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Supplemental Data

Recessive Mutations in the Gene Encoding the Tight Junction Protein Occludin Cause Band-like Calcification with Simplified Gyration and Polymicrogyria

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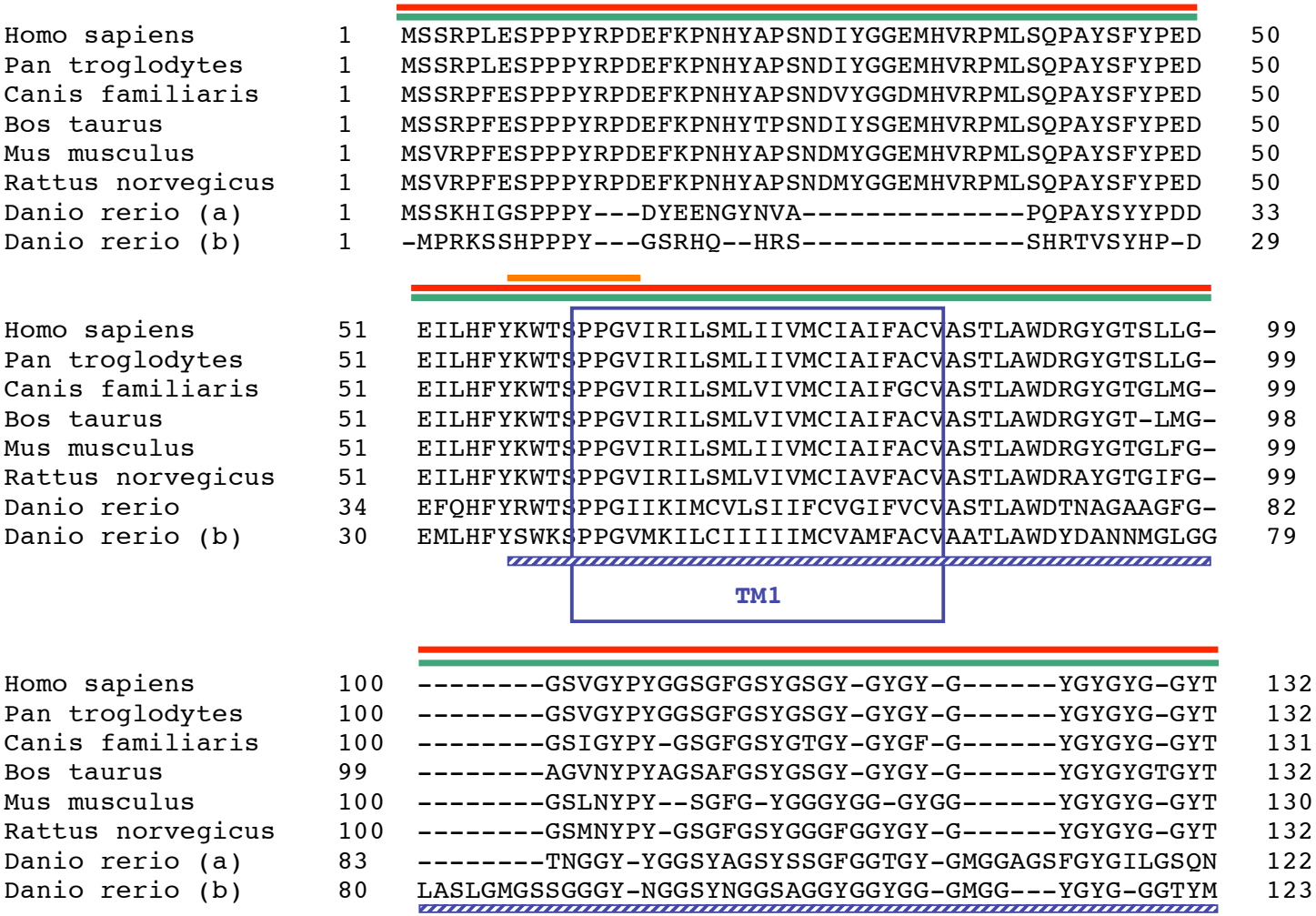
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Danio rerio (a) 1 MSSKHIGSPPPY---DYEENGYNVA--------------PQPAYSYYPDD 33
Danio rerio (b) 1 -MPRKSSHPPPY---GSRHQ--HRS--------------SHRTVSYHP-D 29
Homo sapiens 51 EILHFYKWTSPPGVIRILSMLIIVMCIAIFACVASTLAWDRGYGTSLLG- 99
Pan troglodytes 51 EILHFYKWTSPPGVIRILSMLIIVMCIAIFACVASTLAWDRGYGTSLLG- 99
Canis familiaris 51 EILHFYKWTSPPGVIRILSMLVIVMCIAIFGCVASTLAWDRGYGTGLMG- 99
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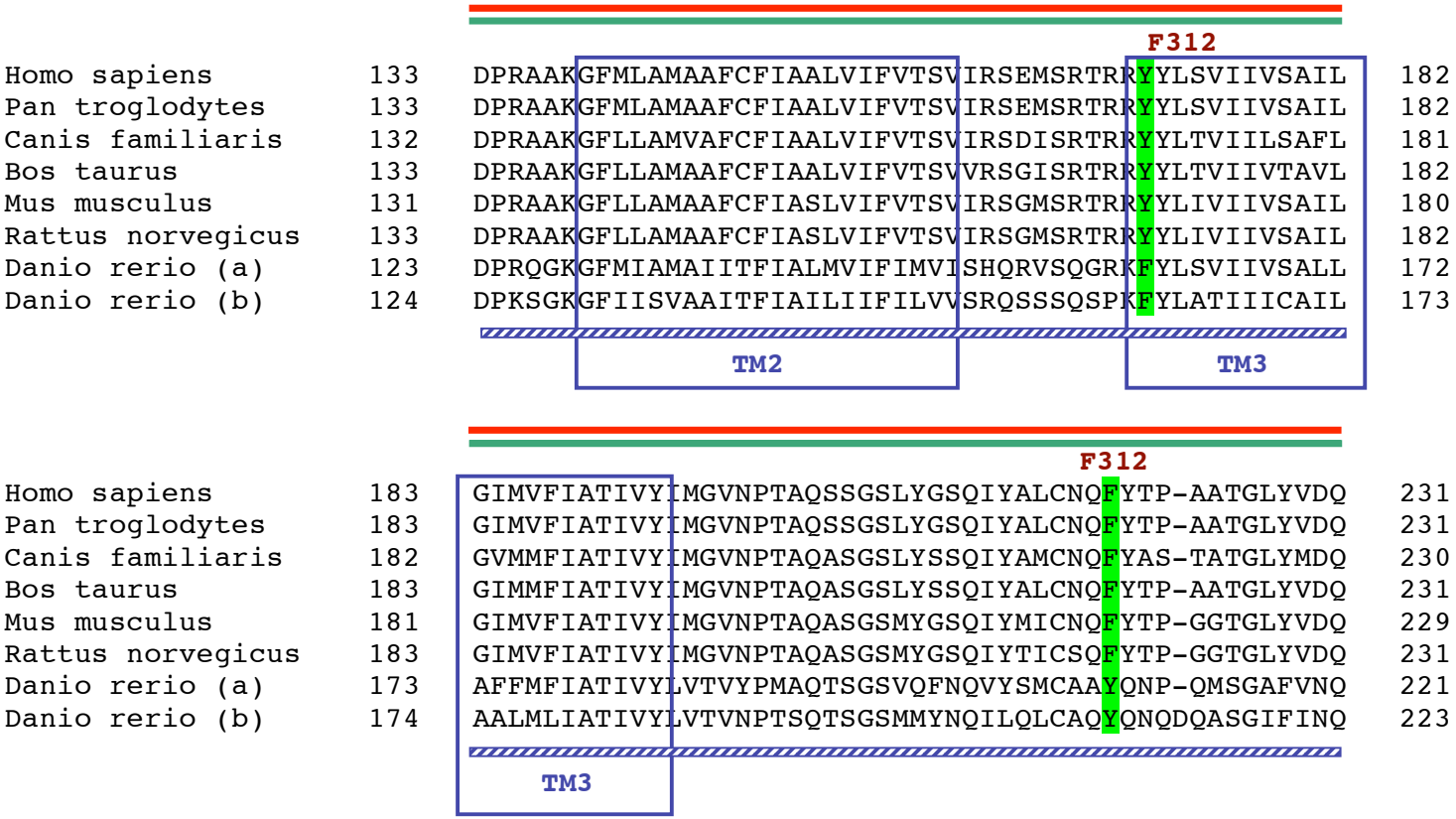
Rattus norvegicus 51 EILHFYKWTSPPGVIRILSMLVIVMCIAVFACVASTLAWDRAYGTGIFG- 99
