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Supplemental Data

Mutations in *CNNM4* Cause Recessive Cone-Rod Dystrophy with Amelogenesis Imperfecta

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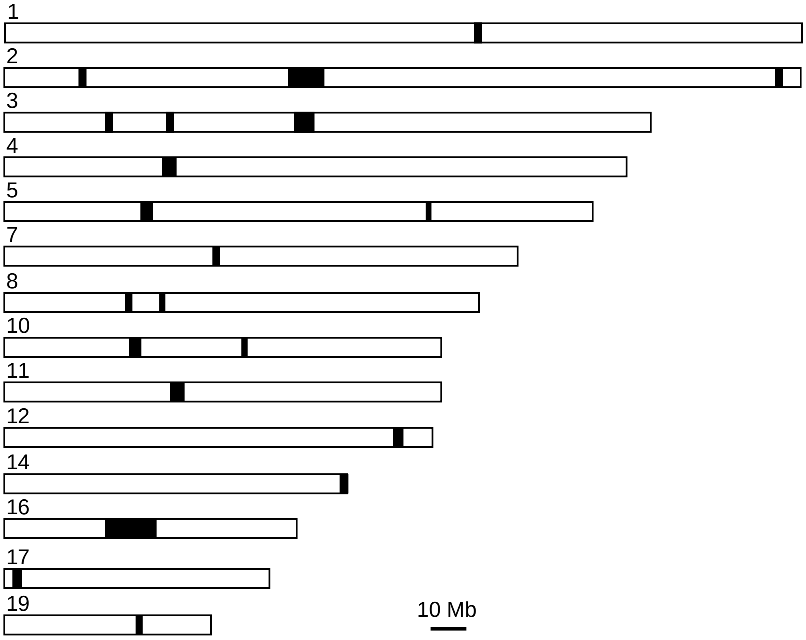
This file contains:

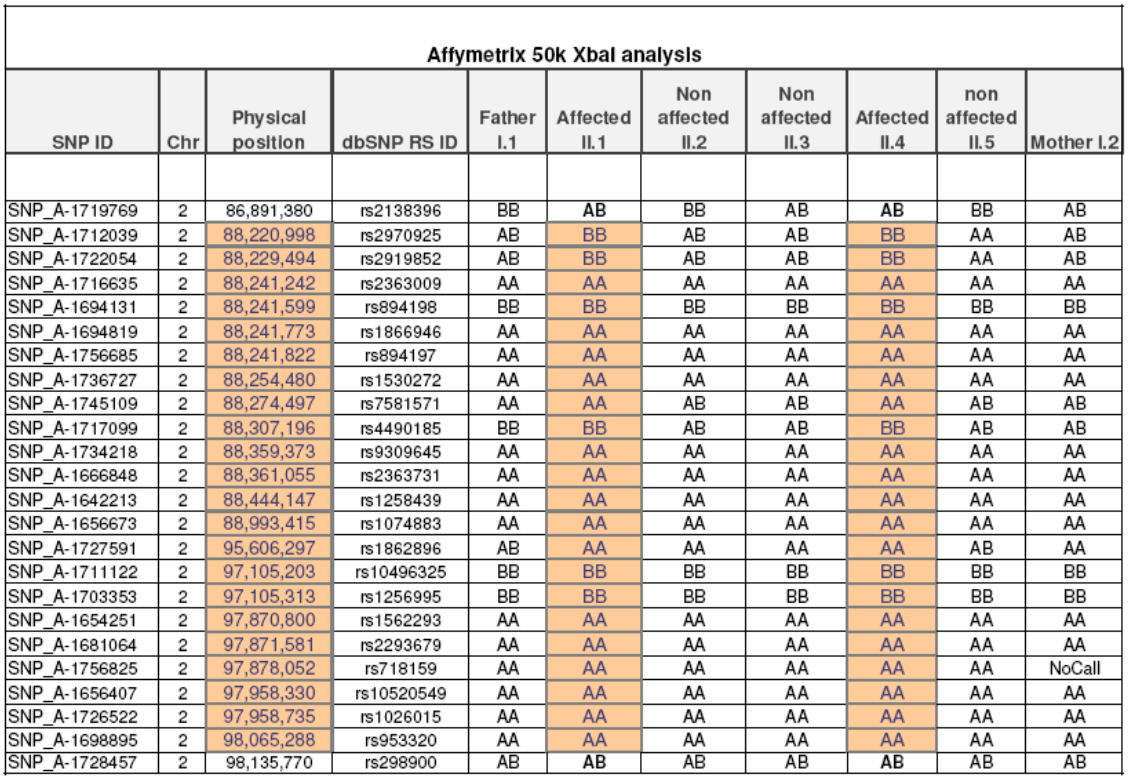
Figure S1

Mapping of homozygous regions. The homozygous regions (black boxes) are schematically represented on human chromosomes.



