American Journal of Human Genetics, Volume *91*

Supplemental Information

Mutations of the Gene Encoding Otogelin Are a Cause of Autosomal Recessive Nonsyndromic Moderate Hearing Impairment

Margit Schraders, Laura Ruiz-Palmero, Ersan Kalay, Jaap Oostrik, Francisco J. del Castillo, Orhan Sezgin, Andy J. Beynon, Tim M. Strom, Ronald J.E. Pennings, Celia Zazo Seco, Anne M.M. Oonk, Henricus P.M. Kunst, María Domínguez-Ruiz, Ana M. García-Arumi, Miguel del Campo, Manuela Villamar, Lies H. Hoefsloot, Felipe Moreno, Ronald J.C. Admiraal, Ignacio del Castillo, and Hannie Kremer

Supplemental Inventory

1. Supplemental Figures and Tables

Figure S1

Figure S2

Figure S3

Table S1

Table S2

2. Supplemental Experimental Procedures

3. Supplemental References

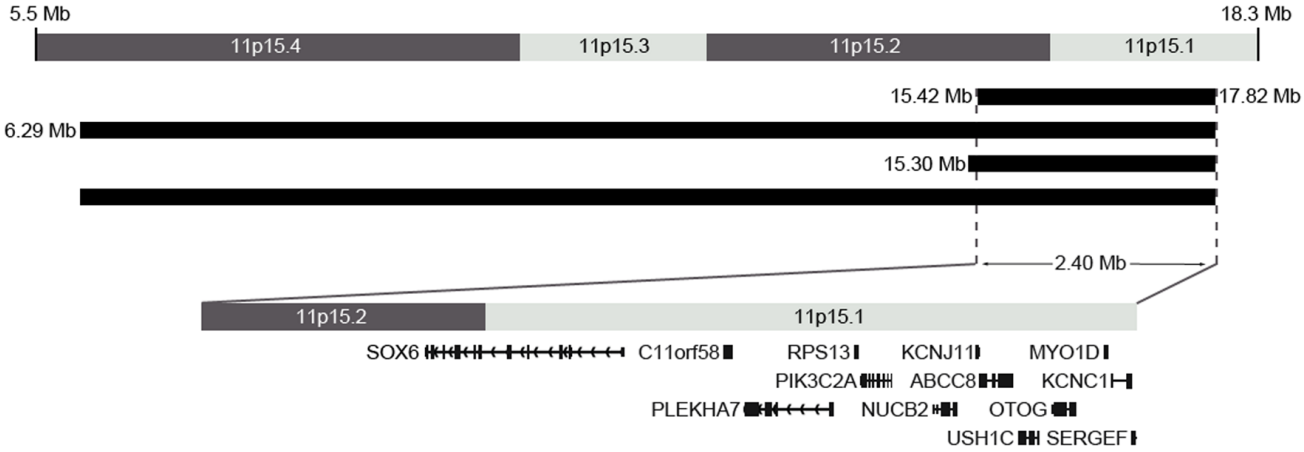

Figure S1. Schematic Representation of Chromosome 11p15.1-p15.4 and the Homozygous Regions in the Affected Sibs
MB positions and chromosome bands were derived from the UCSC genome browser (NCBI Build 37.1; hg19)

