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Supplemental information

Exploring non-coding variants and evaluation of antisense oligonucleotides for splicing redirection in Usher syndrome

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Table S1. Studies performed in the selected patients.

Patient	Family	Gene	Dx	Allele 1	SS	NGS	SEG	Reference
RP-331	**-**	***USH2A***	**ARRP**	**c.2276G>T; p.(Cys759Phe)**	**SP**	**NP**	**NA**	[1]
RPN-645	FRPN-243	*USH2A* *CEP290*	ARRP	c.2276G>T ; p.(Cys759Phe) c.7394_7395del; p.(Glu2465Valfs*2)	SP	P1	NA	[1] [2]
RP-654	**FRP-30**	***USH2A***	**USH**	**c.11146C>T; p.(Gln3716*)**	**SP**	**NP**	**NA**	[3]
RP-681	FRP-49	*CDH23*	USH	c.7279del; p.(Thr2427Leufs*47)	NP	P1	NA	[4]
RPN-803	FRPN-372	*USH2A* *ZNF408*	ARRP	c.2276G>T; p.(Cys759Phe) c.1534dup; p.(Arg512Profs*27)	NP	P1	NA	[1] This study
RP-903	FRP-57	*USH2A*	USH	c.5039A>G; p.(Lys1680Arg)	NP	P2	NA	[5]
RP-904	FRP-58	*USH2A*	USH	c.9799T>C; p.(Cys3267Arg)	SP	NP	NA	[6]
RP-925	-	*USH2A*	USH	c.2276G>T; p.(Cys759Phe)	SP	NP	NA	[1]
RP-963	FRP-140	*USH2A*	USH	c.2276G>T; p.(Cys759Phe)	SP	NP	NA	[1]
RP-1036	FRP-163	*USH2A*	USH	c.2809+1G>A; p.(?)	NP	P1	CC	[7]
RP-1222	FRP-228	*USH1C*	USH	c.1234G>A; p.(Asp412Asn)	NP	P1	NA	[4]
RP-1360	FRP-283	*USH2A*	USH	c.2276G>T; p.(Cys759Phe)	SP	P1	NA	[1]
RP-1387	FRP-320	*PCDH15*	USH	c.3817C>A; p.(Arg1273Ser)	NP	P1	NA	This study
RP-1455	FRP-337	*USH2A*	USH	c.5666A>G; p.(Asp1889Gly)	SP	P1	NA	[8]
RP-1472	**FRP-341**	***USH2A***	**USH**	**c.2299del; p.(Glu767Serfs*21)**	**SP**	**NP**	**NA**	[9]
RP-1485	**FRP-344**	***USH2A***	**USH**	**c.1214del; p.(Asn405Ilefs*3)**	**SP**	**NP**	**NA**	[10]
RP-1494	**FRP-348**	***USH2A***	**USH**	**c.2299del; p.(Glu767Serfs*21)**	**SP**	**NP**	**CC**	[9]
RP-1496	FRP-350	*ADGRV1*	USH	c.3443G>A; p.(Gly1148Asp)	NP	P1	NA	[11]
RP-1541	FRP-367	*USH2A*	USH	c.12067-2A>G; p.?	SP	NP	NA	[12]
RP-1569	**FRP-380**	***USH2A***	**USH**	**c.2299del; p.(Glu767Serfs*21)**	**SP**	**NP**	**NA**	[9]
RP-1595	**FRP-389**	***USH2A***	**USH**	**c.10636G>A; p.(Gly3546Arg)**	**SP**	**NP**	**NA**	[13]
RP-1596				**c.10636G>A; p.(Gly3546Arg)**	**SP**	**NP**	**NA**	
RP-1600	FRP-391	*USH2A*	USH	c.1144G>A; p.(Gln5796*)	SP	P1	NA	[13]
RP-1634	FRP-410	*ADGRV1*	USH	c.17386C>T; p.(Gln5796*)	NP	P1	NA	[14]
RP-1635	FRP-411	*USH2A*	USH	c.2276G>T; p.(Cys759Phe)	SP	NP	NA	[1]
RP-1647	**FRP-417**	***USH2A***	**ARRP**	**c.2276G>T; p.(Cys759Phe)**	**SP**	**NP**	**NA**	[1]
RP-1716	FRP-437	*USH2A*	USH	c.5858C>G; p.(Ala1953Gly)	SP	P1	NA	[15]
RP-1726	FRP-448	*USH2A*	USH	c.13514A>G; p.(Tyr4505Cys)	SP	NP	NA	[16]
RP-1727	FRP-449	*USH2A*	USH	c.1214del; p.(Asn405Ilefs*3)	SP	NP	NA	[10]
RP-1728	FRP-446	*USH2A*	USH	c.13514A>G; p.(Tyr4505Cys)	SP	NP	NA	[16]
RP-1736	FRP-455	*USH2A*	USH	c.2299del; p.(Glu767Serfs*21)	SP	NP	NA	[9]
RP-1741#	**FRP-460**	***USH2A***	**USH**	**c.7595-2144A>G; p.(Lys2532Thrfs*56)**	**SP**	**P1**	**NA**	[3]
RP-1776	**FRP-484**	***USH2A***	**ARRP + SNHL**	**c.2276G>T; p.(Cys759Phe)**	**SP**	**NP**	**NA**	[1]
RP-1815	FRP-505	*USH2A*	USH	c.908G>A; p.(Arg303His)	NP	P2	CC	[17]
RP-1943	FRP-566	*USH2A*	ARRP	c.2276G>T; p.(Cys759Phe)	SP	P1	NA	[1]
RP-1950	FRP-568	*USH2A*	USH	c.2299del; p.(Glu767Serfs*21)	SP	P1	NA	[9]
RP-1953	FRP-570	*USH1C*	USH	c.1859G>T; p.(Arg620Leu)	NP	P1	NA	[18]
RP-1994	FRP-585	*USH2A*	ARRP	c.2299del; p.(Glu767Serfs*21)	SP	P1	NA	[9]