Homozygous regions larger than 3 Mb observed in the genomes of the affected members of family A, as detected by Affymetrix human mapping 50K XbaI array.

Region 1: chromosome 2



Affymetrix 50k XbaI analysis

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1719769	2	86,891,380	rs2138396	BB	**AB**	BB	AB	**AB**	BB	AB
SNP_A-1712039	2	88,220,998	rs2970925	AB	BB	AB	AB	BB	AA	AB
SNP_A-1722054	2	88,229,494	rs2919852	AB	BB	AB	AB	BB	AA	AB
SNP_A-1716635	2	88,241,242	rs2363009	AA	AA	AA	AA	AA	AA	AA
SNP_A-1694131	2	88,241,599	rs894198	BB	BB	BB	BB	BB	BB	BB
SNP_A-1694819	2	88,241,773	rs1866946	AA	AA	AA	AA	AA	AA	AA
SNP_A-1756685	2	88,241,822	rs894197	AA	AA	AA	AA	AA	AA	AA
SNP_A-1736727	2	88,254,480	rs1530272	AA	AA	AA	AA	AA	AA	AA
SNP_A-1745109	2	88,274,497	rs7581571	AA	AA	AB	AB	AA	AB	AB
SNP_A-1717099	2	88,307,196	rs4490185	BB	BB	AB	AB	BB	AB	AB
SNP_A-1734218	2	88,359,373	rs9309645	AA	AA	AA	AA	AA	AA	AA
SNP_A-1666848	2	88,361,055	rs2363731	AA	AA	AA	AA	AA	AA	AA
SNP_A-1642213	2	88,444,147	rs1258439	AA	AA	AA	AA	AA	AA	AA
SNP_A-1656673	2	88,993,415	rs1074883	AA	AA	AA	AA	AA	AA	AA
SNP_A-1727591	2	95,606,297	rs1862896	AB	AA	AA	AA	AA	AB	AA
SNP_A-1711122	2	97,105,203	rs10496325	BB	BB	BB	BB	BB	BB	BB
SNP_A-1703353	2	97,105,313	rs1256995	BB	BB	BB	BB	BB	BB	BB
SNP_A-1654251	2	97,870,800	rs1562293	AA	AA	AA	AA	AA	AA	AA
SNP_A-1681064	2	97,871,581	rs2293679	AA	AA	AA	AA	AA	AA	AA
SNP_A-1756825	2	97,878,052	rs718159	AA	AA	AA	AA	AA	AA	NoCall
SNP_A-1656407	2	97,958,330	rs10520549	AA	AA	AA	AA	AA	AA	AA
SNP_A-1726522	2	97,958,735	rs1026015	AA	AA	AA	AA	AA	AA	AA
SNP_A-1698895	2	98,065,288	rs953320	AA	AA	AA	AA	AA	AA	AA
SNP_A-1728457	2	98,135,770	rs298900	AB	**AB**	AB	AB	**AB**	AB	AB

