HUMAN MUTATION 9:110–117 (1997)

MUTATION UPDATE

Molecular Basis of Choroideremia (CHM): Mutations Involving the Rab Escort Protein-1 (*REP-1*) Gene

J.A.J.M. van den Hurk[1*], M. Schwartz[2], H. van Bokhoven[1], T.J.R. van de Pol[1], L. Bogerd[1], A.J.L.G. Pinckers[3], E.M. Bleeker-Wagemakers[4], I.H. Pawlowitzki[5], K. Rüther[6], H.-H. Ropers[1], and F.P.M. Cremers[1]

[1]*Department of Human Genetics, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands; Fax: 31.24.3540488*
[2]*Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark*
[3]*Department of Ophthalmology, University Hospital Nijmegen, Nijmegen, The Netherlands*
[4]*Department of Ophthalmogenetics, I.O.I., Amsterdam, The Netherlands*
[5]*Institute for Human Genetics, Westfälische Wilhelms-Universität, Münster, Germany*
[6]*Universitäts-Augenklinik, Eberhard-Karls-Universität, Tübingen, Germany*

Communicated by Albert de la Chapelle

Choroideremia (CHM) is an X-linked recessive eye disease that results from mutations involving the Rab escort protein-1 (*REP-1*) gene. In 18 patients deletions of different sizes have been found. Two females suffering from CHM were reported to have translocations that disrupt the *REP-1* gene. In 22 patients, small mutations have been identified. Interestingly, these are all nonsense, frameshift or splice-site mutations; with one possible exception, missense mutations have not been found. This comprises all the known mutations in the disease. Hum Mutat 9:110–117, 1997.

KEY WORDS: **choroideremia (CHM), mutations, rab escort protein-1 (REP-1)**

INTRODUCTION

Choroideremia (CHM) is an X-linked recessive eye disorder characterized by progressive degeneration of the choroid, retinal pigment epithelium (RPE), and retina (Goedbloed, 1942; Waardenburg, 1942). Its incidence is estimated as 1 in 100,000. The clinical manifestation of CHM shows considerable interfamilial as well as intrafamilial variability. Usually, affected males develop night-blindness in their teenage years, followed by progressive constriction of visual fields and complete blindness by middle age. Female carriers generally show no serious visual impairment, but they have patchy pigmentation and degeneration of the RPE suggesting clonal areas of disease attributable to random X-inactivation (Heckenlively and Bird, 1988). Occasionally, female carriers show clinical signs of CHM that can be ascribed to skewed X-inactivation (Fraser and Friedmann, 1968; Harris and Miller, 1968). Manifestation of the disease in females can also result from disruption of the CHM gene by X-autosomal translocations (Kaplan et al., 1989; Siu et al., 1990). In these cases, X-inactivation is nonrandom; the normal X is preferentially inactivated, while both translocation fragments remain active.

The gene for CHM was localized to the Xq13–q22 region by linkage analysis (Lewis et al., 1985; Nussbaum et al., 1985; Lesko et al., 1987; Sankila et al., 1989). This assignment was further refined by the characterization of cytogenetically visible deletions in patients with CHM, mental retardation (MR), and deafness type 3 (DFN3) (Rosenberg et al., 1987; Schwartz et al., 1988; Cremers et al., 1989a). Molecular analysis of deletions in patients with classic CHM (Cremers et al., 1987, 1989b, 1990a,b) formed the basis for the positional cloning of a part of the CHM gene from Xq21.2 (Cremers

Received 26 January 1996; accepted 21 June 1996.
*Correspondence to J.A.J.M. van den Hurk.

et al., 1990c; Merry et al., 1992). Only recently, the complete protein coding region of the gene has been isolated (van Bokhoven et al., 1994b).

STRUCTURE AND FUNCTION OF THE *CHM/REP-1* GENE

The CHM mRNA has a length of approximately 5.6 kb and is expressed in various tissue types, including retina, choroid, and/or RPE (Cremers et al., 1990c, unpublished observations). The open reading frame (ORF) is composed of 15 exons, which span a genomic sequence of at least 150 kb. The exons range in size from 64 nucleotides to 3.4 kb and encode a protein of 653 amino acids (van Bokhoven et al., 1994b).

Insight into the function of the CHM protein came with the biochemical purification of Rab geranylgeranyl transferase (Rab GGTase) from rat brain. This multisubunit enzyme attaches geranylgeranyl (GG) groups to Rab proteins, a modification essential for their action in intracellular vesicular transport. Rab GGTase consists of a catalytic α/β heterodimer and an accessory component, the Rab escort protein (REP). Molecular cloning of rat REP-1 revealed identity with the protein encoded by the *CHM* gene (Seabra et al., 1992a,b, 1993; Andres et al., 1993). REP-1 binds to newly synthesized Rab proteins, presents them to the catalytic part of the enzyme, and delivers the prenylated Rabs to their target membranes (Andres et al., 1993; Alexandrov et al., 1994).

