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UHX1 and *PCTK1*: precise characterisation and localisation within a gene-rich region in Xp11.23 and evaluation as candidate genes for retinal diseases mapped to Xp21.1–p11.2

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The gene for ubiquitin hydrolase on the X chromosome (*UHX1*), cloned and mapped to Xp21.2–p11.2, is a candidate gene for retinal diseases. We used fine mapping techniques to localise *UHX1* between markers DXS1266 and DXS337, where congenital stationary night blindness (XICSNB) and retinitis pigmentosa type 2 (RP2) are also located. Reevaluation of the *UHX1* gene structure demonstrated five new exons, for a total of 21 exons and a predicted protein product of 963 amino acids. Evaluation of patients revealed no *UHX1* mutations using SSCP (10 CSNB1 and 20 XLRP) or deletion screening with cDNA hybridisation (13 CSNB1 and 43 XLRP). Likewise, no aberrations were found in the nearby *PCTAIRE1* (*PCTK1*) gene in 13 CSNB1 and 43 XLRP patients by deletion screening. Thus mutations of *UHX1*, and probably *PCTK1*, do not appear to cause common X-linked eye diseases. *UHX1*'s role in patients with mental retardation may be appropriate for further investigations into *UHX1* function.

Keywords: UHX1; PCTK1; retinal disease; mental retardation

Introduction

Ubiquitin hydrolases are a group of enzymes found in multiple tissues and involved in regulation of cellular protein function through cleavage of ubiquitin from its conjugated forms. These enzymes thus play an important role in protein degradation and reversible protein modification. A novel gene encoding a ubiquitin C-terminal hydrolase on the X chromosome (*UHX1*) has recently been described.[1] A widespread tissue expression with a five- to ten-fold higher expression in the retina was shown. Because of this high retinal expression and evidence suggesting abnormalities in protein processing as the etiology of several retinal diseases,[1] *UHX1* was proposed as a candidate gene for retinal diseases. Another gene in this region, *PCTAIRE*-1 (*PCTK1*), is a member of *PCTAIRE*, a subfamily of the cdc2-related serine-threonine specific

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protein kinase family and involved in cell cycle regulation.[2] *UHX1*, ubiquitin activating enzyme E1 *(UBE1)* and *PCTK1*[3] have all previously been mapped to Xp21.2–p11.2, a region known to contain several retinal diseases, including X-linked congenital stationary night blindness,[4–7] X-linked retinitis pigmentosa types 2 and 3 (RP2, RP3)[8–11] and X-linked cone dystrophy (XLCOD),[12] (for review see Rosenfeld *et al.*[13]).

In addition to *UHX1*'s possible retinal function, recent data suggest a role for ubiquitin C-terminal hydrolase in long-term facilitation in *Aplysia*.[14] This investigation into learning and memory pathways in a mollusk model might carry important implications for causation of mental retardation, learning disorders and memory disturbances in humans. Interestingly, several X-linked mental retardation families also map to this region of the X chromosome (for review see Lubs *et al*.[15]) including one family with XLMR and XLRP.[16] Thus the actual function(s) of *UHX1* in neurologic and possibly other pathways is at present unclear.

Our investigation explores several aspects of *UHX1* and *PCTK1* gene structure and function. The possible role of *UHX1* involvement in learning and memory is still preliminary and beyond the scope of this investigation. We concentrated instead on the possible causation of retinal disease by *UHX1* and *PCTK1*. First, we performed fine mapping of these two genes to determine exactly which of the retinal diseases localised to this region of the X chromosome might be caused by *UHX1* or *PCTK1* mutations. Next, determination of the precise genomic structure was done to allow accurate identification of gene defects. Finally, mutation screening was performed to examine the possible causative role of *UHX1* and *PCTK1* in CSNB1 or RP2, the retinal diseases located in the same region.