Region 2: chromosome 16

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1647134	16	31,637,053	rs2141349	AA	**AB**	AA	AA	**AB**	AB	AB
SNP_A-1705117	16	31,687,619	rs10492807	AA	AA	AA	AA	AA	AA	AA
SNP_A-1704587	16	34,493,676	rs2404821	AB	AA	AA	AB	AA	AA	AA
SNP_A-1745037	16	45,293,764	rs370159	AB	BB	BB	AB	BB	BB	BB
SNP_A-1743163	16	45,704,219	rs10518838	AB	AA	AA	AB	AA	AA	AA
SNP_A-1669317	16	45,716,367	rs9328707	BB	BB	BB	BB	BB	BB	BB
SNP_A-1728975	16	46,068,824	rs9328592	AA	AA	AA	AA	AA	AA	AA
SNP_A-1679126	16	46,222,649	rs10521162	AA	AA	AA	AA	AA	AA	AA
SNP_A-1655423	16	46,288,947	rs10521163	BB	BB	BB	BB	BB	BB	BB
SNP_A-1696880	16	46,306,307	rs9328600	AA	AA	AA	AA	AA	AA	AA
SNP_A-1733078	16	46,716,800	rs1345425	AA	AA	AA	AA	AA	AA	AA
SNP_A-1710265	16	46,723,079	rs939362	AA	AA	AA	AA	AA	AA	AA
SNP_A-1669247	16	46,754,604	rs8050306	AA	AB	AA	AA	AB	AB	AB

Region 3: chromosome 3

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1758263	3	89,193,457	rs10511144	BB	**AB**	BB	AB	**AB**	AB	AB
SNP_A-1746931	3	89,708,135	rs763900	AA	AA	AA	AA	AA	AA	AA
SNP_A-1726472	3	90,045,737	rs1915734	AA	AA	AA	AA	AA	AA	AA
SNP_A-1684696	3	95,122,010	rs4133534	AA	AA	AB	AA	AA	AA	AB
SNP_A-1726912	3	95,569,562	rs2087734	AA	AA	AB	AA	AA	AA	AB
SNP_A-1655477	3	95,595,165	rs1388639	BB	BB	AB	BB	BB	BB	AB
SNP_A-1657287	3	95595591	rs7622335	AA	AB	AA	AB	AB	AB	AB

Region 4: chromosome 4

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1714577	4	48,758,929	rs10517230	AB	**BB**	BB	BB	**AB**	BB	BB
SNP_A-1669019	4	48,759,323	rs10517231	AA	AA	AA	AB	AA	AB	AB
SNP_A-1758758	4	52,525,819	rs2319743	BB	BB	BB	BB	BB	BB	BB
SNP_A-1686342	4	52,555,439	rs6851073	AA	AA	AA	AA	AA	AA	AA
SNP_A-1734028	4	52,625,606	rs6852236	BB	BB	BB	BB	BB	BB	BB
SNP_A-1660315	4	52,737,579	rs178724	AA	AA	AA	AA	AA	AA	AA
SNP_A-1729816	4	52,737,718	rs225172	AA	AA	AA	AA	AA	AA	AA
SNP_A-1725508	4	52,870,613	rs10517245	BB	BB	BB	BB	BB	BB	BB
SNP_A-1740259	4	52881109	rs713222	AB	BB	BB	BB	AB	BB	BB

Region 5: chromosome 11

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1722254	11	51,319,510	rs521874	AB	**AB**	AB	AB	**AB**	AB	AB
SNP_A-1730120	11	51,333,408	rs1694610	AA	AA	AA	AA	AA	AA	AA
SNP_A-1735639	11	51,383,331	rs2078611	BB	BB	BB	BB	BB	BB	BB
SNP_A-1738999	11	51,383,437	rs3886724	BB	BB	BB	BB	BB	BB	BB
SNP_A-1729270	11	54,916,541	rs4316524	AA	AA	AA	AA	AA	AA	AA
SNP_A-1758699	11	54,945,728	rs1237929	AB	BB	AB	AA	BB	AB	AB
SNP_A-1700728	11	55,187,106	rs295603	BB	BB	BB	BB	BB	BB	BB
SNP_A-1688446	11	55,248,049	rs10501337	AA	AA	AA	AA	AA	AA	AA
SNP_A-1663756	11	55,439,371	rs10501340	AA	AA	AA	AA	AA	AA	AA
SNP_A-1646156	11	55,466,760	rs10501342	BB	BB	BB	BB	BB	BB	BB
SNP_A-1677524	11	55,467,285	rs7939844	AB	AB	BB	BB	AB	BB	BB

