DNA

ism1	823	ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCACCCTGCAC	882
uninjected_emb1		ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCACCCTGCAC	
ism1_T3_emb1.1		ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCACCCTGCAC	
ism1_T3_emb1.2		ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCACCCTGCAC	
ism1_T3_emb1.3		ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCCTCATACAG	
ism1_T3_emb1.4		ATGTTGCGACTGGCAGCGGAGCTTCTGCTTCTCCTGGGACTGCTCCTCCTCCTCATACAG	

ism1	883	ATCACTGTGCTCCGAGGCAGCCCCGATAGCTCCTCCAACTCCAGCCACAGCCTCATACAG	942
uninjected_emb1		ATCACTGTGCTCCGAGGCAGCCCCGATAGCTCCTCCAACTCCAGCCACAGCCTCATACAG	
ism1_T3_emb1.1		ATCACTGTGCTCCGAG------------------------CCAGCCACAGCCTCATACAG	
ism1_T3_emb1.2		ATCACTGTGCTCCGAGGCAGCCCCGAT----------ACTCCAGCCACAGCCTCATACAG	
ism1_T3_emb1.3		GTCAGTCCATTCCATTCCA---CATACAGGTCAGTCCATTCCACATACAGGT-----CAG	
ism1_T3_emb1.4		GTCAGTCCATTCCATTCCA---CATACAGGTCAGTCCATTCCACATACAGGT-----CAG	

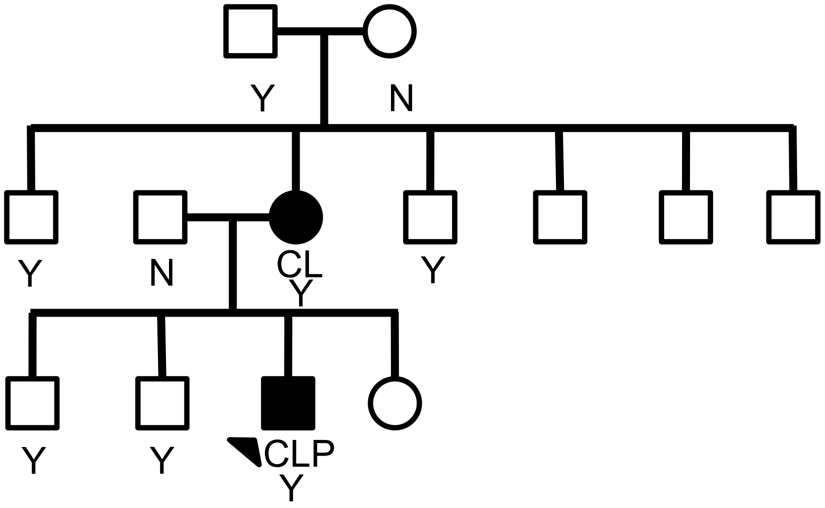
ism1	943	GTCAGTCCATTCCACATACAG	964
uninjected_emb1		GTCAGTCCATTCCTCATACAG	
ism1_T3_emb1.1		GTCAGTCCATTCCACATACAG	
ism1_T3_emb1.2		GTCAGTCCATTCCACATACAG	
ism1_T3_emb1.3		TCCATTCCATTCCACATACAG	
ism1_T3_emb1.4		TCCATTCCATTCCACATACAG	

mRNA

ism1	1	MLRLAAELLLLLGLLLLTLHITVLRG^SPDSSSNSSHSLIQVSPFHIQ…
uninjected_emb1		MLRLAAELLLLLGLLLLTLHITVLRG^SPDSSSNSSHSLIQVSPFHIQ…
ism1_T3_emb1.1		MLRLAAELLLLLGLLLLTLHITVLRV-------SSHSLIQVSPFHIQ…
ism1_T3_emb1.2		MLRLAAELLLLLGLLLLTLHITVLRG^SPD---YSSHSLIQVSPFHIQ…
ism1_T3_emb1.3		MLRLAAELLLLLGLLLLLIQVSP^FHSISYRSVHSTYRF-QSIPFHIQ
ism1_T3_emb1.4		MLRLAAELLLLLGLLLLLIQVSP^FHSISYRSVHSTYRF-QSIPFHIQ
Human ISM1 (signal seq.)		MVRLAAELLLLLGLLLLTLHITVLRG^SGA

Supplemental Figure 1: Representative sequencing results for embryos injected using a CRISPR/Cas9 strategy. Shown here are sequencing results of multiple independent clones (ism1_T3_embl1.1-4) from a single CRISPR/Cas9-mutated embryo compared to an uninjected control showing multiple genetic aberrations of *ism1* per embryo. Guide RNA site is underlined. ^Indicates predicted signal peptide cleavage site.