In yeast, both the α/β catalytic component of Rab GGTase (encoded by the BET4 and BET2 genes) and REP (encoded by the MRS6/MSI4 gene) are essential for cell viability (Waldherr et al., 1993; Jiang et al., 1993, 1994; Benito-Moreno et al., 1994; Fujimura et al., 1994; Ragnini et al., 1994). A complete loss of REP activity would likely be lethal in humans too. Lymphoblasts of CHM patients have a markedly decreased but still detectable Rab GGTase activity (Seabra et al., 1993), suggesting that there is compensation for the deficiency of REP-1. Indeed, an autosomal homologue of the *REP-1* gene has been identified that was designated *CHML* for choroideremia-like. The gene is located in 1q42-qter and, like the *REP-1* gene, is expressed in a wide variety of tissues (Cremers et al., 1992; van Bokhoven et al., 1994c). The CHML protein, or REP-2, was demonstrated to perform a function similar to that of REP-1. It was therefore hypothesized that REP-2 substitutes for the loss of REP-1 activity in choroideremia patients, thereby preventing symptoms in tissues and organs other than the eye (Cremers et al., 1994). The apparent insufficient compensation in the eye might be due to the presence of a Rab or Rabs that depend preferentially on REP-1 for geranylgeranylation. Recently, Seabra et al. (1995) identified an unprenylated Rab protein in lymphoblasts from CHM patients that was prenylated more efficiently by REP-1 than by REP-2. This protein, Ram/Rab27, is also present at high levels in the RPE and choriocapillaris, suggesting that CHM may result from the deficient geranylgeranylation of Ram/Rab27 or a closely related protein.

REP-1 GENE DEFECTS

Deletions

About one-fourth of European patients with classic choroideremia have large deletions that involve the *REP-1* gene.[1] By contrast, no deletions have been found in the North American population yet (Merry et al., 1992). The size of the deletions varies widely and ranges from a few kilobases removing a single exon, to 5–15 Mb spanning the complete *REP-1* gene (Fig. 1). Only 3 out of 18 deletions are located entirely within the *REP-1* gene, while the others have at least one endpoint outside the gene. Deletion breakpoints show a random distribution except for 5 deletions that all end distally between exon 1 and pJ7.6B. These clustered endpoints have not been characterized in detail and the physical size of the chromosomal segment between exon 1 and pJ7.6B is not known. Apart from this region there are no obvious hotspots for the induction of chromosomal breakpoints near or within the *REP-1* gene.

Translocations

X-autosome translocations that involve the *REP-1* gene have been reported in two females suffering from choroideremia. One female carried an (X;13)(q21.2;p12) translocation and the other an (X;7)(q21.2;p12) translocation. Both females also showed ovarian dysgenesis, a common feature in X-autosomal translocations involving the proximal part of Xq (Teboul et al., 1989). The X-chromosomal breakpoint of the X;13 translocation was mapped to *REP-1* intron 12 (Cremers et al., 1990c). The X;7 translocation breakpoint is located either in intron 3 or in exon 4 of the *REP-1* gene (van Bokhoven et al., 1994b) (Fig. 1).

[1]This is based on the preliminary outcome of *REP-1* mutation screening in a large group of unrelated CHM patients. In 39 of these patients, the entire REP-1 protein coding has been screened: 10 patients have deletions, 16 patients have subtle mutations, and in the remaining 13 patients no mutations have been identified.