Region 6: chromosome 5

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1642403	5	42,405,670	rs950812	AB	**NoCall**	BB	BB	**AB**	BB	BB
SNP_A-1656759	5	42,431,038	rs1858138	AB	AA	AB	BB	AA	BB	AB
SNP_A-1667432	5	42,431,058	rs1858137	BB	BB	BB	BB	BB	BB	BB
SNP_A-1720391	5	42,443,617	rs1509461	AB	BB	AB	AA	BB	AA	AB
SNP_A-1751275	5	42,761,386	rs719756	AB	AA	AB	AB	AA	AB	AA
SNP_A-1647578	5	42,783,879	rs722678	AA	AA	AA	AA	AA	AA	AA
SNP_A-1725364	5	42,816,054	rs1364026	AA	AA	AA	AA	AA	AA	AA
SNP_A-1717547	5	42,835,028	rs230814	AB	AA	AB	AB	AA	AB	AA
SNP_A-1677326	5	42,836,481	rs6413428	AB	BB	AB	AB	BB	AB	BB
SNP_A-1728275	5	42,843,181	rs230820	AB	AA	AB	AB	AA	AB	AA
SNP_A-1687096	5	42,874,313	rs10512806	BB	BB	BB	BB	BB	BB	BB
SNP_A-1659378	5	43,047,397	rs2329995	AB	AA	AB	BB	AA	BB	AB
SNP_A-1675097	5	43,063,978	rs9292862	AB	BB	AB	AA	BB	AA	AB
SNP_A-1666636	5	43,248,347	rs10512821	AB	AA	AB	BB	AA	BB	AB
SNP_A-1672582	5	43,248,972	rs2330090	AB	AA	AB	BB	AA	BB	AB
SNP_A-1681086	5	43,403,823	rs10512827	AB	BB	AB	AB	BB	AB	BB
SNP_A-1749125	5	43,404,047	rs10512828	AB	AA	AB	AB	AA	AB	AA
SNP_A-1745444	5	43,419,155	rs779850	AB	AA	AB	AB	AA	AB	AA
SNP_A-1683874	5	43,731,198	rs16873496	BB	BB	BB	BB	BB	BB	BB
SNP_A-1752721	5	44,264,182	rs10512836	AA	AA	AA	AA	AA	AA	AA
SNP_A-1669433	5	44,269,535	rs726941	BB	BB	BB	AB	BB	AB	AB
SNP_A-1749265	5	44,329,760	rs1374977	BB	BB	BB	AB	BB	AB	AB
SNP_A-1652106	5	44,335,755	rs9292903	BB	BB	BB	AB	BB	AB	AB
SNP_A-1724370	5	44,377,093	rs10512845	AA	AA	AA	AB	AA	AB	AB
SNP_A-1735280	5	44,377,640	rs1037181	BB	BB	BB	BB	BB	BB	BB
SNP_A-1669085	5	44,419,757	rs10512848	AA	AA	AA	AA	AA	AA	AA
SNP_A-1749017	5	44,419,980	rs10512849	AA	AA	AA	AA	AA	AA	AA
SNP_A-1687196	5	44,421,083	rs10512851	BB	BB	BB	BB	BB	BB	BB
SNP_A-1699509	5	44,423,294	rs2973646	BB	BB	BB	AB	BB	AB	AB
SNP_A-1726740	5	44,429,326	rs10512852	BB	BB	BB	BB	BB	BB	BB
SNP_A-1682656	5	44,514,499	rs1482684	AA	AA	AA	AB	AA	AB	AB
SNP_A-1689683	5	44,516,774	rs10512853	BB	BB	BB	BB	BB	BB	BB
SNP_A-1758245	5	45,285,361	rs10512876	AA	AA	AA	AA	AA	AA	AA
SNP_A-1712103	5	45,286,487	rs10512875	BB	BB	BB	BB	BB	BB	BB
SNP_A-1735883	5	45,589,144	rs1384732	AA	NoCall	NoCall	NoCall	NoCall	NoCall	NoCall
SNP_A-1747517	5	45,775,824	rs10512877	BB	BB	BB	BB	BB	BB	BB
SNP_A-1725158	5	45,776,880	rs2337953	AA	AA	AA	AA	AA	AA	AA
SNP_A-1730659	5	49,596,616	rs1328254	AA	AB	AB	AB	AB	AB	BB