Materials and Methods

Probes and Hybridisation

A probe for the *ZNF 157* gene[17] was obtained with primers published by Carrel *et al*.[3] The 5'-*UHX1* probe was derived with nested primers: X1F: AAGAAAGATGGCACTTGGCCC, X1R: TCGTCCTCATCATCTGAGTTGGG, X1aF: ACAGCTGCATGTCATGAAcAAC, X1aR: TGGTCACGTAGCGTGAGAGC. The 3'*UHX1* probe was derived with the following nested primers: X2F: ATGCTTTTTGGACACCCCCTC, X2R: ATCCATGAACTCAGAGCTGGG, X2aF: ACCGCTTCACCTGGGAGGG, X2aR: AGCTGCAGGCAGGGGAGGC. The *PCTK1* probes were derived with the following nested primer pairs: Pct1F: ATGAAGAAGATCAAACGGCAGC, Pct1R: TGTGGCATCCCTCCAGCCG, Pct2F: ATAGACAAGACCAATGGTGCC, Pct2R: TCTGTGGCTTAGAACTCGGTG. A probe for the *DXS8237E* gene was produced using the primer pairs: F2: CCAGTCTCG GACTTGGTTG, R4: AGGCTGGGGGCGAGAGAAAG; F3: GGCTGAGCTGGG AGAGTTGG, R5: TGCATCGACACAGAGTCGG. The NCBI clones R41358 and D86969 were kindly provided by Reference Library Data Base (RLDB), Berlin-Charlottenberg:[18] inserts from both clones were obtained by using vector-specific primers. Hybridisation of patient DNA was carried out in Church buffer with stringent washing conditions (0.1 × SSC).

Mapping Panels and PACs

Radiation-induced somatic cell hybrids A19D9 and A19E8 were generated as described.[19] Polymorphic microsatellites used for characterisation are contained in the Généthon human linkage map.[20] The ICRF- and CEPH YACs used as mapping resources (Figure 1) were obtained from RLDB, Berlin.[18] PACs containing the markers DXS1264, DXS1003, DXS1266, DXS337, and ELK1 were isolated from the deJong PAC library by the Sanger Centre (http://www.sanger.ac.uk/HEP/Chr.X/; Xctg311, Xctg448, Xctg597) and distributed by RLDB, Berlin; PACs containing *DXS8237E* and/or *UHX1* were isolated by hybridisation from the deJong PAC library and provided by RLDB, Berlin.

Identification and Analysis of Genomic Clones

The Lawrence Livermore X cosmid library distributed by RLDB, Berlin, was hybridised with a partial *UHX1* cDNA. Hybridisation with the 5' and 3' parts of the published *UHX1* cDNA revealed a complete representation of *UHX1* on cosmid LLNL c110E2210Q. This cosmid was sequenced after subcloning in pUC vectors. The pUC clones were shotgun-sequenced using dye-terminator chemistry. The sequences were assembled and edited with the XGAP program.[21] Gaps were closed using custom-made primers on pUC templates, PCR-products or cosmid DNA. The finished sequence revealed a contig of 41536 base pairs (GDB acc. no. U62534). Exon-intron boundaries were obtained by comparing the genomic sequence with the published cDNA sequence and by exon-prediction programs MC-VECTOR, FEXH AND XGRAIL 1a/2.

PCR Reactions and SSCP Analysis

Annealing temperatures for the primer pairs are listed in Table 1. All 21 exons of the *UXH1* gene were amplified under the following conditions: after an initial denaturation for 5 min at 94°C, denaturation was at 94°C for 1 min, annealing at the exon-specific temperature for 1 min and extension at 72°C for 35 cycles. Amplified fragments from all exons were analysed by SSCP[22] using Hydrolink™ (AT Biochem., Malvern, PA, USA) or SERDO-gels (Boehringer-Ingelheim Bioproducts Partnership, Heidelberg). Staining was performed with Sybrgreen (Molecular-Probes Europe BV, Leiden, The Netherlands), and band visualisation was done with Fluorlmager (Molecular Dynamics GmbH, Krefeld, Germany), both according to the manufacturer's recommendations.

DNA samples

DNA was extracted from peripheral blood samples from unrelated patients with X-linked retinal diseases: 43 affected individuals from RP families without *RPGR* gene mutations (phenotypes as described by Meindl *et al.*,[23] and 13 affected