RP-2034	FRP-614	*USH2A*	USH	c.2299del; p.(Glu767Serfs*21)	SP	P1	CC	[9]
RP-2055	**FRP-619**	***USH2A***	**USH**	**c.8435_8438del; p.(Thr2812Metfs*17)**	**SP**	**NP**	**NA**	[19]
RP-2106	FRP-645	*USH2A*	USH	c.5933C>T; p.(Pro1978Leu)	SP	NP	NA	This study
RP-2131	FRP-667	*MYO7A*	USH	c.3G>A; p.(?)	NP	P1	NA	[4]
RP-2140	FRP-676	*CDH23*	USH	c.6393del; p.(Ile2132Serfs*11)	NP	P1	NA	[20]
RP-2159	FRP-675	*USH2A*	USH	c.3637_3642dup; p.(Phe1213_Ala1214dup)	SP	P1	NA	This study
RP-2213	FRP-719	*MYO7A*	ARRP	c.640G>A; p.(Gly214Arg)	NP	P1	NA	[21]
RP-2214	FRP-720	*USH2A*	ARRP	c.2431_2432del; p.(Lys811Aspfs*11)	NP	P1	NA	[6]
RP-2224	**FRP-722**	***USH2A***	**USH**	**c.2431_2432del; p.(Lys811Aspfs*11)**	**SP**	**NP**	**NA**	[6]
RP-2232	FRP-722	*USH2A*	USH	c.1898C>A; p.(Ser633*)	SP	P1	NA	[7]
RP-2237	FRP-724	*USH2A*	ARRP	c.12575G>A; p.(Arg4192His)	SP	NP	NA	[22]
RP-2238	FRP-725	*USH2A* *PDE6H*	ARRP	c.1724G>A; p.(Cys575Tyr) c.134+2T>C; p.(?)	SP	NP	NA	[23] This study
RP-2239	FRP-726	*USH2A*	ARRP	c.7061G>A; p.(Arg2354His)	SP	P1	NA	This study
RP-2241	FRP-728	*USH2A*	ARRP	c.2276G>T; p.(Cys759Phe)	SP	NP	NA	[1]
RP-2262	FRP-730	*ADGRV1*	USH	c.1875G>A; p.(Asp6252Asn)	NP	P1	NA	This study
RP-2264	FRP-732	*USH2A*	USH	c.1055C>T; p.(Thr352Ile)	SP	P2	NA	[24]
RP-2266	FRP-734	*USH2A*	USH	c.9799T>C; p.(Cys3267Arg)	SP	NP	NA	[6]
RP-2268	FRP-736	*USH2A*	ARRP	c.10073G>A; p.(Cys3358Tyr)	NP	P2	NA	[15]
RP-2269	FRP-737	*USH2A*	ARRP	c.1214del; p.(Asn405Ilefs*3)	NP	P2	NA	[10]
RP-2270	FRP-738	*USH2A*	ARRP	c.12574C>T; p.(Arg4192Cys)	NP	P2	NA	[25]
RP-2275	FRP-740	*USH2A*	USH	c.5776+1G>A; p.(?)	SP	P1	NA	[26]