Danio rerio 34 EFQHFYRWTSPPGIIKIMCVLSIIFCVGIFVCVASTLAWDTNAGAAGFG- 82
Danio rerio (b) 30 EMLHFYSWKSPPGVMKILCIIIIIMCVAMFACVAATLAWDYDANNMGLGG 79
TM1
Homo sapiens 100 --------GSVGYPYGGSGFGSYGSGY-GYGY-G------YGYGYG-GYT 132
Pan troglodytes 100 --------GSVGYPYGGSGFGSYGSGY-GYGY-G------YGYGYG-GYT 132
Canis familiaris 100 --------GSIGYPY-GSGFGSYGTGY-GYGF-G------YGYGYG-GYT 131
Bos taurus 99 --------AGVNYPYAGSAFGSYGSGY-GYGY-G------YGYGYGTGYT 132
Mus musculus 100 --------GSLNYPY--SGFG-YGGGYGG-GYGG------YGYGYG-GYT 130
Rattus norvegicus 100 --------GSMNYPY-GSGFGSYGGGFGGYGY-G------YGYGYG-GYT 132
Danio rerio (a) 83 --------TNGGY-YGGSYAGSYSSGFGGTGY-GMGGAGSFGYGILGSQN 122
Danio rerio (b) 80 LASLGMGSSGGGY-NGGSYNGGSAGGYGGYGG-GMGG---YGYG-GGTYM 123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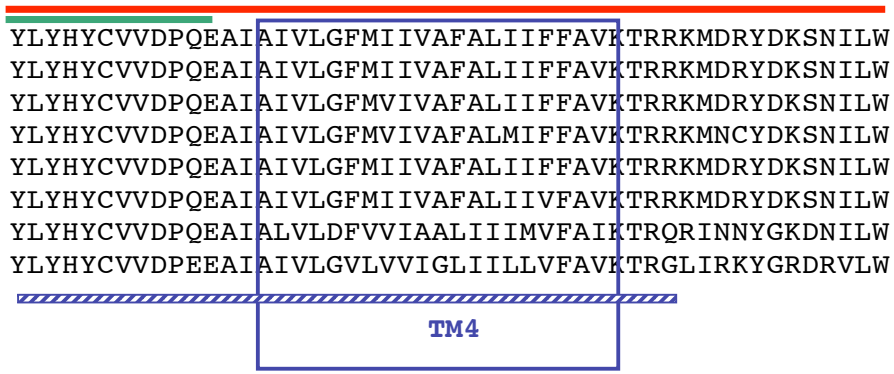
Canis familiaris 132 DPRAAKGFLLAMVAFCFIAALVIFVTSVIRSDISRTRRYYLTVIILSAFL 181
Bos taurus 133 DPRAAKGFLLAMAAFCFIAALVIFVTSVVRSGISRTRRYYLTVIIVTAVL 182