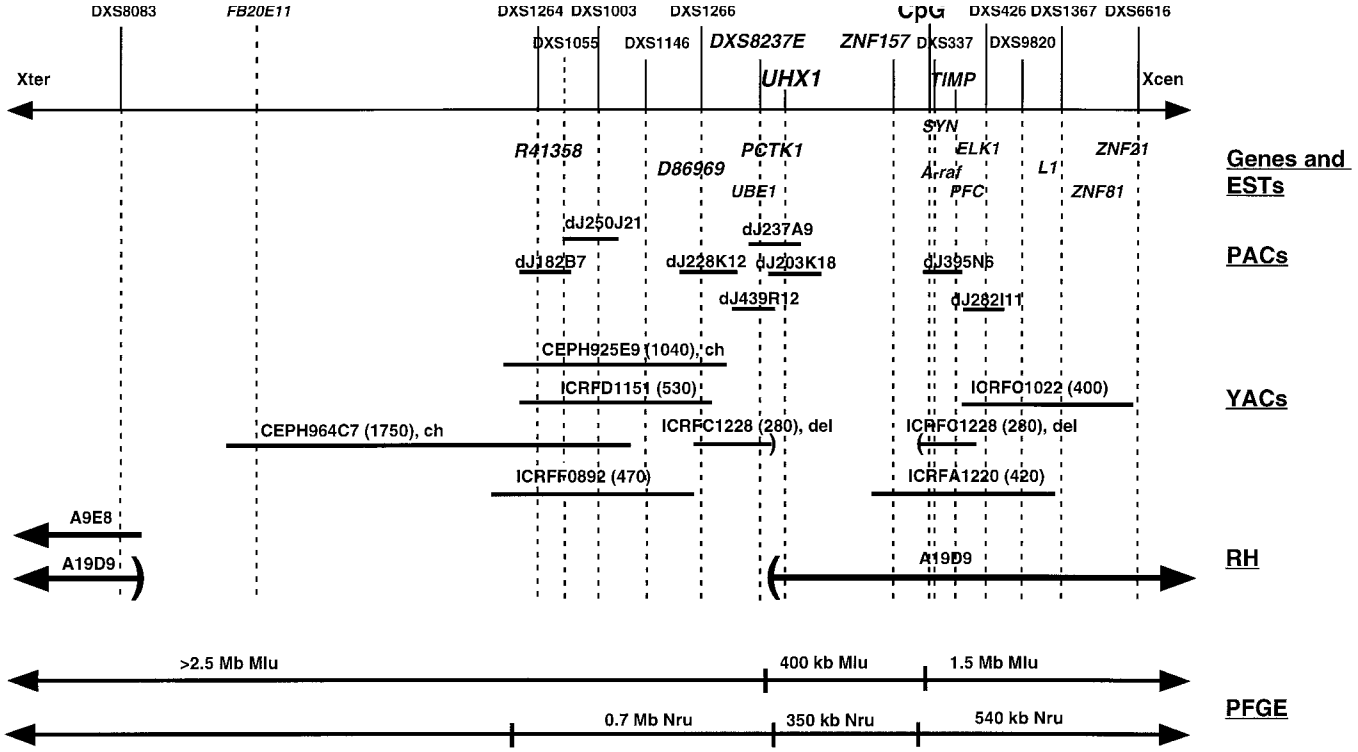

Figure 1 *Fine mapping of* UHX1 *between DXS1266 and DXS337. (**a**) A schematic representation of a physical map between DXS8083 and DXS6616 is shown. A19E8 and A19D9 (RH) are radiation hybrids,[19] PACs from the P deJong library (RPCI – 1 and 3) were obtained either from Sanger Centre or RLDB, Berlin; YACs (sizes in brackets) were either from the ICRF or CEPH libraries (http://www.ceph.fr/cgi-bin/http_infoclones) and provided by RLDB, Berlin. R41368 and D86969 are NCBI ESTs initially mapped between DXS1201 and DXS1039 (http://www.ncbi.nlm.nih.gov/cgi-bin/ SCIENCE 96). DNA markers given above are published.[26,27] Deletion of ICRF YAC y900C1228 was shown by PFGE analysis.*

individuals from X-linked CSNB families (E Zrenner, unpublished data, 1997). The 43 RP families contained only a single family identified as recombinant with the RP3 interval, whilst the remaining 42 could be either RP2 or RP3 families based on linkage studies.