Pedigree 1

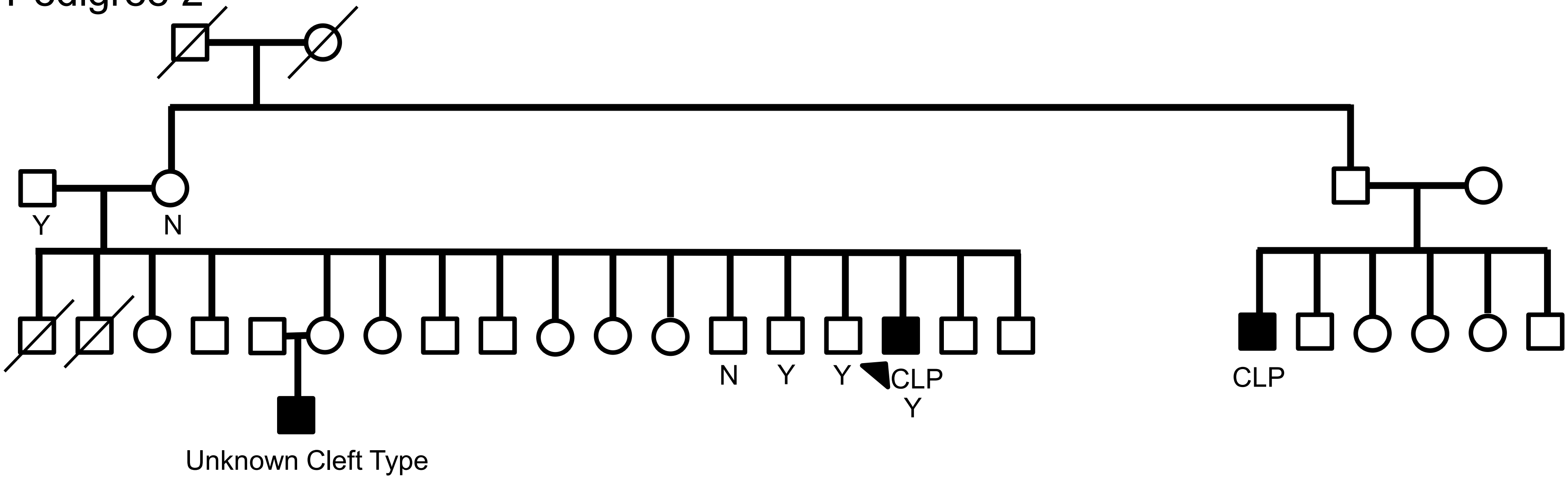


Key

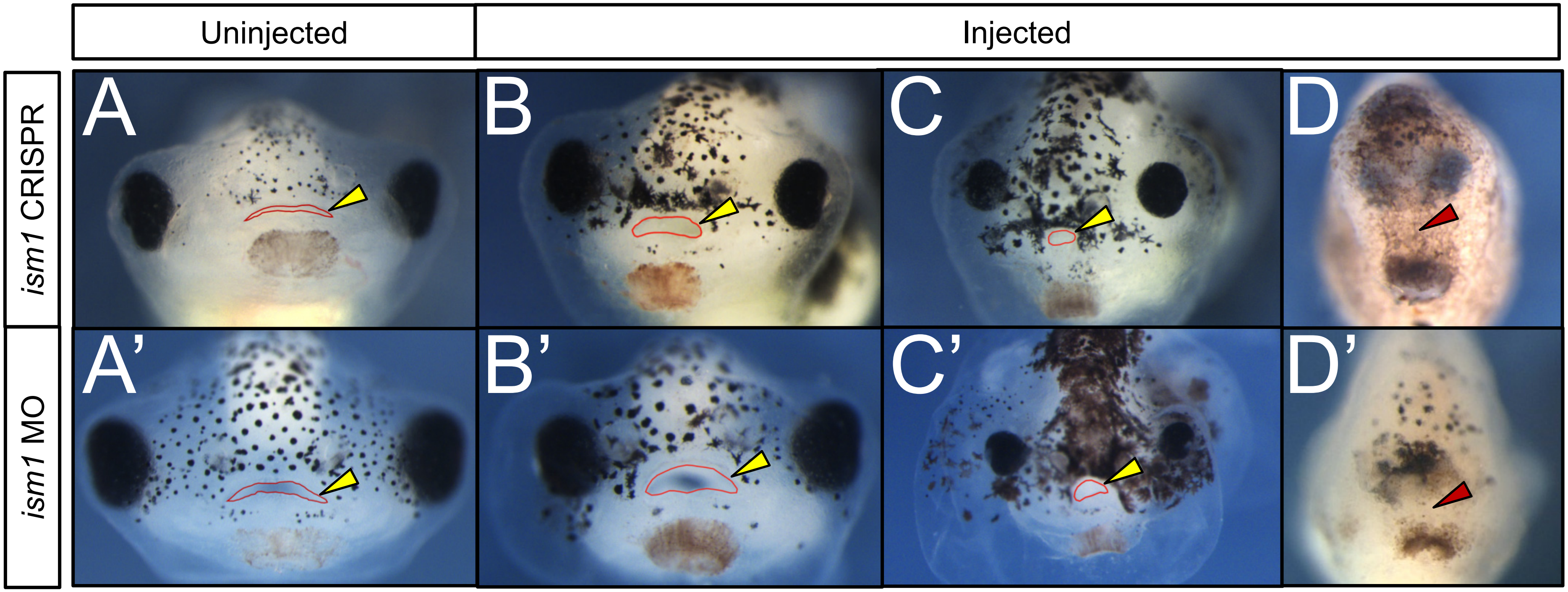
Y = Deletion Detected

N = No Deletion Detected

Pedigree 2



Supplemental Figure 2: Pedigrees of individuals harboring the *ISM1* deletions. (Pedigree 1) The 12kb deletion 3’ of *ISM1* was detected in the proband’s mother who is affected with a cleft lip (CL), unaffected brothers, maternal grandfather and one maternal uncle. (Pedigree 2) The 603kb deletion is paternally inherited and was identified in two unaffected brothers. Two additional individuals are affected in the pedigree but due to lack of samples they could not be tested.


Uninjected
Injected
ism1 CRISPR
ism1 MO
A
B
C
D
A'
B'
C'
D'

Supplemental Figure 3: Comparison of morpholino and CRISPR/Cas9 injected embryos. Embryos injected with a high-dose (48ng) of translation blocking *ism1* morpholino (MO) exhibit similar phenotypes as embryos injected with CRISPR/Cas9 oligos targeting *ism1*. Yellow arrowheads indicate mouths of the embryos while red arrowheads indicate complete absence of mouth development.

Chromosome Region (HG19)	Individual ID	Event	Length (bp)	Cytoband	% of Overlap with CNV in DGV or Seg Dup	Probe Median	Probes	Count of Gene Symbols	Count of Exons	Gene Symbols
chr1:161631150-161844639	CleftProband_66	Gain	213490	1q23.3	12.41	0.53	164	6	122	*FCGR2B, RPL31P11, FCRLA, FCRLB, DUSP12, ATF6*
chr1:212006532-212214302	CleftProband_58	Gain	207771	1q32.3	0.00	0.55	163	3	110	*LOC102723727, INTS7, DTL*
chr1:226161393-226171960	CleftProband_105	Loss	10568	1q42.12	0.00	-0.96	8	1	1	*SDE2*
chr1:226161393-226171960	CleftProband_84	Loss	10568	1q42.12	0.00	-0.82	8	1	1	*SDE2*
chr1:234491011-234564609	CleftProband_13	Loss	73599	1q42.2	0.00	-0.90	62	3	24	*LOC101927765, COA6, TARBP1*
chr1:245172706-245187353	CleftProband_68	Gain	14648	1q44	0.12	0.53	12	1	3	*EFCAB2*
chr1:245172706-245187353	CleftProband_106	Gain	14648	1q44	0.12	0.61	12	1	3	*EFCAB2*
chr1:247108435-247122814	CleftProband_137	Loss	14380	1q44	0.00	-0.97	11	2	3	*ZNF695, ZNF670-ZNF695*
chr1:27719038-27742111	CleftProband_73	Gain	23074	1p36.11	25.86	0.82	18	2	9	*GPR3, WASF2*
chr1:39377221-39405248	CleftProband_30	Loss	28028	1p34.3	0.00	-0.78	23	1	5	*RHBDL2*
chr1:40452270-40510508	CleftProband_92	Loss	58239	1p34.2	0.31	-0.74	43	1	3	*CAP1*