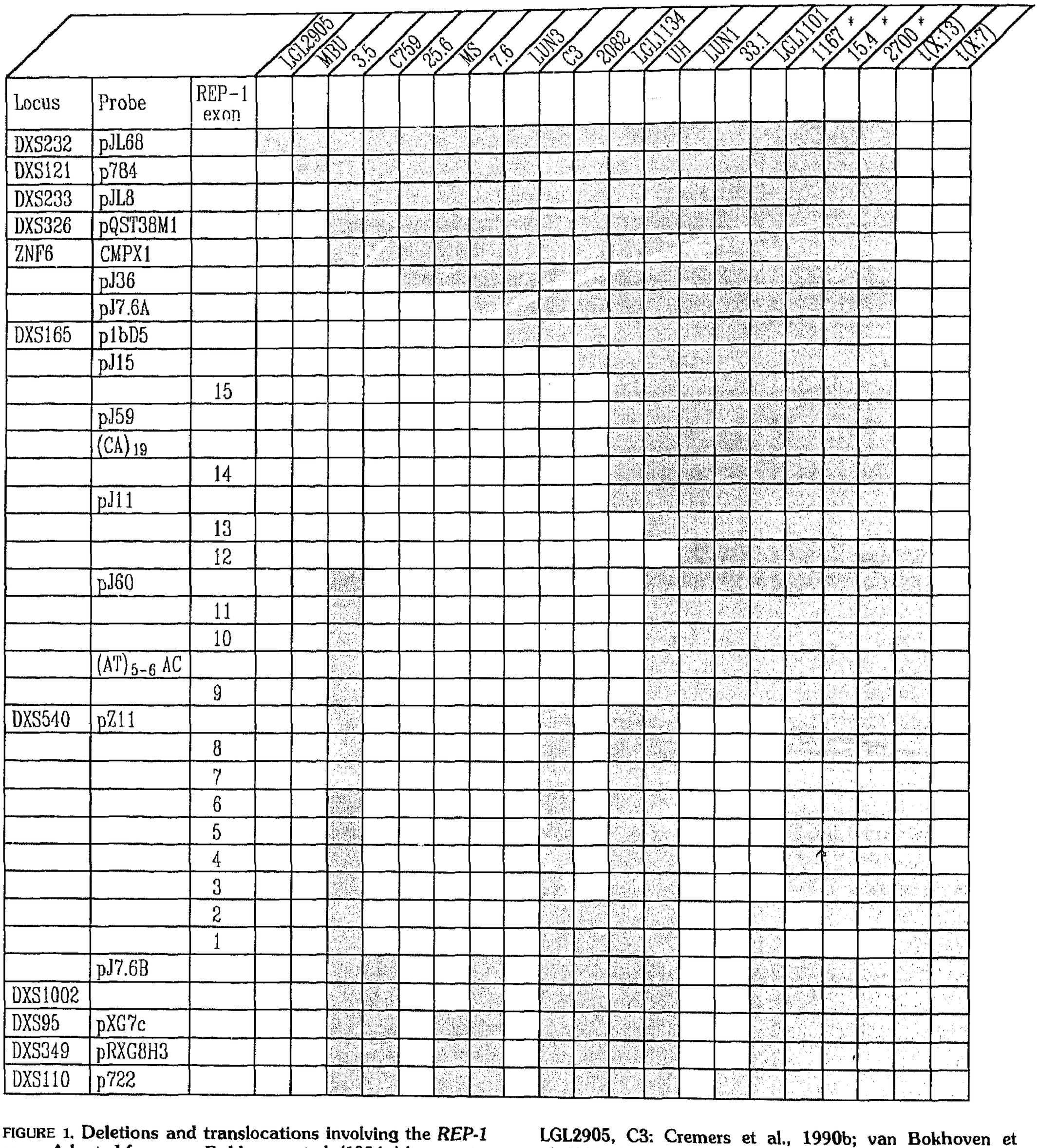

FIGURE 1. Deletions and translocations involving the *REP-1* gene. Adapted from van Bokhoven et al. (1994a) by permission of Oxford University Press. Asterisks mark deletions that have not been described previously. UH and MS are numbered 1 and 6, respectively, in the study by Schwartz et al (1993). Shaded areas indicate chromosomal regions which are present in the patient's DNA. The translocation breakpoints were mapped using human:hamster hybrid cell lines. (References for the deletions and translocations: LGL2905, C3: Cremers et al., 1990b; van Bokhoven et al.,1994a; MBU, LUN1, LUN3: van Bokhoven et al., 1994a; 3.5, 25.6, 7.6, LGL1134, 33.1, LGL1101: Cremers et al., 1990b, 1990c; van Bokhoven et al., 1994a; C759, 2082: Cremers and Ropers, 1994; van Bokhoven et al., 1994a; MS, UH: Schwartz et al., 1993; van Bokhoven et al., 1994a; 1167, 2700, 15.4: This study; t(X;13): Cremers et al., 1989b, 1990c; t(X;7): Philippe et al., 1993; van Bokhoven et al., 1994b).

Point Mutations and Small Insertions/Deletions

Nucleotide substitutions and small insertions/deletions that have been reported in the *REP-1* gene to date are listed in Table 1. These mutations were identified by single strand conformation analyses and heteroduplex analyses of the protein coding region of the *REP-1* gene.

TABLE 1. Nucleotide Substitutions and Small Insertions/Deletions in the *REP-1* Gene

Patient code	Position[a]		Mutation	Effect	References
Nonsense mutations					
1165	Exon 6	829	C→T	Arg267Stop	This study
LN	Exon 7	907	C→T	Arg293Stop	Van Bokhoven et al., 1994a
P20.854	Exon 8	1030	C→T	Gln334Stop	This study
2084	Exon 11	1388–1389	CC→G	Ser453Stop	Van den Hurk et al., 1992
2.1	Exon 12	1501	G→T	Glu491Stop	Van den Hurk et al., 1992
17.1	Exon 12	1514	C→A	Ser495Stop	Van den Hurk et al., 1992
TN[b]	Exon 12	1527	C→A	Cys499Stop	Schwartz et al., 1993
Frameshift mutations					
FK	Exon 5	555–556	delAG	Frameshift	Van Bokhoven et al., 1994a
BO	Exon 5	610	insA	Frameshift	Van Bokhoven et al., 1994a
P23.807	Exon 6	779	delA	Frameshift	This study
JS	Exon 8	1183	insC	Frameshift	Van Bokhoven et al., 1994a
1.2	Exon 12	1476	delA	Frameshift	Van den Hurk et al., 1992
2086; H; JW[b]	Exon 13	1614–1617	delTGTT	Frameshift	Van den Hurk et al., 1992; Pascal et al., 1993; Schwartz et al., 1993
CN[b]	Exon 14	1680–1681	delTT	Frameshift	Schwartz et al., 1993
Splice site mutations					
P21.551	Intron 9	1274 + 1	G→A	5′ splice signal	This study
P21.420	Intron 10	1379 + 2	insGGT	Aberrant splicing	This study
M	Exon 11	1442	A→T	5′ splice signal; Gln471Leu[c]	Donnelly et al., 1994
FC[b]	Intron 12	1541 − 2	A→G	Aberrant splicing	Schwartz et al., 1993
CHM*Sal	Intron 13	1639 + 2	insT	Aberrant splicing	Sankila et al., 1992
10.1	Intron 14	1801-1	G→A	3′ splice signal	This study

[a]The nucleotide positions correspond to the sequence published by Van Bokhoven et al. (1994b). The proposed nomenclature of Beaudet and Tsui (1993) is used for mutations in intron sequences.
[b]Patients CN, TN, FC, and JW are numbered 2, 3, 4, and 5, respectively, in the study by Schwartz et al. (1993).
[c]See text.