Region 7: chromosome 10

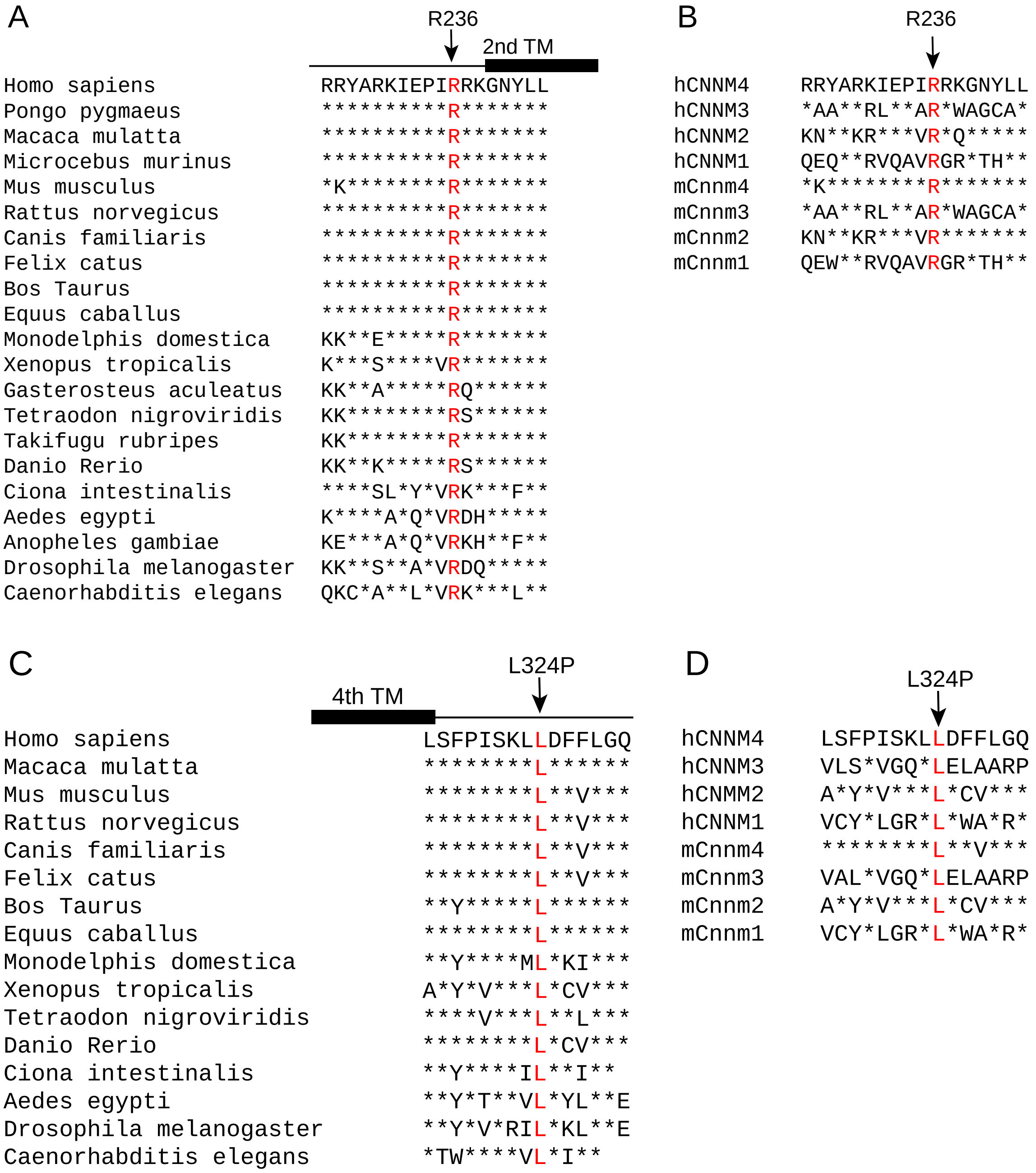
SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1643876	10	38,578,777	rs1740752	AB	**AB**	AB	AB	**AB**	AA	AA
SNP_A-1693319	10	38,584,925	rs10508864	BB	BB	BB	BB	BB	BB	BB
SNP_A-1647840	10	42,276,475	rs10508867	BB	BB	BB	BB	BB	BB	BB
SNP_A-1695273	10	42,332,699	rs210236	AB	AB	AB	AB	AB	AA	AA