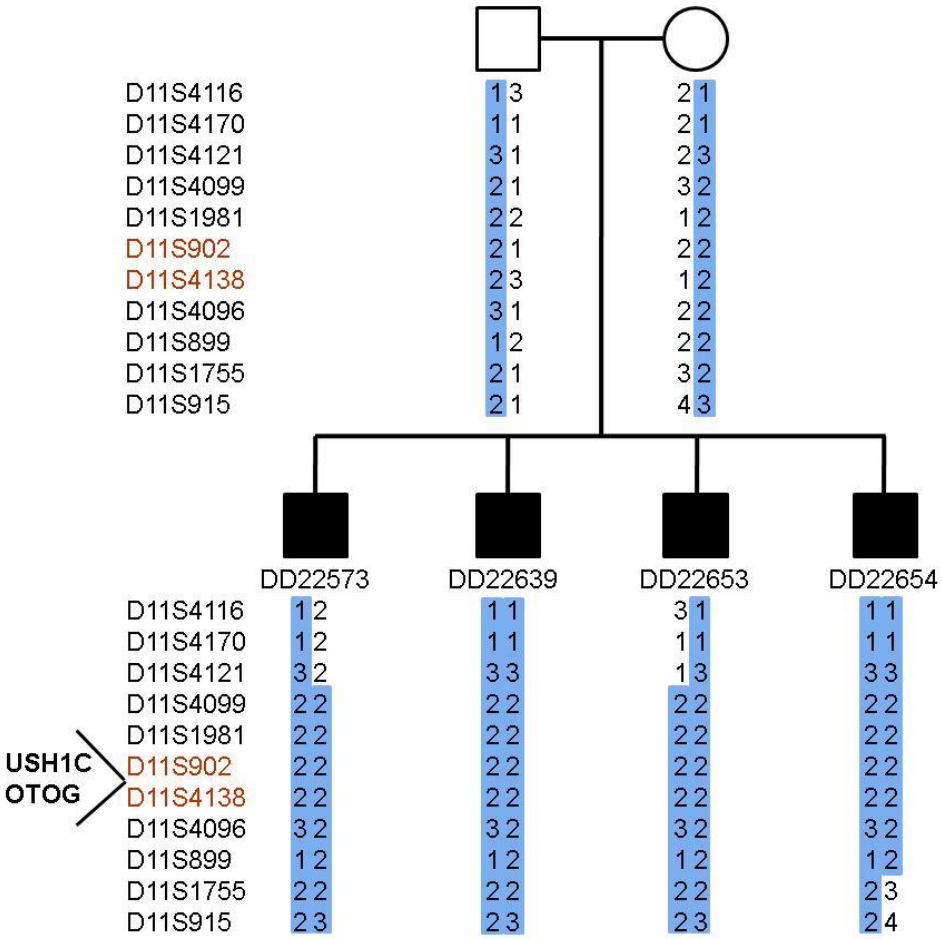

Figure S2. Microsatellite Marker Analysis for Family W00-384
Microsatellite markers were genotyped under standard PCR conditions and were analyzed on an ABI Prism 3730 Genetic Analyzer and with the GeneMapper program according to the manufacturer's protocol. (Applied Biosystems, Foster City, CA).

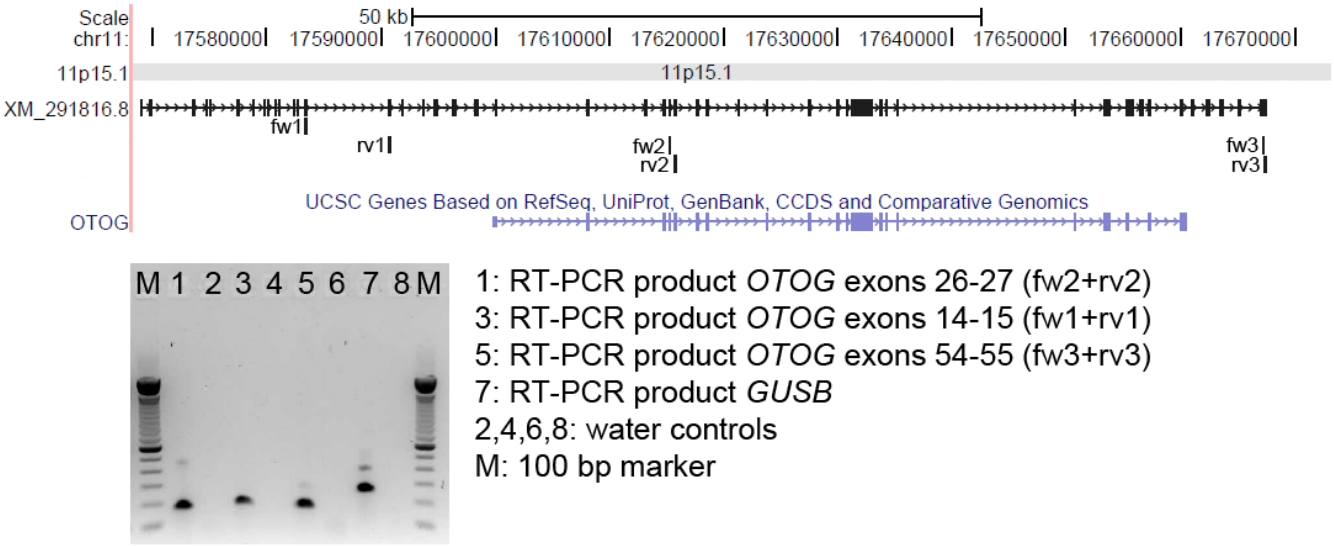

Figure S3. RT-PCR to Detect *OTOG* Transcripts in Fetal Inner Ear
cDNA was synthesized from fetal inner ear RNA using the IScript cDNA Synthesis kit (Biorad) according to the manufacturer's protocol. Primers were designed with Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and the reference sequence XM_291816.8. Primer sequences and PCR conditions are provided in Supplemental Table 1.