Nonsense Mutations and Frameshift Mutations

So far, seven nonsense mutations have been detected in the *REP-1* gene. In addition, seven different deletions and insertions were identified that result in translational frameshifts and premature termination codons (Table 1). One frameshift mutation, a deletion of the tetranucleotide TGTT in exon 13, was found in three apparently unrelated patients from Germany, France, and Denmark. It is of note that in the normal *REP-1* gene the TGTT tetranucleotide is duplicated, which may render this sequence particularly susceptible to mutation (e.g., by polymerase slippage during replication).

Splice Site Mutations

Included in Table 1 are six mutations that affect splice sites. Patient P21.420 has a 3-bp insertion at the 5′ splice site in intron 10. Reverse transcriptase PCR (RT-PCR) analysis (Fig. 2: primers 5 and 6) showed that in about 80% of the patient's *REP-1* mRNA exon 10 is absent, while the remaining 20% lacks exons 10 and 11. Skipping of exon 10 maintains the reading frame, but deletion of exons 10 and 11 causes a frameshift resulting in a premature termination codon (T.J.R.v.d.Pol and J.A.J.M. v.d.Hurk, unpublished observations).

Patient FC carries an acceptor splice site mutation in intron 12. RT-PCR analysis (Fig. 2: primers A and B) revealed that this mutation results in deletion of exon 13 from the *REP-1* transcript thereby introducing an inappropriate stop codon at the border of exons 12 and 14 (M. Schwartz, unpublished observations).

The single nucleotide insertion into the donor splice site in intron 13 in a large family from the Salla region of Northern Finland (CHM*Sal) leads to two aberrantly spliced mRNAs. One mRNA results from the use of a cryptic splice site downstream of the altered splice site and the other from the skipping of exon 13. Both mRNA products contain premature termination codons (Sankila et al., 1992).

In patient P21.551 a mutation was identified that alters a strictly conserved nucleotide of the splice site donor sequence (Mount, 1982; Ohshima and Gotoh, 1987) in intron 9. Patient 10.1 carries a mutation in the invariant AG dinucleotide of the acceptor splice site in intron 14. No formal proof that these mutations cause abnormal splicing has been obtained.

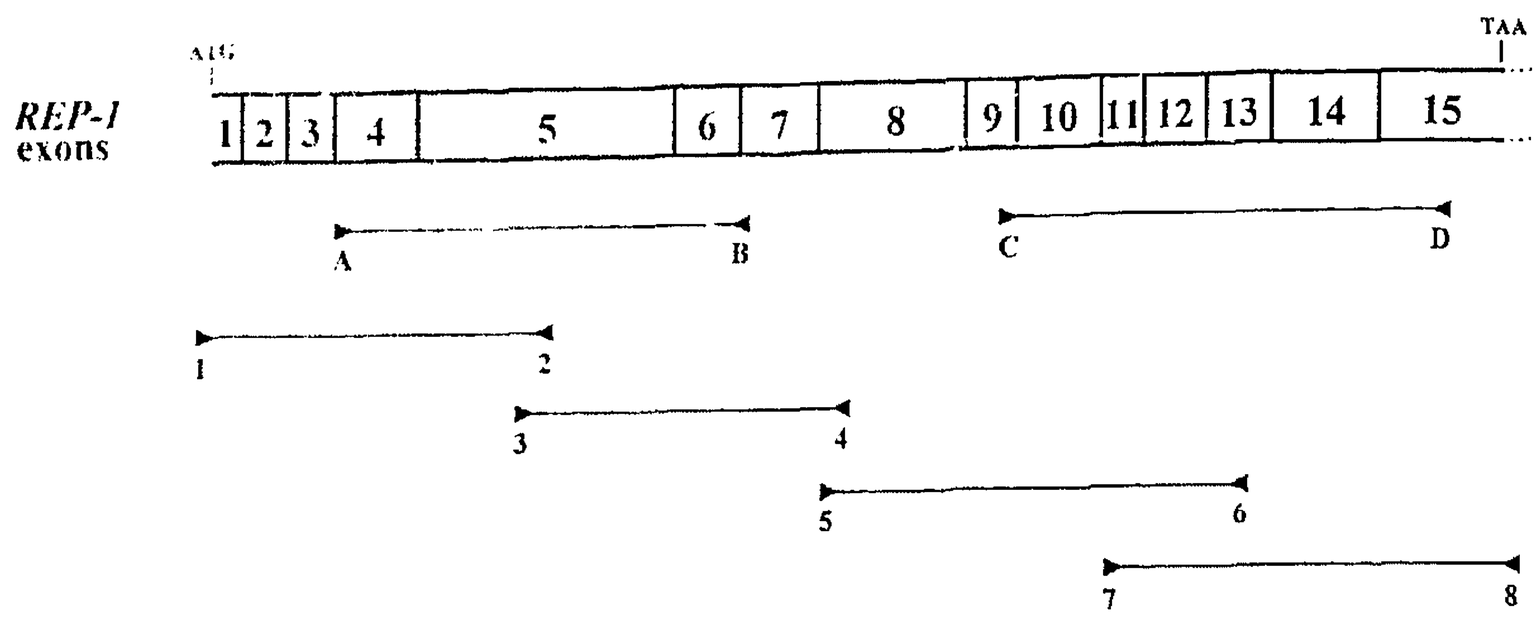

FIGURE 2. Primers for RT-PCR analysis of the *REP-1* mRNA. Primer A: GGAAAACAGTGACATTGTAAGT; primer B: CA-CGGAACCTGTTCCACTCTCG; primer C: GTGGACAAA-GAATCCAGAAA; primer D: AAGCCTCTGGCTGTAAA-CTG; primer 1: AGTCACATGACACGTTTCCCG; primer 2: GTCACTTCAGCACCATTTAC; primer 3: GATC-CAGAG-AATGCGCTAG; primer 4: TGGGGGTTAATTTTTGAGTC; primer 5: GATATGAAGAGATCACATTTT; primer 6: CT-GATTCTAAATCTTCTCTTGC; primer 7: GCAGATCTCCA-GGGCAGTG; primer 8: GGGTATCCAGTTTGGTGTATA)