Region 8: chromosome 2

SNP ID	Chr	Physical position	dbSNP RS ID	Father I.1	Affected II.1	Non affected II.2	Non affected II.3	Affected II.4	non affected II.5	Mother I.2
SNP_A-1665344	2	237,924,688	rs4129307	AA	**AB**	AA	AB	**AB**	AA	AB
SNP_A-1668757	2	238,068,613	rs1826873	AB	AA	AA	AB	AA	AB	AA
SNP_A-1642069	2	238,380,821	rs3754728	BB	BB	BB	BB	BB	BB	BB
SNP_A-1718535	2	238,421,192	rs903371	AA	AA	AA	AA	AA	AA	AA
SNP_A-1647472	2	238,659,740	rs1446924	AA	AA	AA	AA	AA	AA	AA
SNP_A-1758503	2	238,779,956	rs1374386	AA	AA	AB	AA	AA	AB	AB
SNP_A-1681506	2	238,798,933	rs878133	BB	BB	BB	BB	BB	BB	BB
SNP_A-1682860	2	240,067,248	rs843451	AA	AA	AA	AA	AA	AA	AA
SNP_A-1746364	2	240,068,225	rs709274	BB	BB	BB	BB	BB	BB	BB
SNP_A-1677650	2	240,504,012	rs2412029	AB	AA	AA	AB	AA	AB	AA
SNP_A-1754047	2	241,058,775	rs2352852	AA	AA	AA	AA	AA	AA	AA
SNP_A-1648538	2	241,524,068	rs4129624	AA	AA	AA	AA	AA	AA	AA
SNP_A-1677230	2	241,601,632	rs4130554	AB	AB	BB	AA	AB	AB	AB

Figure S2

Cross-species alignment of mutation-harbouring *CNNM4* regions (A,C) and paralog comparison (B,D) of human (h) and mouse (m) *CNNM* genes. Black boxes above sequence comparisons represent the beginning of the second transmembrane (TM) domain (A), respectively the end of the fourth TM domain (C). * denotes amino acid identity.



A

Homo sapiens	RRYARKIEPIRRKGNYLL
Pongo pygmaeus	**********R*******
Macaca mulatta	**********R*******
Microcebus murinus	**********R*******
Mus musculus	*K********R*******
Rattus norvegicus	**********R*******
Canis familiaris	**********R*******
Felix catus	**********R*******
Bos Taurus	**********R*******
Equus caballus	**********R*******
Monodelphis domestica	KK**E*****R*******
Xenopus tropicalis	K***S****VR*******
Gasterosteus aculeatus	KK**A*****RQ******
Tetraodon nigroviridis	KK********RS******
Takifugu rubripes	KK********R*******
Danio Rerio	KK**K*****RS******
Ciona intestinalis	****SL*Y*VRK***F**
Aedes egypti	K****A*Q*VRDH*****
Anopheles gambiae	KE***A*Q*VRKH**F**
Drosophila melanogaster	KK**S**A*VRDQ*****
Caenorhabditis elegans	QKC*A**L*VRK***L**

B

hCNNM4	RRYARKIEPIRRKGNYLL
hCNNM3	*AA**RL**AR*WAGCA*
hCNNM2	KN**KR***VR*Q*****
hCNNM1	QEQ**RVQAVRGR*TH**
mCnnm4	*K********R*******
mCnnm3	*AA**RL**AR*WAGCA*
mCnnm2	KN**KR***VR*******
mCnnm1	QEW**RVQAVRGR*TH**

C

Homo sapiens	LSFPISKLLDFFLGQ
Macaca mulatta	********L******
Mus musculus	********L**V***
Rattus norvegicus	********L**V***
Canis familiaris	********L**V***
Felix catus	********L**V***
Bos Taurus	**Y*****L******
Equus caballus	********L******
Monodelphis domestica	**Y****ML*KI***
Xenopus tropicalis	A*Y*V***L*CV***
Tetraodon nigroviridis	****V***L**L***
Danio Rerio	********L*CV***
Ciona intestinalis	**Y****IL**I**
Aedes egypti	**Y*T**VL*YL**E
Drosophila melanogaster	**Y*V*RIL*KL**E
Caenorhabditis elegans	*TW****VL*I**