chr1:40452270-40511345	CleftProband_5	Loss	59076	1p34.2	0.31	-0.70	44	1	3	*CAP1*
chr1:49784601-50014768	CleftProband_32	Loss	230168	1p33	36.54	-0.87	169	2	4	*AGBL4, AGBL4-IT1*
chr1:54911801-55014065	CleftProband_6	Gain	102265	1p32.3	15.28	0.69	71	1	2	*ACOT11*
chr10:134630599-134646935	CleftProband_130	Gain	16337	10q26.3	35.79	1.17	9	1	1	*CFAP46*
chr10:17334656-17606693	CleftProband_131	Loss	272038	10p12.33	1.01	-0.99	212	2	27	*ST8SIA6, ST8SIA6-AS1*
chr10:19853134-19863618	CleftProband_71	Loss	10485	10p12.33 - p12.32	0.00	-1.12	9	1	1	*MALRD1*
chr10:19853134-19863618	CleftProband_70	Loss	10485	10p12.33 - p12.32	0.00	-1.10	9	1	1	*MALRD1*
chr10:23612640-23622814	CleftProband_32	Gain	10175	10p12.2	0.00	0.59	6	1	1	*C10orf67*
chr10:514552-541103	CleftProband_6	Gain	26552	10p15.3	1.02	0.65	23	1	2	*DIP2C*
chr10:51513243-51550524	CleftProband_52	Gain	37282	10q11.23	20.60	0.60	28	2	2	*MSMB, TIMM23B*
chr10:51513243-51552073	CleftProband_107	Gain	38831	10q11.23	19.78	0.64	29	2	2	*MSMB, TIMM23B*
chr10:5409736-5449369	CleftProband_131	Gain	39634	10p15.1	6.30	0.72	32	2	9	*UCN3, TUBAL3*
chr10:69779004-69974628	CleftProband_136	Gain	195625	10q21.3	0.64	0.60	153	2	137	*HERC4, MYPN*

chr10:90948510-91396619	CleftProband_18	Gain	448110	10q23.31	6.27	0.61	344	11	88	_CH25H, LIPA, IFIT2**, IFIT3, IFIT1B, IFIT1, IFIT5, SLC16A12-AS1, SLC16A12, MIR107, PANK1_
chr11:365864-377721	CleftProband_17	Gain	11858	11p15.5	0.00	0.87	7	1	14	_B4GALNT4_
chr11:5509812-5519989	CleftProband_26	Loss	10178	11p15.4	0.00	-0.95	9	2	1	_OR51B5_, _OR52D1_
chr11:60549417-60586456	CleftProband_6	Loss	37040	11q12.2	1.67	-1.12	28	1	8	_MS4A10_
chr11:8414939-8462604	CleftProband_136	Loss	47666	11p15.4	5.20	-1.07	34	1	16	_STK33_
chr12:179170-234115	CleftProband_16	Gain	54946	12p13.33	27.13	0.57	46	1	3	_IQSEC3_
chr12:360068-464833	CleftProband_76	Gain	104766	12p13.33	0.00	0.65	79	3	31	_SLC6A13, LOC102723544, KDM5A_
chr12:4719026-4772107	CleftProband_124	Gain	53082	12p13.32	19.31	0.84	45	3	27	_DYRK4, AKAP3, NDUFA9_
chr12:72173086-72188140	CleftProband_24	Gain	15055	12q21.1	20.45	1.11	13	1	3	_RAB21_
chr12:72173086-72276664	CleftProband_134	Gain	103579	12q21.1	9.56	0.89	79	3	21	_RAB21_, _MRS2P2_, _TBC1D15_
chr12:72173086-72283834	CleftProband_53	Gain	110749	12q21.1	8.94	0.47	86	3	24	_RAB21_, _MRS2P2_, _TBC1D15_
chr12:72173086-72283834	CleftProband_127	Gain	110749	12q21.1	8.94	0.56	86	3	24	_RAB21_, _MRS2P2_, _TBC1D15_

chr12:72174000-72281874	CleftProband_113	Gain	107875	12q21.1	9.18	0.47	83	3	24	*RAB21, MRS2P2, TBC1D15*
chr12:72190776-72283834	CleftProband_24	Gain	72198	12q21.1	5.80	1.00	53	2	6	*MRS2P2, TBC1D15*
chr12:7586838-7607779	CleftProband_111	Loss	20942	12p13.31	9.47	-0.99	17	1	4	*CD163L1*
chr12:7586838-7607779	CleftProband_109	Loss	20942	12p13.31	9.47	-0.91	17	1	4	*CD163L1*
chr13:67355803-67501956	CleftProband_72	Loss	146154	13q21.32	2.22	-0.87	126	2	8	*PCDH9, PCDH9-AS2*
chr13:98081584-98092624	CleftProband_50	Gain	11041	13q32.1	0.00	0.49	10	1	1	*RAP2A*
chr14:25367816-26852615	CleftProband_8	Gain	1484800	14q12	1.82	0.45	1158	1	7	*STXBP6*
chr14:44749975-45023734	CleftProband_120	Loss	273760	14q21.3	0.00	-0.96	191	2	4	*FSCB, LOC105370473*
chr14:44822079-45023734	CleftProband_127	Loss	201656	14q21.3	0.00	-1.03	138	2	4	*FSCB, LOC105370473*
chr15:51344424-51356893	CleftProband_69	Loss	12470	15q21.2	0.00	-0.89	11	2	2	*TNFAIP8L3, MIR4713HG*
chr15:57107766-57297943	CleftProband_60	Gain	190178	15q21.3	0.00	0.55	145	2	48	*LOC145783, TCF12*
chr15:93626518-93637718	CleftProband_98	Loss	11201	15q26.1	0.00	-0.78	10	1	2	*RGMA*
chr16:216901-237122	CleftProband_52	Loss	20222	16p13.3	43.47	-0.86	15	3	9	*HBA2, HBA1, HBQ1*