In family M, an A→T change at position 1442 in exon 11 predicts the substitution of a leucine for a glutamine and was therefore reported as a possibly causative missense mutation (Donnelly et al., 1994). However, this nucleotide alteration also affects the 5′ splice junction in exon 11. The similarity to the consensus sequence is reduced from 86% for the authentic splice site (AG/gtaaat) to 78% for the mutated splice site (TG/gtaaat) (according to Shapiro and Senepathy, 1987). Whether this actually results in splicing artifacts has not been studied.

Van Bokhoven et al. (1994a) reported another potential splice acceptor mutation, a T→G change at position −14 in intron 4, in patient PU. However, RT-PCR analysis (Fig. 2: primers C and D) showed that this substitution does not affect *REP-1* RNA processing (M. Schwartz, unpublished observations).

POLYMORPHISMS

Table 2 lists the known polymorphisms in the *REP-1* gene. The two intragenic highly polymorphic microsatellite markers have a combined heterozygosity index of 0.96.

DISCUSSION

The deletions that are shown schematically in Figure 1 are all expected to result in an absent or a nonfunctional *REP-1* gene product. Not surprisingly there is no obvious correlation between the size or the breakpoints of the deletions and the clinical manifestation of choroideremia in these patients.

The two female patients carrying balanced X-autosome translocations that involve the *REP-1* gene (Fig. 1) show a mild disease course. Usually, in females with reciprocal X-autosomal translocations the normal X is preferentially inactivated while both translocation fragments remain active. However, there is evidence that the *REP-1* gene partly escapes X-chromosome inactivation (Carrel and Willard, 1993; T.J.R.v.d. Pol and J.A.J.M.v.d. Hurk, unpublished observations). The presence of a low level of functional *REP-1* transcript could account for the mild manifestation of choroideremia in these female patients.

Point mutations and small insertions/deletions have been found in exons 5–14 of the *REP-1* gene (Table 1). Recombinant REP-1 protein that lacks the 49 C-terminal amino acids is still able to assist in the geranylgeranylation of Rab proteins (F.P.M. Cremers and M. Seabra, unpublished observations), indicating that mutations in exon 15 may not cause CHM. The reason for the absence of subtle mutations in exons 1–4 is unclear.

The majority (70%) of small alterations in the *REP-1* gene are nonsense and frameshift mutations that result in premature translation termination codons (Table 1). In many instances, the primary consequence of a premature stop codon is severe or complete loss of mRNA from the mutant allele. A normal level may be retained if the inappropriate termination codon occurs toward the 3′ end of the transcript (McIntosh et al., 1993). If any protein is produced from the mutant allele it will be truncated at the C-terminus. Recently, it has been shown that recombinant REP-1 protein that lacks the 70 C-terminal amino acids is unable to function as a subunit of Rab GGTase (F.P.M. Cremers and M. Seabra, unpublished observations). Since the aforementioned nonsense and frameshift mutations in the *REP-1* gene predict C-terminal truncations of more than 70 amino acids, it is clear that

TABLE 2. Polymorphisms in the *REP-1* Gene

Location	Polymorphism	Heterozygosity	References
Exon 5	381 G/A	0.40	This study
Intron 8	pZ11a-*Eco*RV	0.50	Molloy et al., 1992
	pZ11a-*Msp*I	0.43	
	pZ11c-*Eco*RI	0.46	
Intron 9	$(AT)_{5-6}AC$ repeat	0.87	Van Bokhoven et al., 1996
Intron 14	$(CA)_{19}$ repeat	0.67	Van Bokhoven et al., 1994d

any resulting REP-1 proteins will be nonfunctional.

The remaining 30% of small alterations in the *REP-1* gene are mutations that affect splice sites (Table 1). In family M, the A→T change at position 1442 not only alters the 5′ splice site in exon 11 but also predicts a glutamine to leucine substitution. The effect of the point mutation has not been determined directly, yet Western blot analysis failed to detect any REP-1 protein in EBV transformed lymphoblasts of a patient from this family (M. Seabra and O. Pascal, personal communication). Although it cannot be excluded that an amino acid substitution generates a highly unstable protein, it is more likely that the A→T change affects RNA splicing or, since only one third of the coding region was screened, that there is another mutation in the *REP-1* gene in this family. Further studies are required to establish the status and effect of the A→T change. Three splice site mutations listed in Table 1 have been characterized at the transcript level. One of these (P21.420) results in a mixture of in-frame and frameshifted mRNAs (T.J.R.v.d. Pol and J.A.J.M.v.d. Hurk, unpublished observations). The other two splice site mutations (FC and CHM*Sal) lead to aberrantly spliced transcripts containing premature termination codons that predict C-terminal protein truncations of more than 70 amino acids (M. Schwartz, unpublished observations; Sankila et al., 1992).

