Identification of novel genetic defects in cone-rod dystrophy patients from an outbred population by using homozygosity mapping

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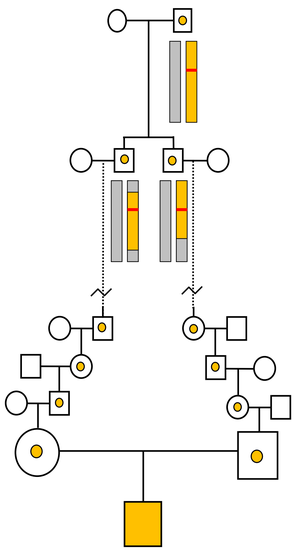
Aim

The purpose of this study was to unravel the causative gene defects in patients with autosomal recessive cone-rod dystrophy.

Background

Cone-rod dystrophy (CRD) is a group of retinal dystrophies in which cones are equally or more severely affected than rods. Main symptoms are reduced visual acuity, photophobia, loss of color vision and visual field defects. ~30% of autosomal recessive cases are caused by mutations in *ABCA4*.

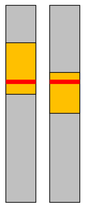
Method



In the outbred Dutch population we previously found homozygous mutations in ~35% of patients with retinal dystrophy. We therefore hypothesize that the parents of these patients share a common ancestor that carries the disease-causing mutation, and that new disease genes can be identified by homozygosity mapping.

In patients with an unknown cause for CRD we searched for sizeable homozygous regions using whole genome SNP arrays. In these regions we analyzed candidate genes for causative mutations.

Patients:
25 CRD patients from 11 families without known mutations in *ABCA4* were genotyped on a 250K SNP-array.



} Size of region: 20 generations → ~5 Mb
10 generations → ~10 Mb

(size region = 100 Mb/number of generations from patient to common ancestor[1])

FIG. 1 *Principle of homozygosity mapping in outbred population in which parents of a patient have a common ancestor. The original region* (in orange) *surrounding the mutation* (red bar) *becomes smaller every generation due to meiotic recombination.*

Conclusions

By unraveling the molecular cause in 4 of 11 multiplex families we show that homozygosity mapping is a powerful tool in identifying novel mutations in patients from an outbred population. The most important result was the identification of a novel retinal dystrophy gene; *EYS*[3]. Furthermore, we show that molecular knowledge of the disease may lead to a better phenotypic understanding.

Results

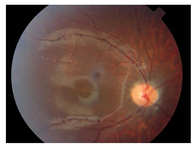
In 4 multiplex CRD families we found the causative mutation; three in known retinal disease genes (*ABCA4*, *PROM1*, *CABP4*) and one in a novel gene (*EYS*). In two families the mutation was located in the largest homozygous region, in two families it was located in the second large region. (table 1)
Clinical re-evaluation led to another diagnosis than cone-rod dystrophy in 3 out of 6 patients. (table 2)

Table. 1 *Three largest overlapping homozygous regions per family. Red boxes show the regions that harbor the causative mutations.*

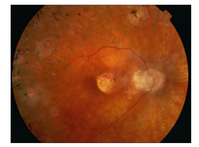
		Family number *(number of affected siblings)*										
		1 *(3)*	2 *(2)*	3 *(2)*	4 *(2)*	5 *(2)*	6 *(2)*	7 *(2)*	8 *(2)*	9 *(3)*	10 *(3)*	11 *(2)*
Size of homozygous region (Mb)	1st	28	2.5	16	19	3	2	19	2	2	4	9
	2nd	2	2	10	2	2	1	5	1	2	1	4
	3rd	1	2	1	2	2	1	2	1	-	1	1

ABCA4
p.C54G/p.C54G
No clinical data available

PROM1
c.1142-1G>A/c.1142-1G>A



***EYS*[3]**
p.Tyr3156X/p.Tyr3156X



***CABP4*[2]**
p.Arg216X/p.Arg216X

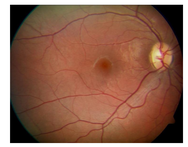


Table. 2 *Overview of clinical data for patients carrying mutations in* PROM1, EYS *and* CABP4.

Mutated gene	Patient (age in years)	Visual acuity		Refractory error*		Nystag-mus	Photo-phobia	Night blind-ness	Color vision	Visual field	Electroretinogram		Phenotype
		RE	LE	RE	LE						Rods	Cones	
PROM1	1 (18)	20/125	20/200	-2.5	-2.75	-	-	+	Abnormal	Central scotoma	↓↓	Non recordable	CRD
	2 (16)	20/125	20/200	-9.5	-9.0	-	-	+	Abnormal	Central scotoma	↓↓	Non recordable	CRD
EYS	3 (61)	20/100	20/40	+3.0	+2.0	-	+	+	?	Constricted	Non recordable	Non recordable	Retinitis pigmentosa
	4 (57)	20/50	20/80	-4.5	-4.25	-	+	+	Abnormal	Central scotoma	↓	↓↓	CRD
											Rods	**Mixed**	**Cones**	
CABP4	5 (12)	20/200	20/200	+ 5.0	+5.5	+	+	-	Abnormal	Normal	Normal	Electronegative	↓↓	Cone-rod synaptic disorder
	6 (10)	20/125	20/200	+4.5	+4.5	+	+	-	Abnormal	Normal	Within 2 SD of normal	Electronegative	↓↓	Cone-rod synaptic disorder

*RE, right eye; LE, left eye; +, present; -, absent; ↓, decreased; ↓↓, severely decreased; SD, standard deviation; * spherical equivalent in diopters.*

References

1. Woods *et al.* AJHG 2006 May;78(5):889-96
2. Littink *et al.* IOVS 2009 May;50(5):2344-50
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