Table S1. Primer Sequences and Annealing Temperatures for Exon Amplification and Sequence Analysis

Fragment	Oligonucleotides	Size (bp)	Annealing Temperature (°C)
	Primers for genomic PCR and sequence analysis		
OTOG exon 1	Forward: 5'-aggtgtgaccctgccttag-3'	392	56
	Reverse: 5'-cccagcctaactttggactc-3'		
OTOG exon 2	Forward: 5'-agcacttagggtgggatagg-3'	260	56
	Reverse: 5'-ctgaacaactccagagaccc-3'		
OTOG exon 3	Forward: 5'-gatgaacaaagagggctgtg-3'	301	58
	Reverse: 5'-aaggagatgccaagcagg-3'		
OTOG exon 4-5	Forward: 5'-tagctgctgaggaatgttgg-3'	640	56
	Reverse: 5'-gaggaaggtgccagtctctc-3'		
OTOG exon 6	Forward: 5'-ataaggtgtgaagctcaggg-3'	313	56
	Reverse: 5'-gtggaaattgctgtttgtcc-3'		
OTOG exon 7	Forward: 5'-ttccttcggtgaagagactg-3'	392	56
	Reverse: 5'-taataccatgaccacgaccc-3'		
OTOG exon 8-9	Forward: 5'-tcagagccttctgtccctc-3'	657	58
	Reverse: 5'-aagaatttcagagccttggg-3'		
OTOG exon 10	Forward: 5'-ttcacctagatgccttcctg-3'	333	56
	Reverse: 5'-acaagacattgagctgaggc-3'		
OTOG exon 11	Forward: 5'-ccctctgggactaggaaatc-3'	357	56
	Reverse: 5'-ccttccacaaacaggtatcttc-3'		
OTOG exon 12-13	Forward: 5'-cggttcagagatgaaagaag-3'	659	56
	Reverse: 5'-tggaactgagtgacatccc-3'		
OTOG exon 14	Forward: 5'-tcctcatacttggagcttgc-3'	277	58
	Reverse: 5'-gcagtaggcaggagtagctg-3'		
OTOG exon 15	Forward: 5'-gggctctgtctgtcattctg-3'	359	56
	Reverse: 5'-tttgccagggtagcagtg-3'		
OTOG exon 16	Forward: 5'-cctcaagcctccccatacc-3'	397	56
	Reverse: 5'-cctaaggggcccatttcc-3'		
OTOG exon 17	Forward: 5'-tggatctatgctatttgtgcc-3'	305	56
	Reverse: 5'-tacaaatttggccagcactc-3'		
OTOG exon 18	Forward: 5'-gagggagagaggctgatcc-3'	399	56
	Reverse: 5'-ggctggaggagactggag-3'		
OTOG exon 19	Forward: 5'-accttgacctgctgtgtgac-3'	405	56
	Reverse: 5'-ccagtctcagatgccctg-3'		
OTOG exon 20	Forward: 5'-tgtccttatgcacctggttg-3'	585	56
	Reverse: 5'-gtcactgcaggacccagac-3'		
OTOG exon 21	Forward: 5'-gggaagtgagtgagggtgtc-3'	586	56
	Reverse: 5'-tccctctgtgaggcgtatc-3'		
OTOG exon 22	Forward: 5'-agtctggtatggaggacgtg-3'	549	58
	Reverse: 5'-cctctctggccattacagc-3'		
OTOG exon 23	Forward: 5'-aaggtccctctgaacctcac-3'	416	58
	Reverse: 5'-acaacacacaaacgcacaac-3'		