chr16:68575089-68774733	CleftProband_84	Loss	199645	16q22.1	9.41	-0.99	141	3	82	*ZFP90, CDH3, CDH1***
chr17:13186916-13531727	CleftProband_80	Gain	344812	17p12	0.87	0.71	293	2	2	*LINC02093, HS3ST3A1*
chr17:26715416-26746199	CleftProband_130	Gain	30784	17q11.2	0.00	0.47	27	2	14	*SARM1, SLC46A1***
chr17:41587266-41772492	CleftProband_124	Gain	185227	17q21.31	0.92	0.61	145	3	151	*DHX8, ETV4, MEOX1*
chr17:57919964-57945360	CleftProband_3	Gain	25397	17q23.1	1.92	0.52	18	1	16	*TUBD1*
chr17:78225607-78326928	CleftProband_134	Gain	101322	17q25.3	4.18	0.60	69	3	55	*SLC26A11, RNF213, LOC100294362*
chr17:967314-1120134	CleftProband_54	Gain	152821	17p13.3	36.45	0.51	115	1	53	*ABR*
chr18:13978807-14132035	CleftProband_103	Gain	153229	18p11.21	18.09	0.61	102	1	7	*ZNF519*
chr18:2795134-2831565	CleftProband_14	Gain	36432	18p11.32	0.00	0.45	24	1	3	*SMCHD1*
chr18:29647247-29677751	CleftProband_66	Gain	30505	18q12.1	19.72	0.95	22	2	5	*RNF125, RNF138*
chr18:44478317-44514278	CleftProband_91	Loss	35962	18q21.1	0.00	-1.00	20	1	37	*PIAS2*
chr19:39338101-39349508	CleftProband_135	Loss	11408	19q13.2	0.00	-1.08	7	1	2	*HNRNPL*
chr19:39338101-39349508	CleftProband_18	Loss	11408	19q13.2	0.00	-0.76	7	1	2	*HNRNPL*

chr19:39339100-39349508	CleftProband_81	Loss	10409	19q13.2	0.00	-0.80	6	1	2	*HNRNPL*
chr19:50041198-50062100	CleftProband_41	Loss	20903	19q13.33	3.07	-1.02	15	2	13	*RCN3, NOSIP***
chr2:10885208-10895660	CleftProband_113	Gain	10453	2p25.1	0.00	0.92	8	1	2	*ATP6V1C2*
chr2:10885208-10895660	CleftProband_47	Gain	10453	2p25.1	0.00	0.94	8	1	2	*ATP6V1C2*
chr2:10885208-10895660	CleftProband_12	Gain	10453	2p25.1	0.00	0.94	8	1	2	*ATP6V1C2*
chr2:10885208-10895660	CleftProband_26	Gain	10453	2p25.1	0.00	1.12	8	1	2	*ATP6V1C2*
chr2:116533395-116676085	CleftProband_125	Loss	142691	2q14.1	0.42	-0.76	110	1	194	*DPP10*
chr2:116533395-116676085	CleftProband_80	Loss	142691	2q14.1	0.42	-0.74	110	1	194	*DPP10*
chr2:116533395-116676085	CleftProband_106	Loss	142691	2q14.1	0.42	-0.71	110	1	194	*DPP10*
chr2:116533395-116676085	CleftProband_42	Loss	142691	2q14.1	0.42	-0.70	110	1	194	*DPP10*
chr2:133915831-134054703	CleftProband_7	Gain	138873	2q21.2	0.01	0.66	115	2	7	*NCKAP5, LOC101928161*
chr2:162746046-162771755	CleftProband_102	Loss	25710	2q24.2	0.08	-1.01	20	1	15	*SLC4A10*
chr2:186538-224408	CleftProband_6	Gain	37871	2p25.3	0.59	0.72	33	1	8	*SH3YL1*
chr2:188915-227936	CleftProband_133	Gain	39022	2p25.3	0.58	0.63	34	1	12	*SH3YL1*

chr2:217489537-217500592	CleftProband_30	Gain	11056	2q35	29.37	0.64	8	1	4	*IGFBP2*
chr2:228665165-228740875	CleftProband_72	Gain	75711	2q36.3	3.38	0.48	60	2	11	*CCL20, DAW1*
chr2:230652913-230917656	CleftProband_126	Gain	264744	2q36.3	1.55	0.59	199	3	129	*TRIP12, FBXO36, SLC16A14*
chr2:44082832-44100834	CleftProband_9	Gain	18003	2p21	4.53	0.83	8	1	2	*ABCG8*
chr2:63868701-64136659	CleftProband_63	Loss	267959	2p15	23.58	-0.92	206	2	26	*UGP2, VPS54*
chr20:12824479-13341144	CleftProband_71	Loss	516666	20p12.1	0.34	-1.00	422	6	37	*LINC01722, LOC102606466, LINC01723, SPTLC3, ISM1-AS1, ISM1*
chr20:25434138-25593810	CleftProband_53	Gain	159673	20p11.21	2.35	0.61	119	2	48	*NINL, NANP*
chr20:29904648-30187906	CleftProband_96	Gain	283259	20q11.21	0.48	0.60	205	10	86	*DEFB118, DEFB119, DEFB121, DEFB122, DEFB123, DEFB124, REM1, LINC00028, HM13, HM13-AS1*
chr20:54947631-54975595	CleftProband_115	Gain	27965	20q13.2 - q13.31	0.22	0.58	24	2	86	*AURKA, CSTF1*
chr21:15555375-15573280	CleftProband_134	Loss	17906	21q11.2	0.00	-1.09	11	1	10	*LIPI*
chr21:46290731-46314521	CleftProband_93	Gain	23791	21q22.3	4.91	0.58	19	2	24	*PTTG1IP, ITGB2*
chr22:19020530-	CleftProband_102	Gain	239438	22q11.21	31.03	0.62	1492	41	917	*DGCR11, DGCR2, TSSK2, ESS2, GSC2,*