Mus musculus 131 DPRAAKGFLLAMAAFCFIASLVIFVTSVIRSGMSRTRRYYLIVIIVSAIL 180
Rattus norvegicus 133 DPRAAKGFLLAMAAFCFIASLVIFVTSVIRSGMSRTRRYYLIVIIVSAIL 182
Danio rerio (a) 123 DPRQGKGFMIAMAIITFIALMVIFIMVISHQRVSQGRKFYLSVIIVSALL 172
Danio rerio (b) 124 DPKSGKGFIISVAAITFIAILIIFILVVSRQSSSQSPKFYLATIIICAIL 173
TM2
TM3
F312
Homo sapiens 183 GIMVFIATIVYIMGVNPTAQSSGSLYGSQIYALCNQFYTP-AATGLYVDQ 231
Pan troglodytes 183 GIMVFIATIVYIMGVNPTAQSSGSLYGSQIYALCNQFYTP-AATGLYVDQ 231
Canis familiaris 182 GVMMFIATIVYIMGVNPTAQASGSLYSSQIYAMCNQFYAS-TATGLYMDQ 230
Bos taurus 183 GIMMFIATIVYIMGVNPTAQASGSLYSSQIYALCNQFYTP-AATGLYVDQ 231
Mus musculus 181 GIMVFIATIVYIMGVNPTAQASGSMYGSQIYMICNQFYTP-GGTGLYVDQ 229
Rattus norvegicus 183 GIMVFIATIVYIMGVNPTAQASGSMYGSQIYTICSQFYTP-GGTGLYVDQ 231
Danio rerio (a) 173 AFFMFIATIVYLVTVYPMAQTSGSVQFNQVYSMCAAYQNP-QMSGAFVNQ 221
Danio rerio (b) 174 AALMLIATIVYLVTVNPTSQTSGSMMYNQILQLCAQYQNQDQASGIFINQ 223
TM3