The (vast majority of) subtle mutations appear(s) to completely inactivate the *REP-1* gene product. This finding explains why there is no apparent correlation between the clinical severity of the disorder and the underlying mutations. The striking absence of missense mutations may indicate that these have no clinical effect. However, this would imply a low pressure on sequence conservation, which is contradicted by the high degree of homology between the human, rat, and mouse sequences, and by the fact that only one polymorphism was ever found in the protein coding region of the human *REP-1* gene. A more likely possibility would be that missense mutations give rise to a different disease phenotype. Alternatively, they may be lethal because of interference with the function of the *REP-2* gene product; for example, the resulting REP-1 proteins may be able to irreversibly bind the α/β catalytic component of Rab GGTase.

The spectrum of *REP-1* gene defects described here enables the definition of the most efficient strategy for future mutation screening. So far, detection of *REP-1* mutations has largely been performed on genomic DNA. In view of the frequent occurrence of gross rearrangements, a preliminary screen for (partial) *REP-1* deletions by polymerase chain reaction (PCR) amplification of exons 1, 8, and 15 would be a logical first step in mutation detection. Subsequently, all exons and adjacent intronic sequences could be PCR-amplified from genomic DNA and scanned for small alterations using single-strand conformation analysis (Orita et al., 1989) or heteroduplex analysis (White et al., 1992). Both methods are attractive because of their technical simplicity and relative sensitivity (Perry et al., 1992; White et al., 1992; Sheffield et al., 1993). Alternatively, mutation analysis could be carried out on mRNA derived from lymphoblasts. This might be a more practical starting material since larger stretches of coding sequences can be screened, and the effect of the mutation on transcript structure can be readily observed. The first step in mRNA analysis, RT-PCR (Fig. 2), detects gross rearrangements and mutations that affect splicing. More subtle mutations in the RT-PCR products can be detected by chemical mismatch cleavage (Cotton et al., 1988) or protein truncation tests (PTT) (Roest et al., 1993). The latter method specifically detects translation termination mutations. Since virtually all nondeletion CHM cases analyzed so far are small mutations that introduce premature termination codons, PTT might be the method of choice for *REP-1* mutation screening.

In approximately one-third of European CHM patients screened for defects in the *REP-1* protein coding region, the causative mutation has not

been identified. It should be stressed that this is generally not a problem for genetic counseling because in most (familial) cases intragenic polymorphisms (Table 2), in particular the two highly polymorphic microsatellites, enable accurate presymptomatic diagnosis and carrier detection. However, in sporadic CHM cases in which the diagnosis is less certain, mutation detection remains inevitable.

ACKNOWLEDGMENTS

This work was supported by the Netherlands Organization for Scientific Research (NWO), the Deutsche Forschungsgemeinschaft (DFG), the British Retinitis Pigmentosa Society (BRPS), "Hotelejer Carl Larsen og Hustru Nicoline Larsens mindelegat", and by the kind donations of several Dutch organizations i.e., De Drie Lichten, Landelijke Stichting voor Blinden en Slechtzienden, Rotterdamse Vereniging Blindenbelangen, Stichting Blindenhulp, Gelderse Blindenvereniging, Stichting Blindenpenning, and the Algemene Nederlandse Vereniging ter Voorkoming van Blindheid. The research of F.P.M.C. has been made possible by a fellowship from the Royal Netherlands Academy of Arts and Sciences.

REFERENCES

Alexandrov K, Horiuchi H, Steele-Mortimer O, Seabra MC, Zerial M (1994) Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes. EMBO J 13:5262–5273.

Andres DA, Seabra MC, Brown MS, Armstrong SA, Smeland TE, Cremers FPM, Goldstein JL (1993) cDNA cloning of component A of Rab geranylgeranyl transferase and demonstration of its role as a Rab escort protein. Cell 73:1091–1099.

Beaudet AL, Tsui L-P (1993) A suggested nomenclature for designating mutations. Hum Mutat 2:245–248.

Benito-Moreno RM, Miaczynska M, Bauer BE, Schweyen RJ, Ragnini A (1994) Mrs6p, the yeast homologue of the mammalian choroideraemia protein: Immunological evidence for its function as the Ypt1p Rab escort protein. Curr Genet 27:23–25.

Carrel L, Willard HF (1993) The X-linked choroideremia gene escapes X chromosome inactivation. Am J Hum Genet 53(suppl):abst 42.

Cotton RGH, Rodrigues NR, Campbell RD (1988) Reactivity of cytosine and thymine in single-base-pair mismatches with hydroxylamine and osmium tetroxide and its application to the study of mutations. Proc Natl Acad Sci USA 85:4397–4401.

Cremers FPM, Ropers H-H (1994) Choroideremia. In Wright A, Jay B (eds): Molecular Genetics of Inherited Eye Disorders. Chur: Harwood Academic Publishers GMBH, pp 303–319.

Cremers FPM, Brunsmann F, van de Pol TJR, Pawlowitzki IH, Paulsen K, Wieringa B, Ropers H-H (1987) Deletion of the DXS165 locus in patients with classical choroideremia. Clin Genet 32:421–423.

Cremers FPM, van de Pol TJR, Diergaarde PJ, Wieringa B, Nussbaum RL, Schwartz M, Ropers H-H (1989a) Physical fine mapping of the choroideremia locus using Xq21 deletions associated with complex syndromes. Genomics 4:41–46.

Cremers FPM, van de Pol TJR, Wieringa B, Collins FS, Sankila E-M, Siu VM, Flintoff WF, Brunsmann F, Blonden LAJ, Ropers H-H (1989b) Chromosomal jumping from the DXS165 locus allows molecular characterization of four microdeletions and a de novo chromosome X/13 translocation associated with choroideremia. Proc Natl Acad Sci USA 86:7510–7514.