21464915			5							*LINC01311, SLC25A1, CLTCL1, HIRA, MRPL40, C22orf39, UFD1, CDC45, CLDN5, LINC00895, SEPT5, SEPT5-GP1BB, GP1BB, TBX1, GNB1L, RTL10, TXNRD2, COMT, MIR4761, ARVCF, MIR185, TANGO2, MIR3618, MIR1306, DGCR8, TRMT2A, MIR6816, RANBP1, SNORA77B, ZDHHC8, CCDC188, LOC284865, LINC00896, MIR1286, RTN4R, DGCR6L,ZNF74, SCARF2, KLHL22, MED15, POM121L4P, TMEM191A, SERPIND1, PI4KA, SNAP29, CRKL, LINC01637, AIFM3, LZTR1, THAP7, THAP7-AS1, TUBA3FP, P2RX6, SLC7A4, MIR649, P2RX6P, LRRC74B, BCRP2*
chr22:50961823-50975550	CleftProband_49	Loss	13728	22q13.33	0.00	-0.76	9	4	66	*NCAPH2, SCO2, TYMP, ODF3B*
chr3:147089763-147111215	CleftProband_71	Gain	21453	3q24	0.00	0.46	19	1	17	*ZIC4*
chr3:154454306-	CleftProband_118	Gain	839402	3q25.2 -	1.90	0.63	582	6	152	*MME, LINC01487,*

155293707				q25.31						*DWORF, LOC100507537, PLCH1-AS2, PLCH1*
chr3:184594308-184629531	CleftProband_124	Gain	35224	3q27.2	0.00	0.90	31	1	8	*VPS8*
chr3:196381991-196392263	CleftProband_40	Loss	10273	3q29	0.00	-1.03	8	1	1	*NRROS*
chr3:28319094-28496647	CleftProband_14	Gain	177554	3p24.1	22.43	0.58	124	3	60	*CMC1, AZI2, ZCWPW2*
chr3:41322324-41638028	CleftProband_18	Loss	315705	3p22.1	8.05	-0.87	214	1	17	*ULK4*
chr3:61011417-61038195	CleftProband_45	Loss	26779	3p14.2	0.00	-1.02	18	1	4	*FHIT*
chr3:63627841-63647102	CleftProband_10	Loss	19262	3p14.2	0.00	-0.94	12	1	3	*SNTN*
chr4:102005257-102052937	CleftProband_124	Gain	47681	4q23	1.19	0.76	36	1	12	*PPP3CA*
chr4:103955997-104097535	CleftProband_6	Gain	141539	4q24	1.60	0.51	120	3	103	*SLC9B2, BDH2, CENPE*
chr4:106532779-106543507	CleftProband_92	Loss	10729	4q24	0.00	-0.76	10	1	2	*ARHGEF38*
chr4:17420017-18685859	CleftProband_93	Gain	1265843	4p15.32	2.71	0.63	945	8	180	*QDPR, CLRN2, LAP3, MED28, FAM184B, DCAF16, NCAPG, LCORL*
chr4:39284028-39549623	CleftProband_107	Gain	265596	4p14	8.05	0.61	188	11	152	*WDR19, RFC1**, MIR1273H, MIR5591, KLB, RPL9, LIAS, LOC401127, UGDH,*

										UGDH-AS1, SMIM14
chr4:52804406-53189333	CleftProband_116	Gain	384928	4q12	1.39	0.62	253	4	77	*LRRC66, SGCB, LINC02480, SPATA18*
chr4:83903642-83942189	CleftProband_96	Gain	38548	4q21.22	0.00	0.46	21	1	14	*LIN54*
chr5:148435735-148447141	CleftProband_8	Loss	11407	5q33.1	0.00	-0.89	10	2	3	*SH3TC2, LOC255187*
chr5:150065365-150076702	CleftProband_45	Gain	11338	5q33.1	0.00	0.60	9	1	7	*RBM22*
chr5:178591822-178720873	CleftProband_10	Gain	129052	5q35.3	2.66	0.68	99	1	6	*ADAMTS2*
chr5:52952870-52974339	CleftProband_23	Gain	21470	5q11.2	0.00	0.59	13	1	4	*NDUFS4*
chr5:53310720-53817429	CleftProband_119	Gain	506710	5q11.2	0.40	0.60	389	5	14	*MIR4459, ARL15, LINC01033, HSPB3, SNX18*
chr5:53310720-53817429	CleftProband_5	Gain	506710	5q11.2	0.40	0.63	389	5	14	*MIR4459, ARL15, LINC01033, HSPB3, SNX18*
chr6:118765650-118993251	CleftProband_140	Gain	227602	6q22.31	6.38	0.60	179	3	35	*BRD7P3, PLN, CEP85L*
chr6:127972290-128091747	CleftProband_127	Gain	119458	6q22.33	4.68	0.60	89	1	8	*THEMIS*
chr6:158303307-159014483	CleftProband_132	Gain	711176	6q25.3	0.66	1.17	412	6	114	*SNX9, SYNJ2-IT1, SYNJ2, SERAC1, GTF2H5, SNORA116, TULP4**, TMEM181*

chr6:39881038-39915446	CleftProband_124	Gain	34409	6p21.2	0.00	0.88	28	1	16	*MOCS1*
chr6:47780655-47794305	CleftProband_98	Loss	13651	6p12.3	0.00	-1.16	12	1	2	*OPN5*
chr6:85465287-85488306	CleftProband_130	Gain	23020	6q14.3	0.00	0.43	19	1	4	*TBX18*
chr7:110244241-110315471	CleftProband_107	Loss	71231	7q31.1	0.00	-1.09	51	1	2	*IMMP2L***
chr7:126333781-126347736	CleftProband_129	Gain	13956	7q31.33	0.00	0.65	12	1	1	*GRM8*
chr7:144075022-144105721	CleftProband_120	Gain	30700	7q35	47.09	0.51	27	2	11	*ARHGEF5, NOBOX*
chr7:35072697-35087865	CleftProband_50	Gain	15169	7p14.3	46.29	0.76	10	1	1	*DPY19L1*
chr7:91771154-91787077	CleftProband_96	Loss	15924	7q21.2	0.00	-0.74	10	2	4	*LRRD1, CYP51A1-AS1*
chr8:118224513-118884227	CleftProband_24	Gain	659715	8q24.11	1.29	0.66	531	2	17	*MED30, EXT1*
chr8:125432724-125484098	CleftProband_8	Gain	51375	8q24.13	2.22	0.62	42	2	2	*TRMT12, RNF139-AS1*
chr8:13121606-13223032	CleftProband_103	Loss	101427	8p22	0.33	-1.02	86	1	3	*DLC1*
chr8:144766006-144796891	CleftProband_7	Gain	30886	8q24.3	7.40	0.51	22	4	78	*ZNF707, BREA2, CCDC166, LOC101928160*
chr8:81874684-81895324	CleftProband_17	Gain	20641	8q21.13	0.00	0.68	18	1	2	*PAG1*