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Pan troglodytes       232   YLYHYCVVDPQEAIAIVLGFMIIVAFALIIFFAVKTRRKMDRYDKSNILW   281
Canis familiaris      231   YLYHYCVVDPQEAIAIVLGFMVIVAFALIIFFAVKTRRKMDRYDKSNILW   280
Bos taurus            232   YLYHYCVVDPQEAIAIVLGFMVIVAFALMIFFAVKTRRKMNCYDKSNILW   281
Mus musculus          230   YLYHYCVVDPQEAIAIVLGFMIIVAFALIIFFAVKTRRKMDRYDKSNILW   279
Rattus norvegicus     232   YLYHYCVVDPQEAIAIVLGFMIIVAFALIIVFAVKTRRKMDRYDKSNILW   281
Danio rerio (a)       222   YLYHYCVVDPQEAIALVLDFVVIAALIIIMVFAIKTRQRINNYGKDNILW   271
Danio rerio (b)       224   YLYHYCVVDPEEAIAIVLGVLVVIGLIILLVFAVKTRGLIRKYGRDRVLW   273

Homo sapiens          282   DKEHIYDEQ--PPNVEEWVKNVSAGTQDVPSPPSDYVERVDSPMAYSSNG   329
Pan troglodytes       282   DKEHIYDEQ--PPNVEEWVKNVSAGTQDVPSPPSDYVERVDSPMAYSSNG   329
Canis familiaris      281   DKEHIYDEQ--PPNVEEWVKNVSAGTQDMPPPPSDYVERVDSPMAYSSNG   328
Bos taurus            282   DKERIYDEQ--PPNVEEWVKNVSAGTQDMPPPLSDYVERVDSPVAYSSNG   329
Mus musculus          280   DKEHIYDEQ--PPNVEEWVKNVSAGTQDMPPPPSDYAERVDSPMAYSSNG   327
Rattus norvegicus     282   DKEHIYDEQ--PPNVEEWVKNVSAGTQDMPPPPSDYAERVDSPMAYSSNG   329
Danio rerio (a)       272   RRVKEFDDQNSPQDVEDWVNNVNGAPEGLLA---------DYPVKFGSRN   312
Danio rerio (b)       274   YDVKTIKDGLTSQGIGEWINNVSGDPEVFV-------------------N   304

