TaqI RFLP at the c-kit oncogene locus (KIT)

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Source/Description: A 1.85 kb cDNA insert comprising the region of the c-kit transcript between nucleotides 500 and 2300 was isolated from a Balb/C mouse brain cDNA library and subcloned into pUC19 (1).

Polymorphism: A two-allele TaqI polymorphism was identified by the presence (D1) or absence (D2) of a 6.5 kb hybridizing fragment. Constant bands were seen at 9.5, 4.3, 3.8 and 2.3 kb.

Chromosomal Localization: 4p11-q22 (HGM 10).

Allelic Frequency: Frequency of the alleles conferring presence (D1)/absence (D2) of 6.5 kb fragment, 0.54 (D1)/0.46 (D2). Calculated from 51 unrelated subjects (41 Caucasian, 3 Asian, 6 Black, and 1 Hispanic). Assumptions: 1) Population is in Hardy-Weinberg equilibrium. 2) It is a 2-allele polymorphism.

Mendelian Inheritance: Co-dominant segregation in 13 nonconsanguineous families.

Probe Availability: See (1).

Comment: Failure to demonstrate polymorphism with TaqI was reported previously using DNA from only 7 individuals, probed with a 0.75 kb fragment of the v-kit gene (2).

Clinical Relevance: The dominant-white spotting locus of the mouse encodes the c-kit proto-oncogene (1). Mice with mutations at this locus may have a macrocytic anemia similar to patients with Diamond-Blackfan Anemia (DBA). Because of the possibility that c-kit is a candidate gene for DBA, fourteen DBA patients (11 sporadic, 2 dominant, 1 consanguineous) and their families were studied. TaqI polymorphism results were similar to the normals. There was no evidence for gross rearrangement or deletions of the c-kit structural gene in the patients.

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G/A polymorphism in an intron of the fibrillin gene FBNI

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Source and Description of Sequence Polymorphism: A G/A nucleotide change at position –5 in the 3' end of the intron in the fibrillin gene FBNI between residues 1075 and 1076 in the published sequence (1). This change does not affect a restriction endonuclease site but the two alleles can be readily distinguished by allele-specific hybridization (2).

Allele-specific Oligonucleotide (ASO) Hybridization

Amplification Oligonucleotides: The oligomers used to amplify genomic DNA are taken from reference (3).

5'-TTTGCCATAACACAGAGGG-3' (ESM-S)

5'-GGCACACTGATACTTCCCT-3' (F2-AS)

Amplification Conditions: 30 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec.

(ASO) Hybridization Probes:

(D1) 5'-GATGTCTGTCGGGAAAATA-3' (G-allele, antisense)

(D2) 5'-TATTTTCCCAACAGACATC-3' (A-allele, sense)

These were labelled and used under the hybridization and wash conditions detailed in (2).

Frequency: Estimated from 83 unrelated normal individuals.

Heterozygosity = 19.3%

D1 G-allele = 0.892

D2 A-allele = 0.108

Mendelian Inheritance: Co-dominant segregation was seen in 2 families.

Chromosomal Localization: 15q21.1.

Other Comments: Fibrillin is a candidate gene for the Marfan syndrome (1, 3).

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