Cremers FPM, Brunsmann F, Berger W, van Kerkhoff EPM, van de Pol TJR, Wieringa B, Pawlowitzki IH, Ropers H-H (1990a) Cloning of the breakpoints of a deletion associated with choroideremia. Hum Genet 86:61–64.

Cremers FPM, Sankila E-M, Brunsmann F, Jay M, Jay B, Wright A, Pinckers AJLG, Schwartz M, van de Pol TJR, Wieringa B, de la Chapelle A, Pawlowitzki IH, Ropers H-H (1990b) Deletions in patients with classical choroideremia vary in size from 45 kb to several megabases. Am J Hum Genet 47:622–628.

Cremers FPM, van de Pol TJR, van Kerkhoff EPM, Wieringa B, Ropers H-H (1990c) Cloning of a gene that is rearranged in patients with choroideraemia. Nature 347:674–677.

Cremers FPM, Molloy CM, van de Pol TJR, van den Hurk JAJM, Bach I, Geurts van Kessel AHM, Ropers H-H (1992) An autosomal homologue of the choroideremia gene colocalizes with the Usher syndrome type II locus on the distal part of chromosome 1q. Hum Mol Genet 1:71–75.

Cremers FPM, Armstrong SA, Seabra MC, Brown MS, Goldstein JL (1994) REP-2, a Rab escort protein encoded by the choroideremia-like gene. J Biol Chem 269:2111–2117.

Donnelly P, Menet H, Fouanon C, Herbert O, Moisan JP, Le Roux MG, Pascal O (1994) Missense mutation in the choroideremia gene. Hum Mol Genet 3:1017.

Fraser GRF, Friedmann AI (1968) Choroideremia in a female. BMJ 2:732–734.

Fujimura K, Tanaka K, Nakano A, Toh-e A (1994) The Saccharomyces cerevisiae MSI4 gene encodes the yeast counterpart of component A of Rab geranylgeranyltransferase. J Biol Chem 269:9205–9212.

Goedbloed J (1942) Mode of inheritance in choroideremia. Ophthalmologica 104:308–315.

Harris GS, Miller JR (1968) Choroideremia. Visual defects in a heterozygote. Arch Ophthalmol 80:423–429.

Heckenlively JR, Bird AC (1988) Choroideremia. In Heckenlively JR (ed): Retinitis Pigmentosa. Philadelphia: JB Lippincott, pp 176–187.

Jiang Y, Ferro-Novick S (1994) Identification of yeast component A: Reconstitution of the geranylgeranyltransferase that modifies Ypt1p and Sec4p. Proc Natl Acad Sci USA 91:4377–4381.

Jiang Y, Rossi G, Ferro-Novick S (1993) Bet2p and Mad2p are components of a prenyltransferase that adds geranylgeranyl onto Ypt1p and Sec4p. Nature 366:84–86.

Kaplan J, Gilgenkrantz S, Dufier JL, Frézal J (1989) Choroideremia and ovarian dysgenesis associated with an X;7 de novo balanced translocation. Human Gene Mapping 10: Tenth International Workshop on Human Gene Mapping. Cytogenet Cell Genet 51:1022.

Lesko JG, Lewis RA, Nussbaum RL (1987) Multipoint linkage analysis of loci in the proximal long arm of the human X chromosome: Application to mapping the choroideremia locus. Am J Hum Genet 40:303–311.

Lewis RA, Nussbaum RL, Ferrell R (1985) Mapping X-linked ophthalmic diseases: Provisional assignment of the locus for choroideremia to Xq13–q24. Ophthalmology 92:800–806.

McIntosh I, Hamosh A, Dietz HC (1993) Nonsense mutations and diminished mRNA levels. Nature Genet 4:219.

Merry DE, Jänne PA, Landers JE, Lewis RA, Nussbaum RL (1992) Isolation of a candidate gene for choroideremia. Proc Natl Acad Sci USA 89:2135–2139.

Molloy CM, van de Pol TJR, Brohet RM, Ropers H-H, Cremers FPM (1992) Three RFLPs for pZ11 (DXS540) in the choroideremia gene at Xq21.2. Nucleic Acids Res 20:1434.

Mount SM (1982) A catalogue of splice junction sequences. Nucleic Acids Res 10:459–472.

Nussbaum RL, Lewis RA, Lesko JG, Ferrell R (1985) Choroideremia is linked to the restriction fragment length polymorphism *DXYS1* at Xq13-21. Am J Hum Genet 37:473–481.

Ohshima Y, Gotoh Y (1987) Signals for the selection of a splice site in pre-mRNA: Computer analysis of splice junction sequences and like sequences. J Mol Biol 195:247–259.

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879.

Pascal O, Donnelly P, Fouanon C, Herbert O, Le Roux MG, Moisan JP (1993) A new (old) deletion in the choroideremia gene. Hum Mol Genet 2:1489.

Perry DJ, Carrell RW (1992) Hydrolink gels: a rapid and simple approach to the detection of DNA mutations in thromboembolic disease. J Clin Pathol 45:158–160.

Philippe C, Cremers FPM, Chery M, Bach I, Abbadi N, Ropers H-H, Gilgenkrantz S (1993) Physical mapping of DNA markers in the q13–q22 region of the human X chromosome. Genomics 17:147–152.

Ragnini A, Teply R, Waldherr M, Voskova A, Schweyen RJ (1994) The yeast protein Mrs6p, a homologue of the rabGDI and human choroideraemia proteins, affects cytoplasmic and mitochondrial functions. Curr Genet 26:308–314.