Homo sapiens          330   KVNDKRFYPESSYKSTP-VPEVVQELPLTSPVDDFRQPRYSSGGNFETPS   378
Pan troglodytes       330   KVNDKRFYPESSYKSTP-VPEVVQELPLTSPVDDFRQPRYSSSGNFETPS   378
Canis familiaris      329   KVNDKRLYPESSYKSTP-VPEVVQELPATSPADDFRQPRYSSSGHLEPPS   377
Bos taurus            330   KVNEKRLYPESSYKSTP-VPEVAQELPLTSPVEDFRQPHYSSSGNLETLS   378
Mus musculus          328   KVNGKRSYPESFYKSTPLVPEVAQEIPLTLSVDDFRQPRYSSNGNLETPS   377
Rattus norvegicus     330   KVNGKRSYPDSLYKSPPLVPEVAQEIPLTLSVDDFRQPRYSSNDNLETPS   379
Danio rerio (a)       313   NLDDN----STSYDKPPLSESPVEILPVRNSVP-------ISSGSEINSS   351
Danio rerio (b)       305   DQNDK----VSAAQPMVYSQKPI-YLP--------------SSASDLTSS   335
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Homo sapiens          379   KRAPAKGRAGRSKRTEQDHYETDYTTGGESCDELEED-WIREYPPITSDQ   427
Pan troglodytes       379   KRAPAKGRAGRSKRTEQDHYETDYTTGGESCDELEED-WIREYPPITSDQ   427
Canis familiaris      378   KRAPSKGRTGRPKRLEQDHYETDYTTGGESCDELEED-WIREYPPITSDQ   426
Bos taurus            379   KRAPAKGRAGKSRRAEQDHYETDYTTGGESCDELEDD-WIREYPPITSDQ   427
Mus musculus          378   KRAPTKGKAGKGKRTDPDHYETDYTTGGESCEELEED-WVREYPPITSDQ   426
Rattus norvegicus     380   KRTPTKGKAGKAKRTDPDHYETDYTTGGESCDELEED-WLREYPPITSDQ   428
Danio rerio (a)       352   VGRPKKRRAGRPRTADGRDYDADY---ASSGDELDDDDFFSEFPPIVNTQ   398
Danio rerio (b)       336   VSGLKGKL---------RAYDA-----GESGDELDTD----EYPPIINEQ   367

Homo sapiens          428   QRQLYKRNFDTGLQEYKSLQSELDEINKELSRLDKELDDYREESEEYMAA   477
Pan troglodytes       428   QRQLYKRNFDTGLQEYKSLQSELDEINKELSRLDKELDDYREESEEYMAA   477
Canis familiaris      427   QRQLYKRNFDTGLQEYKSLQAELDEINKELSRLDKELDDYREESEEYMAA   476
Bos taurus            428   QRQLYKRSFDTGLQEYKSLQAELDEVNKELSRLDKELDDYREESEEYMAA   477
Mus musculus          427   QRQLYKRNFDAGLQEYKSLQAELDDVNKELSRLDKELDDYREESEEYMAA   476
Rattus norvegicus     429   QRQLYKRNFDAGLQEYKSLLAELDEVNKELSRLDRELDDYREESEEYMAA   478
Danio rerio (a)       399   ERDDYKHLFDQDHQEYKDLQAEMDQINKRLAEVDRELDGLQEGSPQFLDA   448
Danio rerio (b)       368   ERLEYKRDFDRDHMVYKRLQAELDDINQGLADADRELDRLEEGSPQFMDV   417