Homozygous and heterozygous patients for deep-intronic variants in USH2A are highlighted in bold. RP/RPN: Patient number; "-": Unkown; RP/FRPN: Family number; Dx: Diagnosis; ARRP: Autosomal Recessive Retinitis Pigmentosa; USH: Usher Syndrome; SS: Sanger Screening; SP: Screening Performed; NP: Not Performed; P1: Panel design one; P2: Panel design two; SEG: Segregation analysis. The "#" symbol refers to the NGS positive control; CC: Cosegregation confirmed.

Table S2. Sequence of antisense oligonucleotides tested for the aberrant splicing modulation caused by three deep-intronic variants.

Variant	Oligonucleotide	Sequence (3'-5')	G-C%
USH2A: c.14134-3169A>G	AON1-PE64	CCAUUUUUCUAGGAGAAGCCC	47.6
	AON2-PE64	CAACUGUUUGUUCACAAGGU	40
USH2A: c.9958+3438A>G	AON1-PE50	UGCAGGUGUAAAAAUGGCCAAG	45.5
	AON7-PE50	UACCUUGACUGGAGUGAUCUC	47.6
USH2A: c.8681+3960A>G	AON1-PE43	CAGUGACAAUUUGGAUGGAGAC	45.5

G-C: Guanine-Cytosine content.

Table S3. Primer sequences used for the minigene assays

Variant	Restriction enzyme	Rectriction site and tails	Primer sequence (5'-3')
USH2A: c.4106C>T	XhoI	AAGAATCTCGAG	gggaaatccagtacatcacc
	NheI	AAGAATGCTAGC	caatatcaactgagtgtggac
USH2A: c.5168-26A>C	XhoI	AAGAATCTCGAG	cactgcctcctatatttacag
	NheI	AAGAATGCTAGC	catctgaaaggagatggaaac
USH2A: c.9958+3438A>G	XhoI	AAGAATCTCGAG	cctgtactatgactaaccag
	NheI	AAGAATGCTAGC	ggtgttctgacttctctttc
USH2A: c.8681+3960A>G	NdeI	AAGAATCATATG	gaagctgccgaagtgagtc
	NheI	AAGAATGCTAGC	gaaacacgtgtcatggaactg