OTOG exon 24	Forward:	5'-ggttggtgtttgttgacctc-3'	362	56
	Reverse:	5'-cacccaattctgcatcaaac-3'		
OTOG exon 25-26	Forward:	5'-tgttcctccacctctgtacc-3'	716	58
	Reverse:	5'-tacagctcacccaaggacac-3'		
OTOG exon 27	Forward:	5'-ccatccctcttcaaactgtg-3'	342	58
	Reverse:	5'-ccctcttccctgaccttg-3'		
OTOG exon 28	Forward:	5'-ggcaaggtataggtcccttc-3'	394	58
	Reverse:	5'-tcttccctggagatctgagc-3'		
OTOG exon 29	Forward:	5'-cttcatgttggtgctggtc-3'	371	56
	Reverse:	5'-cagttggtgcttggtacattc-3'		
OTOG exon 30	Forward:	5'-agccttggctctgtctgtc-3'	256	56
	Reverse:	5'-actgtcagggctctctctgc-3'		
OTOG exon 31	Forward:	5'-catcaagagttcctccttgg-3'	394	58
	Reverse:	5'-tatttctccggccactacag-3'		
OTOG exon 32	Forward:	5'-attaccttcccgaggcttag-3'	636	58
	Reverse:	5'-aggattctgcttcttagggg-3'		
OTOG exon 33	Forward:	5'-ggctgagctctgggactc-3'	324	58
	Reverse:	5'-acggaaagacacagttaccg-3'		
OTOG exon 34	Forward:	5'-aaaggataaggcctgtgctc-3'	276	56
	Reverse:	5'-tgatcttgcttctctgggac-3'		
OTOG exon 35_1	Forward:	5'-agagtgccagtcctcaagc-3'	762	58
	Reverse:	5'-tactgcaggtgtcagcacag-3'		
OTOG exon 35_2	Forward:	5'-atggctccttgcctgttag-3'	726	58
	Reverse:	5'-gtgctatgtgagtgggtgg-3'		
OTOG exon 35_3	Forward:	5'-aaagcctctgtcatcaccac-3'	725	58
	Reverse:	5'-catctagcagggaagaggaag-3'		
OTOG exon 36	Forward:	5'-tacatgtgggaaggatctgg-3'	393	58
	Reverse:	5'-cacaagcgtaggaaatctgc-3'		
OTOG exon 37	Forward:	5'-atctgtgtgatgcgtggtc-3'	398	58
	Reverse:	5'-acatctgtctttgcctctgg-3'		
OTOG exon 38	Forward:	5'-gcccactcacttggagg-3'	359	58
	Reverse:	5'-ccctgacattggtgtgg-3'		
OTOG exon 39	Forward:	5'-gtcagatgatgggtggatg-3'	371	58
	Reverse:	5'-caggaggaattatgtgtggg-3'		
OTOG exon 40-41	Forward:	5'-gggataatggtattattgacctc-3'	753	56
	Reverse:	5'-aatgattaaagctgggaaagc-3'		
OTOG exon 42	Forward:	5'-gtgctgatgacactctgagc-3'	404	56
	Reverse:	5'-gacctgaaaggatgcagatg-3'		
OTOG exon 43	Forward:	5'-catctgcatcctttcaggtc-3'	427	58
	Reverse:	5'-tcctaggccttgtcctatcc-3'		
OTOG exon 44-45	Forward:	5'-gcagttgtccagtgttctcc-3'	565	58
	Reverse:	5'-cagtgagcttgtccttctcc-3'		
OTOG exon 46	Forward:	5'-ctgattccagaacggatcac-3'	436	58
	Reverse:	5'-gacccagcttcttcaggtg-3'		
OTOG exon 47	Forward:	5'-ttagccagtgttgcccttc-3'	247	58
	Reverse:	5'-ccctcaatccctcctcag-3'		