Homo sapiens          478   ADEYNRLKQVKGSADYKSKKNHCKQLKSKLSHIKKMVGDYDRQKT   522
Pan troglodytes       478   ADEYNRLKQVKGSADYKSKKNHCKQLKSKLSHIKKMVGDYDRQKT   522
Canis familiaris      477   ADEYNRLKQVKGSPDYKNKRNYCKQLKSKLSHIKKMVGDYDRQKT   521
Bos taurus            478   ADEYNRLKQVKGSADYKSKRNYCKQLKSKLSHIKKMVGDYDRRKT   522
Mus musculus          477   ADEYNRLKQVKGSADYKSKRNYCKQLKSKLSHIKRMVGDYDRRKP   521
Rattus norvegicus     479   ADEYNRLKQVKGSADYKSKKNYCKQLKSKLSHIKRMVGDYDRRKT   523
Danio rerio (a)       449   MDEYNAIQDQKRSGEYKQKKKRCKYLKAKLNHIKKMVSDYDRRS-   492
Danio rerio (b)       418   MDEYNRLKSLKKSTDYQMKKRKCKRLKSKLSLIKRRVSDYDHRQ-   461
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Figure S1. Multiple alignment of occludin is shown for 8 orthologs [above]. Mutations identified in 5 families are highlighted. Deletions in 4 families are represented by coloured bars; F085 and F275 in green, F386 in red and F375 in orange. The two altered amino acids

in F312 are highlighted in green. The hatched blue bar represents the Marvel domain containing all 4 transmembrane domains [boxed].

TM; transmembrane domain. Human NP_002529.1; Pan troglodytes XP_001158288.1; Canus lupus familiaris NP_001003195.1; Bos taurus; NP_001075902.1; Mus musculus NP_112619.2; Rattus norvegicus NP_112619.2; Danio rerio NP_997997.1; Danio rerio NP_001008618.1.

Table S1. Clinical information for F085, F351 and F386.

Feature	F085a1	F085a2	F351	F386a1	F386a2
Gender	Female	Male	Female	Female	Male
Irritability at birth	+	+	-	-	-
Feeding difficulties at birth	+	-	-	-	-
Age at first seizure	2 weeks	2 weeks	6 weeks	2 days	N/A
Hypotonia	+	+	+	-	-
Hyperreflexia	-	+	+	+	+
Abnormal posturing /dystonia	+	+	-	-	-
Developmental	None	None	None	None	Smiles and babbles

progress										
Visual function and hearing	Roving eye movements no fixation; normal retina and BAEPs		No fixation; multifocal retinal grey lesions of unknown significance		No fixation at 4 months; normal retina		Roving eye movements; no fixation		Mild myopia and astigmatism; no response to light, normal ophthalmological examination	
Structural malformations	-		PDA/PFO Cleft lip		-		PDA; Transient nephrogenic diabetes insipidus secondary to mild renal dysplasia		Umbilical hernia	
Growth	**Age**	**Measurement**	**Age**	**Measurement**	**Age**	**Measurement**	**Age**	**Measurement**	**Age**	**Measurement**
OFC	Birth	30cm [-3SD]	Birth	34cm [-1SD]	Birth	31cm [-2.5SD]	2 days	35cm []	Birth	32cm [-2SD]

	1 year	39cm [-4SD]	6.5 years	44cm [-5SD]	-	-	4.1 years	46cm [-2.5SD]	35 days	32cm [-4.5SD]
Weight	Birth	2.56kg [-1SD]	Birth	3.54kg [+1SD]	Birth	3.2kg [0SD]	Birth	2.4kg []	Birth	3.186kg []
	1.1 year	9.4kg [0SD]	6.5 years	24.6kg [+1SD]	-	-	4.1 years	8.58kg [-5SD]	35 days	3.65kg [-2SD]
Length	1.1 year	82cm [+1.5SD]	6.5 years	118cm [0SD]	-	-	4.1 years	79cm [-6SD]	35 days	52cm [-2SD]
Age at death	2.5 years		7 years		-		-		-	
Congenital infection screen	Negative		Negative		Negative at birth. Repeat testing (4 months): positive IgG and CMV in urine but not blood or CSF		Rubella IgG and negative IgM positive; CMV and toxoplasmosis negative		Negative	

Abbreviations: OFC: occipito-frontal circumference; PDA: patent ductus arteriosus; PFO: patent foramen ovale; SD: standard deviation; N/A: information not available.