Roest PAM, Roberts RG, Sugino S, van Ommen G-JB, den Dunnen JT (1993) Protein truncation test (PTT) for rapid detection of translation-terminating mutations. Hum Mol Genet 2:1719–1721.

Rosenberg T, Niebuhr E, Yang H-M, Parving A, Schwartz M (1987) Choroideremia, congenital deafness and mental retardation in a family with an X chromosomal deletion. Ophthalm Paediatr Genet 8:139–143.

Sankila E-M, Lehner T, Eriksson AW, Forsius H, Kärnä J, Page D, Ott J, de la Chapelle A (1989) Haplotype and multipoint linkage analysis in Finnish choroideremia families. Hum Genet 84:66–70.

Sankila E-M, Tolvanen R, van den Hurk JAJM, Cremers FPM, de la Chapelle A (1992) Aberrant splicing of the CHM gene is a significant cause of choroideremia. Nature Genet 1:109–113.

Schwartz M, Yang H-M, Niebuhr E, Rosenberg T, Page DC (1988) Regional localization of polymorphic DNA loci on the proximal long arm of the X chromosome using deletions associated with choroideremia. Hum Genet 78:156–160.

Schwartz M, Rosenberg T, van den Hurk JAJM, van de Pol TJR, Cremers FPM (1993) Identification of mutations in Danish choroideremia families. Hum Mutat 2:43–47.

Seabra MC, Brown MS, Slaughter CA, Südhof TC, Goldstein JL (1992a) Purification of component A of Rab geranylgeranyl transferase: Possible identity with the choroideremia gene product. Cell 70:1049–1057.

Seabra MC, Goldstein JL, Südhof TC, Brown MS (1992b) Rab geranylgeranyl transferase: A multisubunit enzyme that prenylates GTP-binding proteins terminating in Cys-X-Cys or CysCys. J Biol Chem 267:14497–14503.

Seabra MC, Brown MS, Goldstein JL (1993) Retinal degeneration in choroideremia: Deficiency of Rab geranylgeranyl transferase. Science 259:377–381.

Seabra MC, Ho YK, Anant JS (1995) Deficient geranylgeranylation of Ram/Rab27 in choroideremia. J Biol Chem 270:24420–24427.

Shapiro MB, Senepathy P (1987) RNA splice junctions of different classes of eukaryotes: Sequence statistics and functional implications in gene expression. Nucleic Acids Res 15:7155–7174.

Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM (1993) The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16:325–332.

Siu VM, Gonder JR, Jung JH, Sergovich FR, Flintoff WF (1990) Choroideremia associated with an X-autosomal translocation. Hum Genet 84:459–464.

Teboul M, Mujica P, Chery M, Leotard B, Gilgenkrantz S (1989) Translocations X-autosomes équilibrées et retard mental. Contribution à la cartographie des retards mentaux liés à L'X (à l'exclusion de L'X-FRA). J Genet Hum 37:179–195.

van Bokhoven H, Schwartz M, Andrésson S, van den Hurk JAJM, Bogerd L, Jay M, Rüther K, Jay B, Pawlowitzki IH, Sankila E-M, Wright A, Ropers H-H, Rosenberg T, Cremers FPM (1994a) Mutation spectrum in the CHM gene of Danish and Swedish choroideremia patients. Hum Mol Genet 3:1047–1051.

van Bokhoven H, van den Hurk JAJM, Bogerd L, Philippe C, Gilgenkrantz S, de Jong P, Ropers H-H, Cremers FPM (1994b) Cloning and characterizaton of the human choroideremia gene. Hum Mol Genet 3:1041–1046.

van Bokhoven H, van Genderen C, Molloy CM, van de Pol TJR, Cremers CWRJ, van Aarem A, Schwartz M, Rosenberg T, Geurts van Kessel AHM, Ropers H-H, Cremers FPM (1994c) Mapping of the choroideremia-like (CHML) gene at 1q42-qter and mutation analysis in patients with Usher syndrome type II. Genomics 19:385–387.

van Bokhoven H, van Genderen C, Ropers H-H, Cremers FPM (1994d) Dinucleotide repeat polymorphism within the choroideremia gene at Xq21.2. Hum Mol Genet 3:1446.

van Bokhoven H, van den Hurk JAJM, Bogerd L, van de Pol TJR, Ropers H-H, Cremers FPM (1996) A highly polymorphic microsatellite marker located within the choroideremia gene. Ophthalmic Genetics 17, in press.

van den Hurk JAJM, van de Pol TJR, Molloy CM, Brunsmann F, Rüther K, Zrenner E, Pinckers AJLG, Pawlowitzki IH, Bleeker-Wagemakers EM, Wieringa B, Ropers H-H, Cremers FPM (1992) Detection and characterization of point mutations in the choroideremia candidate gene by PCR-SSCP analysis and direct DNA sequencing. Am J Hum Genet 50:1195–1202.

Waardenburg PJ (1942) Chorioideremie als Erbmerkmal. Acta Ophthalmol 20:235–274.

Waldherr M, Ragnini A, Schweyen RJ, Boguski MS (1993) MRS6—yeast homologue of the choroideraemia gene. Nature Genet 3:193–194.

White MB, Carvalho M, Derse D, O'Brien SJ, Dean M (1992) Detecting single base substitutions as heteroduplex polymorphisms. Genomics 12:301–306.