Mutation and Polymorphism Report

Authors: Eeva-Marja Sankila[1,2], Tarja H. Joensuu[1,2,3], Riikka H. Hämäläinen[1,3], Nina Raitanen[1,3], Olavi Valle[4], Jaakko Ignatius[1,5] , and Bru Cormand[1,3]

Affiliations: [1]Department of Medical Genetics, University of Helsinki, Helsinki; [2]Department of Ophtalmology, Helsinki University Hospital, Helsinki; [3]Department of Molecular Genetics, The Folkhälsan Institute of Genetics, Helsinki; [4]Säästöpankinranta 4 C, Helsinki; [5]Department of Clinical Neurophysiology, Jorvi Hospital, Espoo, Finland

Dr. Eeva-Marja Sankila, Helsinki University Eye Hospital, Haartmaninkatu 4 C, FIN-00029 HUCH, Finland;
Tel: +358-9-4711; Fax: +358-9-47173163;
E-mail: Eeva-Marja.Sankila@helsinki.fi

Title : A CRX mutation in a Finnish family with dominant cone-rod retinal dystrophy

Keywords: CRX; cone-rod dystrophy; E80K

Species: Homo sapiens

Change is: Mutation

Gene/Locus

Name:	Cone-rod homeobox-containing gene
Symbol:	CRX
Genbank accession number:	NM_000554
OMIM accession number:	602225
Locus specific database:	
Chromosomal location:	19q13.3
Inheritance:	Germline; autosomal dominant

Mutation / polymorphism name

Nucleotide change–Systematic name:	c238G>A
Amino acid change–Trivial name:	E80K
Mutation / polymorphism type:	Missense
Polymorphism frequency:	
Detection method:	RFLP (*Hinf* I), SSCP, DNA sequencing
Detection conditions:	PCR and sequence primers were as described by Freund et al. (1997) **SSCP:** The PCR products were heat-denaturated at 95°C for 10 minutes with 50% formamide, 10mM EDTA and electrophoresed in MDE 0.7X, TBE 0.5X gel for 18h at 6W and room temperature. The bands were visualized by silver staining. **RFLP:** PCR products were digested using the restriction enzyme *Hinf* I (Promega, Madison, WI) according to manufacturer's conditions, electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. **SEQUENCE:** PCR products were purified with QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Sequencing was performed with dRhodamine Terminator Cycle Sequencing kit (Perkin Elmer) on a Perkin Elmer 377 automated DNA sequencer.
Diagnosis method developed:	The mutation abolishes a *Hinf* I restriction site in exon 2 PCR product (primers J34 and J35, see Freund et al. 1997). Normal allele: 229+85 bp; mutated allele: 314 bp

Evidence for existence and effect of mutation:

	Yes	No	Don't know
1. Base change found on repeat PCR sample	X		
2. Base change segregates or appears with trait	X		
3. Base change affects conserved residue	X		
4. Expression analysis supports hypothesis for causation			X
5. Normals tested (50 required)	X		

Ancillary data

1. **Haplotype association :**
2. **Ethnic background/Population association**: Finnish
3. **Geographic association :** Southern Finland
4. **Frequency** (of mutation) **in population:** 0/190
5. **Clinical phenotype of proband :** Cone-rod dystrophy (CRD)
6. **Homologous allele** (if recessive trait)**:**
7. **PIC:** (if microsatellite)
8. **Other:**
9. **Present in HGMD listing:** Yes: No: X

Comments

Cone-rod dystrophy (CRD) is a progressive retinal degeneration clinically characterized by early loss of color vision and visual acuity followed by nyctalopia and loss of peripheral vision. Recently, a photoreceptor-specific homeobox gene (CRX) was implicated in CRD (Freund et al. 1997; Swain et al. 1997). We studied a Finnish CRD family with 21 affected members in six generations previously described by Valle et al. (1981). CRD in this family is characterized by early age of onset (childhood to early teens), loss of blue-green color vision and progressive visual failure. Macular pigmentation, bull's eye lesion and later changes resembling retinitis pigmentosa are seen in ophtalmoscopy. Linkage analysis with marker D19S178, close to the CRX locus, showed no recombinations (theta=0.00, Z_{max}=5.39). Sequencing of the CRX gene in the patients revealed a G to A transition resulting in a Glu80Lys substitution. This aminoacid change is likely a disease-causing mutation: It occurs in the first residue of the recognition helix of the homeodomain, a position held by a glutamic acid residue in the majority of known homeodomains (Duboule 1994); it causes a change in the aminoacid charge and structure, which is likely to disrupt a salt bridge between residues R69 and E80 (Freund et al. 1997); previously, a Glu to Ala change in the same aminoacid position has been reported in two dominant CRD families (Freund et al. 1997; Sohocki et al. 1998); the residue is conserved through evolution; and finally, the mutation was not found in the non-affected population.

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References

Duboule D (1994) Guidebook to the Homeobox Genes (Toronto: Oxford University Press)

Freund CL, Gregory-Evans CY, Furukawa T, Papaioannou M, Looser J, Ploder L, Bellingham J, Ng D, Herbrick J-AS, Duncan A, Scherer SW, Tsui L-CT, Loutradis-Anagnostou A, Jacobson SG, Cepko CL, Bhattacharya SS, McInnes RR (1997) Cone-Rod Dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for mantainance of the photoreceptor. Cell 91:543-553

Sohocki MM, Sullivan LS, Mintz-Hittner HA, Birch D, Heckenlively JR, Freund CL, McInnes RR, Daiger SP (1998) A range of clinical phenotypes associated with mutations in CRX, a photoreceptor transcription-factor gene. Am J Hum Genet 63:1307-1315

Swain PK, Chen S, Wang Q-L, Affatigato LM, Coats CL, Brady KD, Fishman GA, Jacobson SG, Swaroop A, Stone E, Sieving PA, Zack DJ (1997) Mutations in the cone-rod homeobox gene are associated with the cone-rod dystrophy photoreceptor degeneration. Neuron 19:1329-1336

Valle O, Erkkilä H, Raitta C (1981) Dominant progressive cone-rod dystrophy. Acta Ophthalmol 59:695-706