Results

Fine Mapping of UHX1 *Between the Markers DXS1266 and DXS337*

A cDNA probe encompassing the 3' part of the published *UHX1* sequence was used to hybridise DNAs from different radiation hybrids,[19] each maintaining different portions of Xp11.4–Xp11.22. *UHX1* was present on radiation hybrid A19D9, while probes for *DXS8237E*,[24], *D86969* and R41358 (NCBI ESTs located between the markers DXS1201 and DXS1039) demonstrated no hybridisation signal. The proximal localisation of *UHX1* was further confirmed by PFGE analysis, which showed the *UHX1* gene together with the *ZNF157* gene[17] on a 350 kb NruI fragment, whilst *DXS8237E* and *D86969* were located on a 700 kb NruI fragment. *DXS8237E* and *UHX1* also segregated with different MluI fragments (Figure 1 and Figure 2). The NCBI EST R41358 was mapped to the PAC dJ182B7, which is part of a contig around DXS1055 and DXS1003. It also identified the 700 kb NruI fragment and 3 Mb MluI fragment in genomic DNA, but was not present in a constructed PAC contig encompassing the region between the markers DXS1266 and *UHX1*. Thus, DXS1055 and DXS1003 were clearly distal to the marker DXS1266. The IMAGE clone D86869, which also identified the large MluI fragment and the 700 kb NruI fragment, was mapped to the PAC dJ228K12, suggesting a localisation of this EST between DXS1003 and *DXS8237E*. PACs containing the markers DXS1264 (dJ182B7), DXS1003 (dJ250J21), DXS1266 (dJ228K12), DXS337 (dJ395N6) and ELK1 (dJ282I11) were identified using the Acedb software of Sanger Centre (X chromosome status map: 45000 kb-47000 kb); PACs containing the genes *DXS8237E*, *PCTK1* and/or *UHX1* were isolated from

Table 1 Exon-intron organisation of the *UHX1* gene

Exon	*Position*	*Exon size*	*Splice acceptor*	*Splice donor*	*Intron size*
1	1–439	439	CTTTCCATGC	GCGAAAGCTGgtgaggctgggctgc	5852
2	440–549	109	ctgtctgggccccagGTTCCTTGTG	CTCTTTCAAGgtacaaggcctttgc	173
3	550–680	130	acccccgccccacagATGAGATAAA	TGAACGCAAGgtataatggatgggg	309
4	681–798	117	cctctactcttacagGTCATAGAGC	GATTCTATTGgtgagtctaagggtc	398
5	799–944	195	catcaccccgcacagGCCTAGTATT	GACTGGGCAGgtaagggtggggagg	137
6	945–1006	61	ctttctccaacccagTTGATCATCA	ZGCATGTCATgtgagcccttggggt	126
7	1007–1109	102	ctcatctaaccccagGAACAACAAC	GGGCCCTGCAGgttgggccattatag	401
8	1110–1283	173	ctctggcctctgcagTGCCTCAGCA	TGTGTTCAAGgtgtgactcaaccct	91
9	1284–1442	158	tgcccatcttcttagAACAAGGTTG	ACCGGATCAGgtaggctgccccgc	383
10	1443–1667	224	tgacactatcaacagGAGGTGGCAC	GCCAGAGCAGgtgtggggcagtggg	133
11	1668–1751	83	ccgttttcccttagCACCGGCTCG	GCCAGAGAGGgtgagactgcagaag	104
12	1752–1846	94	atatcccattcctagATGATGGTGG	ATATCTTCGTgtgagtggggatggc	676
13	1847–2053	206	gtccacccccacagCTATGAGGTG	ACCGGCTCTCgtaagtgtcctcttc	897
14	2054–2106	62	tctgctctgttgcagACGCTACGTG	GATGAGAAAGgtgaggggggctaaca	132
15	2107–2349	242	atcctcctgtcctagAAGATGACGA	GAAGTCCATGgtatttcctttggct	92
16	2350–2420	70	ctccctcacccccagCCCAGCCGTA	AGAGGCTGAGgtaaatgagatccca	284
17	2421–2533	112	ttcacccaatcctagGGCTACGTGA	AAAACCCCTGgtgaggggccagagc	1590
18	2534–2683	149	gggctctgtctgtagGTACTGCCCT	TTCCTATCCGgtcaggggccaggga	83
19	2684–2799	115	tctccccacccacagGGACCTGGAC	GATGGACACTgtatgtccaggctg	192
20	2800–2888	88	ctccttccctttcagACACAACATT	TCAGATCGAGgtgtgacttccatcc	94
21	2889–3423	534	gtcctctccccacagTCCAAGGCAG	TCTGCTCCCC	

the deJong PAC library and provided by RLDB, Berlin (see Figure 1: dJ439R12, dJ237A9, dJ203K18).