OTOG exon 48	Forward:	5'-gttgtgccagcagtgagag-3'	567	58
	Reverse:	5'-tacaaaggagggcaccatac-3'		
OTOG exon 49	Forward:	5'-ggtggcagactgctcctg-3'	307	64
	Reverse:	5'-agagctccaccactgtctgg-3'		
OTOG exon 50	Forward:	5'-ggatgaggagttcatgcacag-3'	398	61
	Reverse:	5'-tggagcacccaaggtctg-3'		
OTOG exon 51-52	Forward:	5'-tcgagcacttacagagggtc-3'	617	56
	Reverse:	5'-cccttcttcctgcactcc-3'		
OTOG exon 53	Forward:	5'-cctgtttctgggatcttgg-3'	257	60
	Reverse:	5'-ccttcatctactcagcccag-3'		
OTOG exon 54-55	Forward:	5'-ccctccagcatgacactaac-3'	587	58
	Reverse:	5'-acacgtgccagatttctacc-3'		
Primers for RT-PCR				
OTOG exon 14-15	Forward:	5'-tgagtgctcagtgactggtg-3'	178	58
	Reverse:	5'-tgacactgactggacacagg-3'		
OTOG exon 26-27	Forward:	5'-agtgggatttttcacactgg-3'	160	58
	Reverse:	5'-ggtcctcatctcattgatgg-3'		
OTOG exon 54-55	Forward:	5'-aagaatgaatgcaggagcag-3'	162	58
	Reverse:	5'-acagaagagctgcacagagc-3'		
Primers for qPCR				
OTOG exon 6-7	Forward:	5’- ctcgggatatgtgtgtcctg-3’	91	60
	Reverse:	5’- tggcagatctcaaacacctc-3’		

Table S2. Variants Identified in the Genes within the Homozygous Region of Family W00-384

Gene	reference sequence	cDNA change	Protein change	rs-number	1000 Genomes Frequency	EVS frequency (EA allele)
PIK3C2A	NM_002645.2	c.270T>C	synonymous	rs214936	G = 0.367/801	G=3973/A=4613
NUCB2	NM_005013.2	c.1207_1209delCAA	p.403delGln*	rs72423941	Unknown	Unknown
KCNJ11	NM_000525.3	c.570C>T	synonymous	rs5218	A=0.275/601	A=2563/G=6023
ABCC8	NM_000352.3	c.207T>C	synonymous	rs1048099	G=0.443/968	G=4381/A=4205
ABCC8	NM_000352.3	C.1686C>T	synonymous	rs1799857	A=0.386/842	A=3876/G=4710
USH1C	NM_153676.3	c.2547-11T>C	-	rs10832795	G=0.386/843	G=3551/A=5035
USH1C	NM_153676.3	c.105-16C>T	-	rs2041027	A=0.451/985	A=3585/G=5001
USH1C	NM_153676.3	c.496+33A>G	-	rs12795083	Unknown	Unknown
USH1C	NM_153676.3	c.522-45delC	-	rs5789990	delC=0.357/779	Unknown
USH1C	NM_153676.3	c.580-27G>A	-	rs6486378	T=0.347/757	T=2542/C=6044
USH1C	NM_153676.3	c.2340C>T	synonymous	rs10832796	A=0.222/485	A=2489/G=6097
USH1C	NM_153676.3	c.2457G>C	p.Glu819Asp	rs1064074	G=0.434/949	G=4803/C=3783
OTOG	XM_291816.8	c.4123G>C	p.Ala1375Pro	rs7934079	G=0.188/410	Unknown
OTOG	XM_291816.8	c.4940C>T	p.Pro1647Leu	rs2041028	T=0.362/791	Unknown
OTOG	XM_291816.8	c.5508delC	p.Ala1838ProfsX31[#]	-	Unknown	Unknown
OTOG	XM_291816.8	c.5840C>T	p.Thr1947Met	rs7111528	T=0.341/745	Unknown
OTOG	XM_291816.8	c.7930+8C>T	-	rs11024353	T=0.032/69	Unknown
OTOG	XM_291816.8	c.7930+33G	-	rs55858137	A=0.026/57	Unknown
OTOG	XM_291816.8	c.3270C>A	synonymous	rs875696	A=0.042/92	Unknown
KCNC1	NM_004976.4	c.744C>T	synonymous	rs2229007	T=0.025/54	T=351/C=8235
SERGEF	NM_012139.2	c.981G>A	synonymous	rs211146	C=0.367/801	T=5264/C=3322

Variants are shown located within exons and in 50 bp within the 50 bases immediately adjacent to an exon. *This variant is not considered to be pathogenic since it is an in-frame deletion of a single amino acid which does not affect any of the conserved domains of the translated protein (as predicted by SMART, http://smart.embl-heidelberg.de). [#]This is considered the causative variant for family W00-384 underlying the deafness.