D

hCNNM4	LSFPISKLLDFFLGQ
hCNNM3	VLS*VGQ*LELAARP
hCNMM2	A*Y*V***L*CV***
hCNNM1	VCY*LGR*L*WA*R*
mCnnm4	********L**V***
mCnnm3	VAL*VGQ*LELAARP
mCnnm2	A*Y*V***L*CV***
mCnnm1	VCY*LGR*L*WA*R*

Figure S3

Cnnm4 mRNA expression is at its highest levels in the mouse retina. Mouse Cnnm1, -2, -3 and -4 mRNA expression was assessed by RT-PCR (Panel A) and quantitative PCR (Panel B) in mouse brain, retina, cornea, lens and RPE with attached choroid (RPE) samples. Pooled total RNA samples of 2 mice were used for each experiment. For RT-PCR, amplified PCR fragments were analyzed on a 2% agarose gel and fragments of the expected size were detected, respectively 440 bp for Cnnm1, 333 bp for Cnnm2, 195 bp for Cnnm3, 280 bp for Cnnm4 and 198 bp for the control gene RL8 (ribosomal protein L8), as indicated by the 1kb Molecular Weight Marker (M; Roche, Basel, Switzerland). Quantitative PCR was performed on three samples in duplicate (+/-SEM) and all Cnnm mRNA expression levels were normalized to RL8 expression. Cnnm4 expression was also analysed in mandibula, upper and lower incisives by RT-PCR (Panel C) and quantitative PCR (Panel D).

