

Sequence tagged site (STS) TaqI RFLP at dopamine β -hydroxylase (DBH)

J.Gelernter, P.V.Gejman¹, S.Bisighini and K.K.Kidd²

Yale University School of Medicine and West Haven VA Medical Center, Psychiatry 116A, West Spring Street, West Haven, CT 06516, ¹National Institute of Mental Health, Bethesda, MD and ²Yale University School of Medicine, Department of Human Genetics, New Haven, CT, USA

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Dopamine β -hydroxylase (DBH) is the enzyme responsible for catalyzing the conversion of dopamine to norepinephrine. As abnormalities in either the dopamine or norepinephrine systems can cause psychiatric abnormalities, DBH is a potential candidate gene for causation of mental illness. We describe here an STS at that locus and an RFLP recognized by the STS PCR product. **SOURCE/DESCRIPTION:** PCR was used to amplify a 370 bp segment of human dopamine β -hydroxylase ((1); sequence from Genbank; accession, X13257) plus a 40-bp GC clamp; total length of PCR product, 410 bp (product called DBH-RGG). The PCR profile is as follows: 30 cycles at 94°C, 45°C, and 72°C, one minute at each step, using the Perkin Elmer Cetus 'GeneAmp' kit (standard buffers and conditions as specified by the manufacturer). The sequences of the oligonucleotide PCR primers (5'-3') are: primer D β 1, a 17-mer: TCC ACC CGT GGG CCC GA (complementary to bases 770–754 of published sequence); primer D β 2, a 57-mer: CGC CCG CCG CGC CCC CGC CCC GTC CCG CCG CCC CCG CCC CTG CAA AGA CAC AGT CAT (bases 401–417 of D β H sequence plus a 40-base GC clamp). (Human genomic DNA was used as template). For hybridization with Southern blots, the 410 bp PCR product was isolated from a low melting point agarose gel and labeled by the random primer method.

POLYMORPHISM: TaqI identifies a two-allele polymorphism with bands at 8.0 and 4.2 kb when probed with DBH-RGG (Figure 1). There is also a weak constant band at about 0.8 kb.

FREQUENCY: Among 38 unrelated Caucasians: 8.0 kb allele, 0.84; 4.2 kb allele, 0.16.

NOT POLYMORPHIC FOR: BamHI; BanI; BclI; BglI; BstNI; DraI; EcoRI; HaeIII; HincII; HindIII; KpnI; MspI; PstI; PvuII; RsaI; SacI; XbaI; and XmnI, using a panel of 12 unrelated individuals.

CHROMOSOMAL LOCALIZATION: Dopamine β -hydroxylase was previously localized to 9q34 (2).

MENDELIAN INHERITANCE: Codominant inheritance was observed in four extended kindreds.

PROBE AVAILABILITY: The primer sequences above identify a sequence tagged site. Identity of the DBH-RGG PCR product can be verified by restriction digestion with SacI, which cleaves the 410 bp product into 325 and 85 bp fragments (Figure 2; the 85 bp fragment is very faint and not included in this photograph).

OTHER COMMENTS: Presumably only the last 17 bp of D β 2 are required for specificity to obtain probe, but this has not been verified. No spurious bands have been observed on ethidium bromide stained gels of the PCR product. Additional validation for the identity of DBH-RGG and DBH itself is provided by preliminary linkage data we have obtained showing linkage of

the RFLP recognized by DBH-RGG and the ABO blood group locus (LOD score >3 at 0% recombination in one family); this genetic linkage has been observed also based on DBH activity (e.g. (3)).

The PCR product has given consistent results when used as a probe on genomic TaqI blots. However, probe band purified from agarose and oligolabeled gave anomalous results on BanII filters: it recognized a mitochondrial band. We cannot provide an exact explanation for this at this time.

The present RFLP is probably the same as the polymorphism reported in (4).

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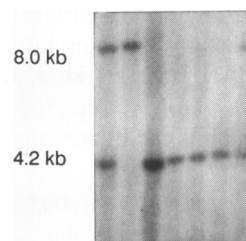


Figure 1. Polymorphic bands seen when a Southern blot of TaqI-digested genomic human DNA is probed with DBH-RGG.

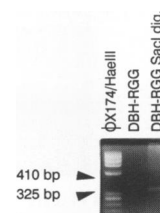


Figure 2. DBH-RGG PCR product, whole and SacI-digested.