The new localisation of *UHX1* between DXS1266 and DXS337 in Xp11.23 excluded it as a candidate gene for RP3 and XLCOD, and narrowed the field of possibly-related retinal diseases to RP2 and CSNB1.[4,6,25]

Genomic structure of the UHX1 *gene*

Close linkage of *UHX1* to *DXS8237E* and *PCTK1* was shown by the hybridisation of these genes to the PAC dJ237A9. All genes were found on this PAC, while PAC dJ203K18 contained only *UHX1* and *PCTK1*, indicating a small distance between these two genes (Figure 1). To determine the genomic structure of the *UHX1* gene, two *UHX1* cosmids from the Lawrence Livermore library (LLXN01) were isolated. Both cosmids were shown by PCR also to contain the *PCTK1* gene which maps close to the ubiquitin activating enzyme E1 gene (*UBE1*). *UHX1* was originally described as a 3121 bp DNA sequence with a 2070 bp open reading frame (ORF), coding for a 690 amino acid sequence (1, Acc.No.:HSU44839). In this report, the ORF was preceded by a 679 bp 5' untranslated region (UTR) and a 410 bp 3'UTR containing an atypical polyadenylation signal and ending with nine adenines. Our data indicate that the previously published cDNA needs to be adjusted at the following positions: G at nucleotide 35 to A, G at nucleotide 47 to C, delete G at nucleotide 55, GC at nucleotides 111 and 112 to CG, insert G at nucleotide 212, insert G at nucleotide 262, insert G at nucleotide 292, insert G at nucleotide 302, and GC at nucleotides 342 and 343 to CG. These inaccuracies led to the prediction of a long 5'UTR and an altered ORF designation. We amplified our own cDNA sequence isolated from brain (GIBCO) encompassing the entire 5'UTR, and reanalysed it for intron-exon boundaries using cosmid sequencing and computer analysis (Table 2, Figure 3). Five previously undescribed exons were thereby identified, extending in the 5' direction. The new findings showed that the ORF frame consisted of 3023 bp with a total coding region of 2889 bp and a 5'UTR 134 bp long. All exons, spread over 16 kb (Figure 3a), could be identified by the gene structure programs XGRAIL2 and FEXH, except exon 1. Exon 1 was recognised by GRAIL1a, although this program did not identify the exon-intron boundaries of the predicted exons accurately. No promoter region could be detected by either XGRAIL 2 or PROMOTER-SCAN II (Figure 3b).

Genomic Structure of the PCTK1 *gene*

Full sequencing of the *UHX1*-containing cosmid revealed a second gene close to the *UHX1* gene (Figure 3). This gene consists of at least 15 exons and a

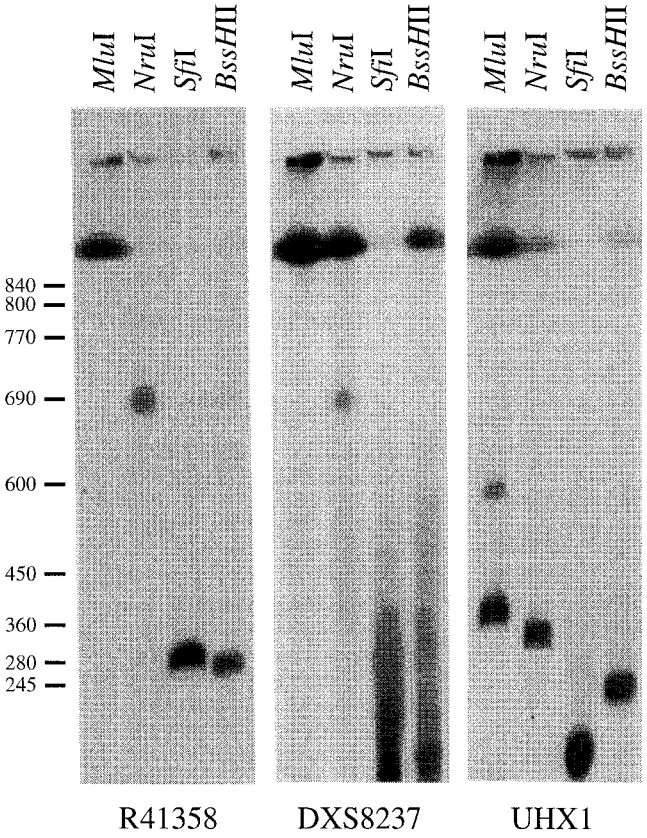

Figure 2 *Mapping of* UHX1, DXS8237E *and R41538 by PFGE analysis. Hybridization was performed under stringent conditions. Sizes are indicated on the left. High resolution PFGE indicates a size for the Mlul fragment identified by DXS8237E and R41358 of >2.5 Mb. PFGE blots were prepared as described in Schindelhauer* et al.[27]