chr8:81874684-81895324	CleftProband_89	Gain	20641	8q21.13	0.00	0.74	18	1	2	*PAG1*
chr8:92115755-92186155	CleftProband_111	Loss	70401	8q21.3	8.28	-0.90	49	1	4	*LRRC69*
chr9:28608502-28744856	CleftProband_23	Loss	136354	9p21.1	24.59	-0.96	70	1	2	*LINGO2*
chr9:28609587-28744856	CleftProband_128	Loss	135270	9p21.1	20.44	-0.94	102	1	3	*LINGO2*
chr9:9934053-9954893	CleftProband_82	Loss	20841	9p23	1.59	-1.14	16	1	1	*PTPRD***
chrX:117775819-117816411	CleftProband_98	Gain	20777565	Xq24	0.00	0.87	32	1	12	*DOCK11*, *IL13RA1*
chrX:117816411-138650338	CleftProband_81	Gain	670973	Xq27.1	4.07	0.55	488	3	23	*FGF13*, *SRD5A1P1*, *F9*
chrX:32711758-32827653	CleftProband_127	Gain	115896	Xp21.1	1.73	0.43	83	1	12	*DMD***

Supplemental Table 1: All CNVs after filtering. 134 CNVs were identified, 51 of which were deletions (Loss) and 83 of which were amplifications (Gain). **Indicates gene associated with clefting (*IFIT2*, *SLC46A1*, *RFC1*, *TULP4*, *IMPP2L*, *PTPRD*, *DMD*, *CDH1*, *NOSIP*).

Gene Symbols	Total CNVs Overlapping Gene	Deletions Overlapping Gene	Amplifications Overlapping Gene	Cytoband	Associated with Clefting in PubMed	MGI Mouth Expression
ABCG8	1	0	1	2p21	0	0
ABR	1	0	1	17p13.3	0	x
ACOT11	1	0	1	1p32.3	0	0
ADAMTS2	1	0	1	5q35.3	0	0
AKAP3	1	0	1	12p13.32	0	0
ARHGEF5	1	0	1	7q35	0	0
ATF6	1	0	1	1q23.3	0	0
AURKA	1	0	1	20q13.2 - q13.31	0	x
AZI2	1	0	1	3p24.1	0	0
B4GALNT4	1	0	1	11p15.5	0	0
BDH2	1	0	1	4q24	0	x
C10orf67	1	0	1	10p12.2	0	
CCDC166	1	0	1	8q24.3	0	0
CCL20	1	0	1	2q36.3	0	0
CENPE	1	0	1	4q24	0	0
CEP85L	1	0	1	6q22.31	0	0
CFAP46	1	0	1	10q26.3	0	0
CH25H	1	0	1	10q23.31	0	0
CLRN2	1	0	1	4p15.32	0	0
CMC1	1	0	1	3p24.1	0	0
CSTF1	1	0	1	20q13.2 - q13.31	0	0
DAW1	1	0	1	2q36.3	0	
DCAF16	1	0	1	4p15.32	0	0
DEFB118	1	0	1	20q11.21	0	0
DEFB119	1	0	1	20q11.21	0	0
DEFB121	1	0	1	20q11.21	0	

DEFB123	1	0	1	20q11.21	0	
DEFB124	1	0	1	20q11.21	0	
DHX8	1	0	1	17q21.31	0	0
DIP2C	1	0	1	10p15.3	0	0
DMD	1	0	1	Xp21.1	x	x
DOCK11	1	0	1	Xq24	0	0
DPY19L1	1	0	1	7p14.3	0	0
DTL	1	0	1	1q32.3	0	0
DUSP12	1	0	1	1q23.3	0	0
DWORF	1	0	1	3q25.2 - q25.31	0	0
DYRK4	1	0	1	12p13.32	0	0
ETV4	1	0	1	17q21.31	0	x
EXT1	1	0	1	8q24.11	0	x
F9	1	0	1	Xq27.1	0	0
FAM184B	1	0	1	4p15.32	0	0
FBXO36	1	0	1	2q36.3	0	0
FCGR2B	1	0	1	1q23.3	0	0
FCRLA	1	0	1	1q23.3	0	0
FCRLB	1	0	1	1q23.3	0	0
FGF13	1	0	1	Xq27.1	0	x
GPR3	1	0	1	1p36.11	0	x
GRM8	1	0	1	7q31.33	0	0
GTF2H5	1	0	1	6q25.3	0	x
HERC4	1	0	1	10q21.3	0	0
HM13	1	0	1	20q11.21	0	0
HS3ST3A1	1	0	1	17p12	0	x
HSFX1	1	0	1	Xq28	0	0
HSFX2	1	0	1	Xq28	0	0
IFIT1	1	0	1	10q23.31	0	x
IFIT1B	1	0	1	10q23.31	0	0

IFIT2	1	0	1	10q23.31	x	0
IFIT3	1	0	1	10q23.31	0	0
IFIT5	1	0	1	10q23.31	0	0
IGFBP2	1	0	1	2q35	0	0
IL13RA1	1	0	1	Xq24	0	0
INTS7	1	0	1	1q32.3	0	0
IQSEC3	1	0	1	12p13.33	0	0
ITGB2	1	0	1	21q22.3	0	x
KDM5A	1	0	1	12p13.33	0	x
KLB	1	0	1	4p14	0	0
LAP3	1	0	1	4p15.32	0	x
LCORL	1	0	1	4p15.32	0	0
LIAS	1	0	1	4p14	0	x
LIN54	1	0	1	4q21.22	0	0
LIPA	1	0	1	10q23.31	0	0
LOC100507537	1	0	1	3q25.2 - q25.31	0	0
LRRC66	1	0	1	4q12	0	0
MAGEA11	1	0	1	Xq28	0	0
MAGEA9	1	0	1	Xq28	0	0
MAGEA9B	1	0	1	Xq28	0	0
MED28	1	0	1	4p15.32	0	x
MED30	1	0	1	8q24.11	0	0
MEOX1	1	0	1	17q21.31	0	0
MME	1	0	1	3q25.2 - q25.31	0	x
MOCS1	1	0	1	6p21.2	0	0
MYPN	1	0	1	10q21.3	0	0
NANP	1	0	1	20p11.21	0	x
NCAPG	1	0	1	4p15.32	0	x
NCKAP5	1	0	1	2q21.2	0	0
NDUFA9	1	0	1	12p13.32	0	x

