A *NotI* RFLP in the human alpha2-C4 adrenergic receptor locus (ADRA2RL2) detected by PFGE

W.-T.Hsieh*, J.L.Barrick, M.R.Hoehe and E.S.Gershon
Clinical Neurogenetics Branch, Bldg 10/Rm 3N218,
NIMH, ADAMHA, Bethesda, MD 20892, USA

Source/Description: Probe pADRA2RL2 (1) containing 1270 bp coding sequence and 240 bp of the 3' flanking region of the human alpha2-C4 adrenergic receptor encoding the putative alpha2B-AR (2).

Polymorphism: A two-allele polymorphism with fragments of 800 kb (B1) and 400 kb (B2) is revealed after hybridization with *NotI* digested human genomic DNA.

Not Polymorphic For: *MluI* and *NruI*.

Frequency: 26 unrelated Caucasians were studied.

B1 allele: 0.37
B2 allele: 0.63

Chromosomal Location: The alpha2-C4 adrenergic receptor has been mapped to the short arm of chromosome 4 just proximal to Huntington's disease gene marker G8 (4p16.3) (M.R.Hoehe *et al.*, in preparation).

Mendelian Inheritance: Co-dominant segregation in 3 families.

Other Comments: The digests were run on a 1% agarose gel in TBE at 330 V using a Pulsaphor (Pharmacia/LKB) with a pulse time of 100 s or 40 s for 40 hours. The probe also hybridizes to an *MluI* fragment larger than 1,000 kb, and to two *NruI* fragments of 750 and 450 kb. This polymorphism is independent of the *Bsu36I* polymorphism reported earlier (1).

Probe Availability: Generously provided on request from Drs. J.W.Regan and R.J.Lefkowitz. Howard Hughes Medical Institute at Duke University Medical Center, Department of Medicine, Biochemistry, and Physiology, Durham, NC 27710.

References: 1. M.R.Hoehe *et al.* (1989) *NAR* 17, 10148. 2. J.W.Regan *et al.* (1988) *PNAS* 85, 6301–6305.

Acknowledgements: We thank Dr. Cassandra Smith for invaluable assistance on PFGE.

Sequence polymorphism in the human alpha-2-macroglobulin (A2M) gene

W.Poller, J.-P.Faber¹ and K.Olek¹

Medizinische Universitätsklinik, Klinikum Bergmannsheil,
Ruhr – Universität Bochum, Gilsingstrasse 14, D-4630
Bochum, FRG and ¹Institut für Klinische Biochemie der
Universität, Sigmund-Freud-Strasse 25, D-5300 Bonn,
FRG

Description: The thiolester site of the human alpha-2-macroglobulin (A2M) is essential for the function of A2M as a proteinase inhibitor (1, 2). This site, which is encoded for by exon 24 of the A2M gene, was amplified by polymerase chain reaction (PCR) using the primer 5'-CTGAAGAATTATCCCTG-AAAC-3' and 5'-TGGTTCCTGCCATATCGCTC-3'. PCRs were performed in 100 µl volumes containing 0.5 µg genomic DNA, 25 pmoles of each primer, 200 µM of each dNTP in 50 mM KCl, 10 mM Tris-HCl, 3.0 mM MgCl₂, 0.01% (w/v) gelatin and 2.5 U Taq polymerase. 30 cycles of amplification were conducted, each consisting of 1 min at 94°C, 1 min at 58°C, 30 sec at 72°C. Sequencing of the 0.8 kb amplification product with the primer 5'-ATTAGGCTCTGCCATGCAA-3' according to the method of Wong *et al.* (3) showed a sequence polymorphism of the gene.

Polymorphism: A point mutation near the thiol ester site changing codon 1000 from GTC to ATC with a corresponding amino acid exchange Val¹⁰⁰⁰ to Ile¹⁰⁰⁰ creates a new *Sau3A* restriction site (Fig. 1).

Frequency: Frequencies of the two alleles among 30 unrelated Caucasians:

GTC (Val¹⁰⁰⁰), *Sau3A* –: 0.30
ATC (Ile¹⁰⁰⁰), *Sau3A* +: 0.70

Chromosomal Localization: 12 p13.3–p12.3

Mendelian Inheritance: Co-dominant segregation shown in one informative family.

References: 1. Kan, C.-C. *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82, 2282. 2. Feldman, S.R. *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82, 5700. 3. Wong, C. *et al.* (1987) *Nature* 330, 384.

Acknowledgement: This work was supported by grant 01KE8903/6 from the Bundesministerium für Forschung und Technologie, FRG.

* To whom correspondence should be addressed