homology search demonstrated identity with the previously published *PCTK1* cDNA.[3] Exon-intron boundaries of the 15 exons are described in Table 2. Again all exons, except exon 2 and 5, could be predicted by FEXH and XGRAIL1a/2 computer analysis. No promoter region was identified, probably due to insufficient sequence data in the 5' direction (Figure 3a). A combination of our data with that of Carrel *et al*[3] indicates that the *UHX1* gene is located 17 kb from the *UBE1* gene.

Mutation Analysis of UHX1 *and* PCTK1 *in Patients with Retinal Disorders*

To examine the possible causative role of *UHX1* in retinal diseases mapped to Xp21.2–p11.2, DNA samples from patients with XLRP (43 cases) and XLCSNB (13 cases) were digested with EcoRI and PstI and hybridised with the *UHX1* cDNA probe. From the 43 XLRP families linked to the RP3 and RP2 region, only 30–40% can be expected to be of the RP2 type, even after exclusion of RPGR mutations.

No aberrations of the *UHX1* gene were detected in any of the patient samples using this technique. Hybridisation revealed one fragment of 9 kb with the 5' probe and two fragments of 3 and 12 kb with the 3' probe. In 20 of the 43 XLRP and 10 of the 13 XLCSXIB patients, all 21 exons from the *UHX1* gene were amplified with 18 primer pairs (Table 3). No sequence changes could be detected in any patient analysed by SSCP analysis. All 43 XLRP and 13 XLCSNB patients were also screened for intragenic deletions in the *PCTK1* gene using a cDNA probe. A single 20 kb EcoRI fragment and two Pstl fragments of 2 kb and

Table 2 Exon-intron organisation of the *PCTK1* gene

Exon	*Position*	*Exon size*	*Splice acceptor*	*Splice donor*	*Intron size*
1	1–325	325	TTCCATCGCT	TCTGCACCAGgtgggtccactggct	626
2	326–459	133	tctccctgccacaagAGATTGTGCA	CTCCACTGAGgtgcttgaccccgtc	100
3	460–585	125	tgccttacctgctagGACATCAACA	TGTCAGCCTAgtaagcaccttctgt	91
4	586–642	56	tcatttgtcccacagTCTGAGATTG	ACTGGGCGAGgtgagaggcaaatag	98
5	643–757	114	ttcctcacattccagGGTTACCTAT	ATCCGGGAAGgtacacaccccatc	670
6	758–852	94	ccatgcttcctgcagTGTCCCTGCT	TGAGTACCTGgtaaggttgagtggc	99
7	853–915	62	ctcttctttcctcagGACAAGGACC	CAACGTGAAAgtgggtgtggggcag	252
8	916–1039	123	ttcctgggtccccagCTGTTCCTGT	GCTGACTTTGgtaccactggcctcc	181
9	1040–1160	120	ttctctgatttccagGCCTGGCCCG	TTGACATGTGgtaaggacaggtgga	109
10	1161–1258	97	gccctccctgcccagGGGTGTGGGC	CGTATCTTAGgtgaggaggcatggg	85
11	1259–1364	105	ctttttccctactagGAACCCCAAC	ACGCACCCCGgtgaggatggtgggt	82
12	1365–1407	42	ttgtccttgtggcagACTTGATAGC	GCTGTTGCAGgtgagaccaccttgg	156
13	1408–1498	90	cgcacccccactcagTTTGAGGGTC	CTTCCTGACAgtgagtggagctggg	1098
14	1499–1576	77	ctcccccatctgtagCTACTTCCAT	CCTGACTCAGgtaggtatagcccct	87
15	1577–1745	168	tgctttcccccacagGCAGGCCAGC	CCACTTGTCC	

3.7 kb could be detected with the full-length cDNA. This is in agreement with the partial restriction map for *PCTK1* previously published.[3] No deletions or RFLPs could be found in 43 RP and 13 XLCSNB patients from unrelated families. Since deletion screening and SSCP have only a limited sensitivity, we cannot fully exclude mutations in the *UHX1* and *PCTK1* gene in the analysed patients.