NDUFS4	1	0	1	5q11.2	0	x
NINL	1	0	1	20p11.21	0	0
NOBOX	1	0	1	7q35	0	0
PANK1	1	0	1	10q23.31	0	x
PLCH1	1	0	1	3q25.2 - q25.31	0	0
PLN	1	0	1	6q22.31	0	x
PPP3CA	1	0	1	4q23	0	0
PTTG1IP	1	0	1	21q22.3	0	x
QDPR	1	0	1	4p15.32	0	0
RAP2A	1	0	1	13q32.1	0	0
RBM22	1	0	1	5q33.1	0	0
REM1	1	0	1	20q11.21	0	x
RFC1	1	0	1	4p14	x	x
RNF125	1	0	1	18q12.1	0	0
RNF138	1	0	1	18q12.1	0	x
RNF213	1	0	1	17q25.3	0	0
RPL9	1	0	1	4p14	0	0
SARM1	1	0	1	17q11.2	0	0
SERAC1	1	0	1	6q25.3	0	0
SGCB	1	0	1	4q12	0	0
SLC16A12	1	0	1	10q23.31	0	0
SLC16A14	1	0	1	2q36.3	0	0
SLC26A11	1	0	1	17q25.3	0	0
SLC46A1	1	0	1	17q11.2	x	0
SLC6A13	1	0	1	12p13.33	0	x
SLC9B2	1	0	1	4q24	0	x
SMCHD1	1	0	1	18p11.32	0	0
SMIM14	1	0	1	4p14	0	x
SNX9	1	0	1	6q25.3	0	x
SPATA18	1	0	1	4q12	0	0

STXBP6	1	0	1	14q12	0	0
SYNJ2	1	0	1	6q25.3	0	x
TBX18	1	0	1	6q14.3	0	x
TCF12	1	0	1	15q21.3	0	x
TMEM181	1	0	1	6q25.3	0	0
TMEM185A	1	0	1	Xq28	0	0
TPD52L1	1	0	1	6q22.31	0	0
TRIP12	1	0	1	2q36.3	0	0
TRMT12	1	0	1	8q24.13	0	0
TUBAL3	1	0	1	10p15.1	0	0
TUBD1	1	0	1	17q23.1	0	0
TULP4	1	0	1	6q25.3	x	x
UCN3	1	0	1	10p15.1	0	0
UGDH	1	0	1	4p14	0	x
VPS8	1	0	1	3q27.2	0	0
WASF2	1	0	1	1p36.11	0	0
WDR19	1	0	1	4p14	0	x
ZCWPW2	1	0	1	3p24.1	0	0
ZIC4	1	0	1	3q24	0	0
ZNF519	1	0	1	18p11.21	0	0
ZNF707	1	0	1	8q24.3	0	0
AGBL4	1	1	0	1p33	0	0
ARHGEF38	1	1	0	4q24	0	0
CDH1	1	1	0	16q22.1	x	x
CDH3	1	1	0	16q22.1	0	x
COA6	1	1	0	1q42.2	0	0
DLC1	1	1	0	8p22	0	x
FHIT	1	1	0	3p14.2	0	0
HBA1	1	1	0	16p13.3	0	0
HBQ1	1	1	0	16p13.3	0	0

IMMP2L	1	1	0	7q31.1	x	0
ISM1	1	1	0	20p12.1	0	x
LIPI	1	1	0	21q11.2	0	x
LRRC69	1	1	0	8q21.3	0	0
LRRD1	1	1	0	7q21.2	0	
MS4A10	1	1	0	11q12.2	0	0
NCAPH2	1	1	0	22q13.33	0	0
NOSIP	1	1	0	19q13.33	x	
NRROS	1	1	0	3q29	0	0
ODF3B	1	1	0	22q13.33	0	
OPN5	1	1	0	6p12.3	0	0
OR51B5	1	1	0	11p15.4	0	0
OR52D1	1	1	0	11p15.4	0	0
PCDH9	1	1	0	13q21.32	0	x
PIAS2	1	1	0	18q21.1	0	x
PTPRD	1	1	0	9p23	0	x
RCN3	1	1	0	19q13.33	0	0
RGMA	1	1	0	15q26.1	0	0
RHBDL2	1	1	0	1p34.3	0	x
SCO2	1	1	0	22q13.33	0	x
SH3TC2	1	1	0	5q33.1	0	0
SLC4A10	1	1	0	2q24.2	0	0
SNTN	1	1	0	3p14.2	0	0
SPTLC3	1	1	0	20p12.1	0	x
ST8SIA6	1	1	0	10p12.33	0	0
STK33	1	1	0	11p15.4	0	0
TARBP1	1	1	0	1q42.2	0	0
TNFAIP8L3	1	1	0	15q21.2	0	0
TYMP	1	1	0	22q13.33	0	x
UGP2	1	1	0	2p15	0	x

ULK4	1	1	0	3p22.1	0	0
VPS54	1	1	0	2p15	0	0
ZFP90	1	1	0	16q22.1	0	0
ZNF695	1	1	0	1q44	0	0
ARL15	2	0	2	5q11.2	0	
EFCAB2	2	0	2	1q44	0	0
HSPB3	2	0	2	5q11.2	x	x
MSMB	2	0	2	10q11.23	0	0
PAG1	2	0	2	8q21.13	0	0
SH3YL1	2	0	2	2p25.3	0	
SNX18	2	0	2	5q11.2	0	0
THEMIS	2	0	2	6q22.33	0	0
TIMM23B	2	0	2	10q11.23	0	x
CAP1	2	2	0	1p34.2	0	0
CD163L1	2	2	0	12p13.31		
FSCB	2	2	0	14q21.3	0	0
LINGO2	2	2	0	9p21.1	0	0
MALRD1	2	2	0	10p12.33 - p12.32	0	
SDE2	2	2	0	1q42.12		
HNRNPL	3	3	0	19q13.2	0	0
ATP6V1C2	4	0	4	2p25.1	0	0
DPP10	4	4	0	2q14.1	0	
RAB21	5	0	5	12q21.1	0	x
TBC1D15	5	0	5	12q21.1	0	0

Supplemental Table 2: Genes overlapped by CNVs within the cohort after filters. We identified 200 genes overlapped by deletions and amplifications passing our filtering pipeline in the case cohort. Genes were annotated for known association with clefting in PubMed and expression within the murine mouth in MGI. Highlighted genes were selected as plausible novel clefting genes after assessment for low haploinsufficiency scores (≤10), having a higher frequency of CNV overlap in cases than controls, and craniofacial expression in the mouse (MGI).