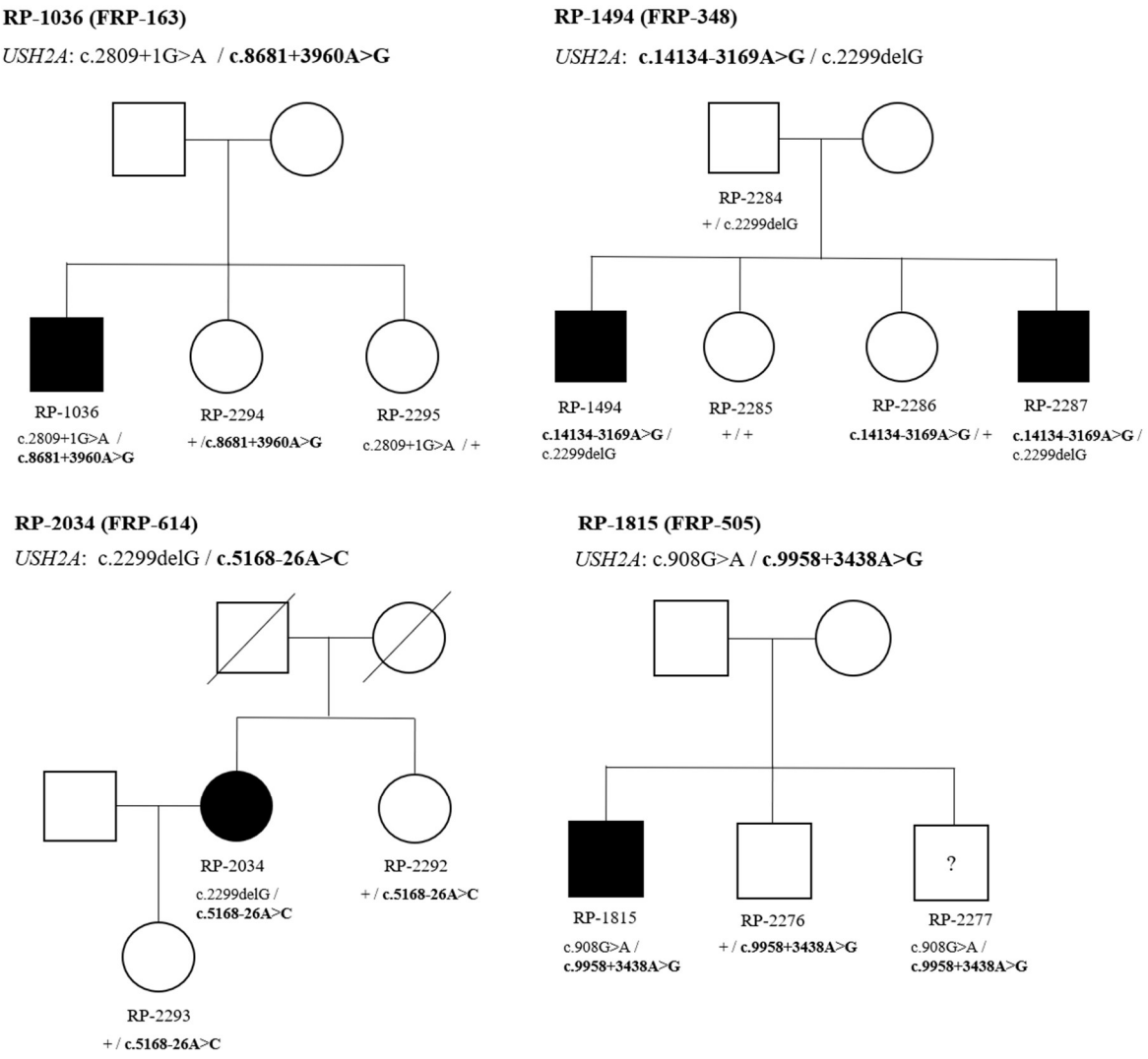

Figure S1. Family pedigrees in which segregation analysis were assessed. Variants identified in this study are highlighted in bold.

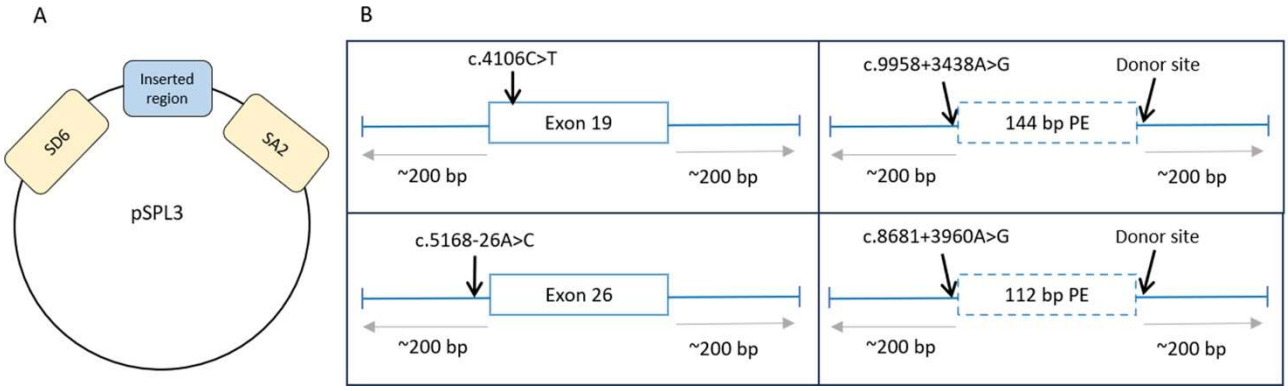

Figure S2. Minigene constructs for the analysis of *USH2A* variants. Regions inserted are depicted in blue in both sections A and B. In section A, constitutive exons are represented in yellow (SD6 and SA2). Each square in section B represents designs performed for each variant. PE: Pseudoexon; bp: base pair.

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