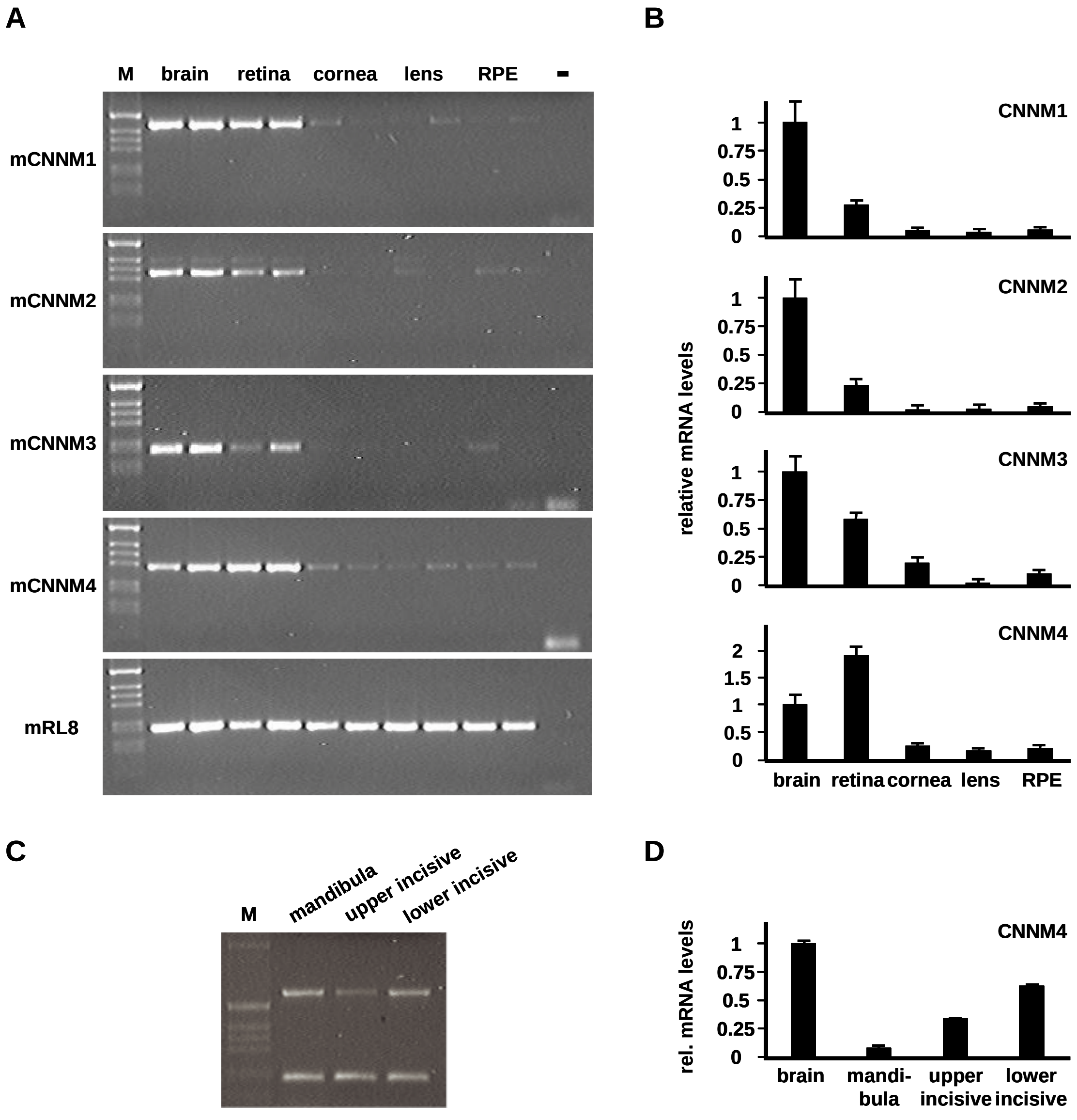


Table S1

List of sequenced genes in the homozygous region of chromosome 2

Gene ID	Physical position on Chr 2 (start)	Number of exons	OMIM
TEKT4	94'900'959	6	
MAL	95'055'156	4	188860
PROM2	95'303'972	24	
CSEN	95'326'799	9	604662
TRIM43	95'621'493	7	
FAHD2A	95'432'203	8	
ARID5A	96'566'195	6	611583
LMAN2L	96'735'396	8	609552
SEMAC4C	96'889'201	15	604462
COX5B	97'628'953	4	123866
ACTR1B	97'638'863	11	605144
STARD7	96'214'324	8	
CNNM4	96'790‘366	7	607805
CNNM3	96'845'718	8	607804

Table S2

Primers and PCR conditions for human *CNNM4*

Exon	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing T°
1a	CTGCTCCACCTTAAGCGACT	TAGACAACCTCATCGGGTCC	60°C + betaine
1b	TAATTACGGTGCTGCTGGTG	GAACGATTTTGGAGCGGTAG	60°C
2 + 3	CCCCATCCTGGTGACTTATG	CATAGGACGAGGGCTGCAG	60°C + betaine
4	CGGGAGTCGTCTGAGCAC	CCCACTGTCCCTTGTTCCT	58°C + betaine
5	GAGGTGCATGGCTTCCCT	CGACACCAGAACTGAGCATG	60°C + betaine
6	CTTCCATGGGATGAGGTGAG	AAACCCGAGATGCCTTTTTC	58°C
7	TGTAAGGGCTTCCAGAGACG	GGGTAGCTGTGATCAGGGAC	60°C + betaine
dupC	CTATACTCGCATCCCGGTGT	GAACGATTTTGGAGCGGTAG	60°C + betaine
R236Q	CAACATCTCCAGCAACCTGA	CTTCTCTGCTCGTTGCTCCT	60°C + betaine
L324P	ACCTCAGGCCCTGTGCTC	TATTGGACTGCTCGTCTTCG	60°C

WAVE primers and conditions for human *CNNM4*

Mutation	Temperature	%B buffer
dupC	60°C	58%
R236Q	62.9°C	60.5%
L324P	60.4°C	59%

Table S3

Primers for RT-PCR and real-time PCR of the mouse *Cnnm* genes

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing T°
Cnnm1	GATCACACGACAACAGTACC	TCAAGGTTCTCAGCAGCTTC	58
Cnnm2	TCTCTGCCTTTAAGCAGACG	CCATCACACCATAGTAGGAG	58
Cnnm3	ACATCGTGGACATGCTCTAC	CCTCATTGTTCACCTTCTGC	58
Cnnm4	AGCAACCAGTTTGGCAGCTG	GATCCTCGAGTCAGATGGTTTCCTCTTCGGAG	58

Primers for RT-PCR of mouse *Cnnm4* in mandibula and teeth

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing T°
Cnnm4	CTCTAGGAGGCACTCTTCTGC	ATCCTCAGCCAGCCATGC	60