FILIPINOS

Variant	Nucleotide Change	Chromosome Position	Exon	Fil. Ctrl. Alleles	Fil. Cleft Alleles	Fil. Ctrl. Alleles Percent	Fil. Cleft Alleles Percent	Fil. Ctrl. vs Case Fold Change	Fil. Cleft vs Fil. Ctrl. Fold Change	PhyloP LRT (UCSC)	PhastCon (UCSC)	Prediction Algorithm (Damaging)
E89K	c.265G>A	chr20:13,251,277	2	1/628	0/538	0.16	0	N/A	0	6.22	1	MT; FMK
S102P	c.304T>C	chr20:13,251,316	2	1/632	0/538	0.16	0	N/A	0	4.62	1	P; MT; FMK
Q126E	c.376C>G	chr20:13,251,388	2	1/628	1/538	0.16	0.19	0.86	1.17	7.25	1	P; MT; FMK
D157A	c.470A>C	chr20:13,260,372	3	3/676	2/516	0.44	0.39	1.14	0.87	6.60	1	P; MT; FMK
R159Q	c.476G>A	chr20:13,260,378	3	0/688	0/516	0	0	N/A	N/A	4.23	1	MT; FMK
D186N	c.556G>A	chr20:13,260,458	3	1/688	0/516	0.15	0	N/A	0	5.84	1	MT; FMK
N188S	c.563A>G	chr20:13,260,465	3	0/688	0/516	0	0	N/A	N/A	8.41	1	P; MT; FMK
P193R	c.578C>G	chr20:13,260,480	3	47/688	37/528	6.83	7.01	0.97	1.03	4.13	0.99	MT; FMK
S305N	c.914G>A	chr20:13,279,625	6	4/168	0/550	2.15	0	N/A	0	6.43	1	P; MT; FMK

EUROPEANS

Variant	Nucleotide Change	Chromosome Position	Exon	Fil. Ctrl. Alleles	Fil. Cleft Alleles	Fil. Ctrl. Alleles Percent	Fil. Cleft Alleles Percent	Fil. Ctrl. vs Case Fold Change	Fil. Cleft vs Fil. Ctrl. Fold Change	PhyloP LRT (UCSC)	PhastCon (UCSC)	Prediction Algorithm (Damaging)
E89K	c.265G>A	chr20:13,251,277	2	0/8400	0/480	0	0	N/A	0	6.22	1	MT; FMK
S102P	c.304T>C	chr20:13,251,316	2	320/8400	8/480	3.81	1.67	2.29	0.44	4.62	1	P; MT; FMK
Q126E	c.376C>G	chr20:13,251,388	2	0/8400	0/480	0	0	N/A	0	7.25	1	P; MT; FMK
D157A	c.470A>C	chr20:13,260,372	3	0/8400	0/465	0	0	N/A	0	6.60	1	P; MT; FMK
R159Q	c.476G>A	chr20:13,260,378	3	4/8400	1/456	0.02	0.22	0.11	9.21	4.23	1	MT; FMK
D186N	c.556G>A	chr20:13,260,458	3	0/8400	0/456	0	0	N/A	0	5.84	1	MT; FMK
N188S	c.563A>G	chr20:13,260,465	3	0/8400	1/456	0	0.22	0	>18	8.41	1	P; MT; FMK
P193R	c.578C>G	chr20:13,260,480	3	6/8400	0/456	0.07	0	N/A	0	4.13	0.99	MT; FMK
S305N	c.914G>A	chr20:13,279,625	6	1/8400	1/489	0.01	0.20	0.06	17.14	6.43	1	P; MT; FMK

COMBINED

Variant	Nucleotide Change	Chromosome Position	Exon	Fil. Ctrl. Alleles	Fil. Cleft Alleles	Fil. Ctrl. Alleles Percent	Fil. Cleft Alleles Percent	Fil. Ctrl. vs Case Fold Change	Fil. Cleft vs Fil. Ctrl. Fold Change	PhyloP LRT (UCSC)	PhastCon (UCSC)	Prediction Algorithm (Damaging)
E89K	c.265G>A	chr20:13,251,277	2	1/9028	0/1018	0.01	0	N/A	0	6.22	1	MT; FMK
S102P	c.304T>C	chr20:13,251,316	2	321/9032	8/1018	3.55	0.79	4.49	0.22	4.62	1	P; MT; FMK
Q126E	c.376C>G	chr20:13,251,388	2	1/9028	1/1018	0.01	0.10	0.1	10	7.25	1	P; MT; FMK
D157A	c.470A>C	chr20:13,260,372	3	3/9076	2/981	0.03	0.20	0.15	6.67	6.60	1	P; MT; FMK
R159Q	c.476G>A	chr20:13,260,378	3	4/9088	1/972	0.04	0.10	0.40	2.25	4.23	1	MT; FMK
D186N	c.556G>A	chr20:13,260,458	3	1/9088	0/972	0.01	0	N/A	0	5.84	1	MT; FMK
N188S	c.563A>G	chr20:13,260,465	3	0/9088	1/972	0	0.10	0	N/A	8.41	1	P; MT; FMK
P193R	c.578C>G	chr20:13,260,480	3	53/9088	37/984	0.58	0.04	14.5	0.07	4.13	0.99	MT; FMK
S305N	c.914G>A	chr20:13,279,625	6	5/8568	1/1039	0.06	0.10	0.6	1.67	6.43	1	P; MT; FMK

Supplemental Table 3: ***ISM1*** **sequencing results.** We sequenced 520 NSCL/P subjects and identified nine missense variants in *ISM1*. Four were not reported in 1000 Genomes or EVS, and three (R159Q, N188S, S305N; highlighted in red) were overrepresented in European cases versus controls, and four (D157A, R159Q, N188S, S305N; highlighted in red) were overrepresented in cases versus controls when all variants in Europeans and Filipinos were assessed. (P – Polyphen; MT- MutationTaster; FMK – FATHMM MKL)