethidium bromide for 20 min at 200 V and detected directly under ultraviolet light. (Incomplete digestion may sometimes occur, which can be avoided by a purification step of the PCR product before enzymatic digestion. However, this does not interfere with the scoring of the alleles.) The PCR-amplified digestion products are shown in Fig. 1B along with representative α_2M genotypes. In a preliminary study of 367 individuals genotyped by this method, the allele frequency of one or two α_2M alleles was 19.1% in patients with sporadic late-onset Alzheimer disease compared with 13.8% in age-matched unaffected individuals. We have encountered no difficulties in the samples tested, and we found this method to be well-suited to high-throughput routine clinical screening.

References

1. Borth W. Alpha 2-macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J 1992;6:3345–53.
2. Du Y, Ni B, Glinn M, Dodel RC, Bales KR, Zhang Z, et al. Alpha2-macroglobulin as a beta-amyloid peptide-binding plasma protein. J Neurochem 1997;69:299–305.
3. Hughes SR, Khorkova O, Goyal S, Knaeblein J, Heroux J, Riedel NG, et al. Alpha2-macroglobulin associates with beta-amyloid peptide and prevents fibril formation. Proc Natl Acad Sci U S A 1998;95:3275–80.
4. Du Y, Bales KR, Dodel RC, Liu X, Glinn MA, Horn JW, et al. Alpha2-macroglobulin attenuates beta-amyloid peptide$_{1-40}$ fibril formation and associated neurotoxicity of cultured fetal rat cortical neurons. J Neurochem 1998;70:1182–8.
5. Blacker D, Wilcox MA, Laird NM, Rodes L, Horvath SM, Go RCP, et al. Alpha-2 macroglobulin is genetically associated with Alzheimer disease. Nat Genet 1998;19:357–60.
6. Matthijs G, Marynen P. A deletion polymorphism in the human alpha-2-macroglobulin (A2 M) gene. Nucleic Acids Res 1991;19:5102.

Richard C. Dodel[1]
Kelly R. Bales[2]
Martin R. Farlow[3]
Thomas Gasser[4]
Steven M. Paul[2]
Yansheng Du[1*]

Departments of
[1]Pharmacology and Toxicology
and [3] Neurology
Indiana University School of Medicine
Indianapolis, IN 46285

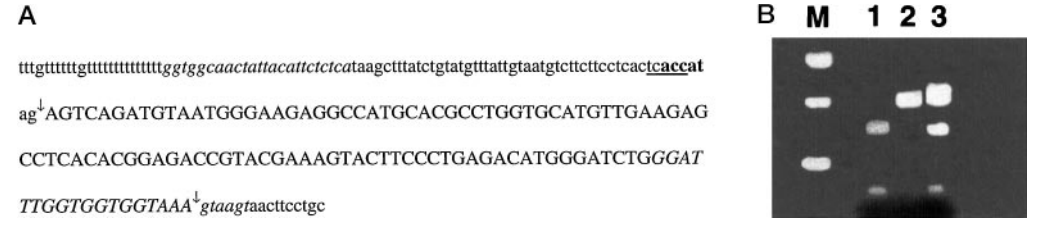

Fig. 1. Sequence (*A*) and amplification products (*B*) of exon 18 of the gene coding for α_2M.

(*A*) Sequence of exon 18 and partial sequence of flanking introns of the human α_2M gene (the primer sequence is in *italics*; the pentanucleotide polymorphism in the 5′ splicing site of exon 18 is in *bold*; the restriction site of *Hph*I is *underlined*; *arrows* indicate start/end of coding region of exon 18; *capital letters* indicate the coding region of exon 18. (*B*) Photograph of a 2% agarose gel stained with ethidium bromide and viewed through ultraviolet light. *Lane M*, molecular markers (100, 200, and 400 bp). *Lanes 1–3*, *Hph*I restriction fragment from three possible α_2M genotypes: *lane 1*, wild-type allele (130 and 66 bp); *lane 2*, homozygote for α_2M polymorphism deletion (191 bp); *lane 3*, heterozygote for α_2M polymorphism deletion (191, 130, and 66 bp).