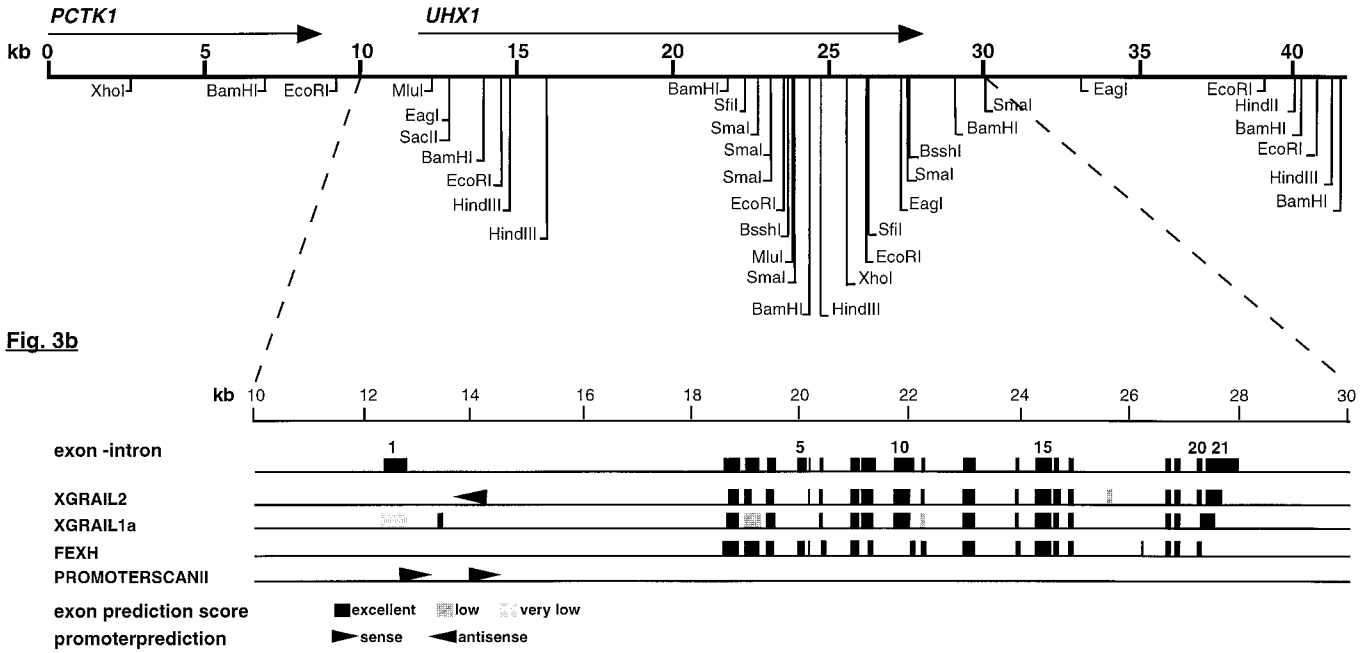

Figure 3 *(**a**) Sequence based restriction map of cosmid E2210Q containing the genes for* PCTK1 *and* UXH1. *(**b**) UHX1 exons and results of computer-generated intron-exon boundary determinations. Exons are represented by black boxes. Predicted excellent exons by the used gene structure programs are indicated by black boxes, low and very low predicted exons are shaded grey. Promoter motifs are shown by triangles.*