Table S2. OCLN oligonucleotide primer sequences.

Exon	Primer name	Direction	Sequence	Length (bp)	Tm (^{0}C)
2	OCLNx2F	Forward	gggtgggatgaagaaaagaa	20	59.0
2	OCLNx2R	Reverse	gcaaacacttaaagtttcaacca	23	58.4
3	OCLNx3F	Forward	ccaaataagttgtgttctttctgc	24	59.3
3	OCLNx3R	Reverse	ccagagtgattctatcacatatctcaa	27	59.9
4	OCLNx4F	Forward	tgtagagggtgaattgtgattaag	24	57.4
4	OCLNx4R	Reverse	gacacaaatttggggcttta	20	57.6
5	OCLNx5F	Forward	ccccctttcattacaagataaat	24	58.8
5	OCLNx5R	Reverse	gcaaatactcatttttacactcagtc	26	57.7
6	OCLNx6F	Forward	gtgcagatgtctgctggtgt	20	59.9
6	OCLNx6R	Reverse	caacacctggttggtctcct	20	60.0
7	OCLNx7F	Forward	gagcattgaattttatgtctgct	23	57.6
7	OCLNx7R	Reverse	ggatgctgtacctccacaga	20	58.7
8	OCLNx8F	Forward	cataagctgtcatttttagctcca	24	59.8
8	OCLNx8R	Reverse	gaaaagctcttccyccagatg	21	58.1
9	OCLNx9F	Forward	cccagcagacctgttttcat	20	60.1
9	OCLNx9R	Reverse	ggggttatggtccaaagtca	20	59.6

Table S3. Homozygous segments (>5Mb in size) detected using AutoSNPa software and results of SNP array.

Patient	Chromosome	Position	Size
F351	1	23225040-39268760	16Mb
F351	2	197051600-211193600	14.14Mb
F375a1	2	158427700-173759800	15.33Mb
F085a2	3	34355650-54003860	16.65Mb
F351	4	6988317-16894910	9.91Mb
F351	4	20502520-37166220	16.66Mb
F351	5	66092530-115792100	49.70Mb
F085a1	5	53643760-74142270	20.50Mb
F085a2	5	53643760-75313620	21.67Mb
F275	5	37997960-75229940	37.23Mb
F375a1	5	68154750-79393880	11.24Mb
F375a2	5	68154750-86808550	18.65Mb
F085a1	6	102280700-138037600	35.76Mb
F085a1	6	162096700-167501600	5.4Mb
F351	7	95899670-105006100	9.11Mb

F375a1	7	152287000-158787600	6.5Mb
F351	8	41746400-62115350	20.37Mb
F085a1	9	14955090-86714350	71.76Mb
F085a1	9	90773460-95847350	5.07Mb
F085a2	9	14955090-39309750	24.35Mb
F375a1	11	46884030-56214360	9.33Mb
F085a1	11	1745827-37297360	35.55Mb
F375a2	11	48144890-56371960	8.23Mb
F375a1	12	50803560-67885550	17.08Mb
F375a2	12	48588440-67885550	19.03Mb
F375a2	12	20467460-29674340	9.21Mb
F375a2	12	30927630-41339610	10.41Mb
F085a1	13	52534140-68471840	15.94Mb
F351	14	47603580-69443730	21.84Mb
F085a2	16	1688439-10889550	9.20Mb
F275	17	5438682-13823320	8.38Mb

F351	18	44818930-65667260	20.85Mb
F375a1	18	11625170-38706840	27.08Mb
F351	19	17596520-40072490	2.48Mb
F275	19	17688070-35037140	17.35Mb
F375a2	19	34252640-59312250	25.06Mb
F375a1	21	29269400-46940180	17.67Mb
F375a2	21	29698160-46940180	17.21Mb
F351	X	232264670-36351700	13.09Mb
F085a1	X	44550940-109168400	64.62Mb