Table 3 SSCP primers for *UHX1* screening

Exon	*Forward sequence*	*Reverse sequence*	*Annealing T°C*
1	TGTGCACGGGGCCATTTC	ACCGCAGCCCAGCCTCACC	60
2	TATAGCTAACCCTCAGCTACC	AAGAACTCCAGGGCTAGG	51
3	TTCTTCCTCTGCCACCTCC	TTTCTTACTACGCACTCCCC	51
4	ATAGCTAACCCTCAGCTACC	AAGAACTCCAGGGCTAGGG	55
5	TCTCGAATGTACTCCATCACC	TGAACAGAAAAGCCCTCCCC	55
6/7	AACCCAGTTGATCATCATGG	AGACACAACTATAATGGCCC	55
8	TCTGCCTCATTCACCTGGTC	AGAGCCAAGAGACGTAGGG	60
9	TCTGATGACCCTGCCCATC	TTGGTGATGTTAATTGGACTGC	55
10	TCTTACCCTGGGCAAGCCC	GTGTCCATCATCATCTCCCC	55
11/12	TTTGTGGATACCGTTTTTCCC	ACCAAGTTCCTGAGGAACCAC	55
13	TGATCAGGTGTGTGCCTGCTGTC	AAGTCGGAGTTCAGACCCC	55
14	ATCTTAAGTAAAATCCCGGGC	ACCTAACCCAGAGCCCCC	55
15	TCCCATCTCTGACATCCTCCTG	AGCCCTCGCTGATAGCCAAAGG	55
16	ATGACCACCTCTCCCTCAC	AAGAGCTGGAAGTACCCCC	55
17	ATCTGGTTGTCTGTTCACCC	TGAAACCCCCACACACAGG	55
18	AAGTCCGTTTGCTGACTCGG	ATCCTCTCCCTGGCCCC	52
19	TACCAGTATTAACCCTCTC	AGCCTGGCACATACAGTGTCC	54
20/21	CGAGGATAACTCTCCTTCCC	TTTCTGTGGCAGGACCCAG	57

Discussion

Precise knowledge of the fine mapping and genomic organization of the *UHX1* gene is necessary to allow investigation into its function. Complete sequencing of a cosmid (LLNLc110E2210Q) containing the entire *UHX1* gene revealed differences in the cDNA that shortened the open reading frame in the original study. Genomic sequencing revealed five more exons in the 5' sequence, yielding a gene of about 16 kb with 21 exons. The predicted ubiquitin-related enzymatic function was not altered.

The second gene found on the cosmid, *PCTK1*, has already been cloned[1] and mapped adjacent to *UBE1*.[3] *PCTK1* belongs to the cdc2-related protein family, which is involved in the regulation of mitosis.[2] So far, no diseases associated with mutations of the *PCTAIRE* subfamily have been described.

Comparison of the mapping databases did not allow construction of a consensus map for the region around *UXH1*. Preliminary PFGE mapping experiments indicated *UHX1* as the most centromeric marker, followed by *PCTK1* and *UBE1*. Two new findings helped determine the orientation of the markers: the location of markers DXS1003 and DXS1055 telomeric to *UXH1* and *DXS8237E*, and *UHX1*'s centromeric position relative to *DXS8237E*. The proximity of *UHX1* to *PCTK1*, and *TIMP1* to *ARAF1* suggested a different orientation from that published by Carrel *et al*,[3] possibly because their study used a YAC (ICRF-YAC y900C1228)[26] since shown to be deleted [own data].

The localisation of *UHX1* and *PCTK1* limited their candidacy to RP2 and CSNB1 and excluded RP3 and XLCOD. No aberrations could be detected for *UHX1* by SSCP analysis, or by deletion screening for *UHX1* and *PCTK1*. Evaluation for point mutations in *PCTK1* is underway. Thus, *UHX1*, and to a lesser extent *PCTK1*, are unlikely to cause retinal diseases. We did not exclude other mechanisms by which retinal diseases might still be caused by these two genes, such as the possibilities of either mutations in the as yet unidentified promoter regions for the two genes or another retinal disease mapping to Xp21.2–p11.2. In addition, the finding of four different genes (*UHX1*, *PCTK1*, *UBE1* and *EST DXS8237E*) within 70 kb indicates a gene-rich region, leaving a number of candidate genes for RP2 and CSNB1 to be explored.

A recent report by Hegde *et al*[14] indicates that ubiquitin C-terminal hydrolase is essential for long-term facilitation in the mollusc *Aplysia*. Therefore, *UHX1* might be a candidate for investigations of human learning and memory, especially syndromal and non-specific X-linked mental retardation (XLMR) already mapped to this region.[15,16] The role of *UHX1* in the regulation of normal growth and cancer development, suggested by Swanson *et al*,[1] may also be appropriate for future studies.

In summary, we present important new data which enabled the precise genetic structure of *UHX1*, and the fine mapping, which is an important prerequisite to establish a sequence ready map for the RP2 region. Abnormalities in *UHX1* and *PCTK1* are unlikely to be the cause of XLRP2 or XLCSNB. Other possible functions of *UHX1* in memory, learning, and normal and neoplastic growth remain to be explored.

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