A Population-Based Study of Effects of Genetic Loci on Orofacial Clefts

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Appendix

Study Population and Samples

Norway

We included two Norwegian OFC studies in our analysis. The first sample is from the Norway Facial Clefts Study (NCL), which included the majority of children born with OFC in 1996-2001 in Norway and identified from records of two hospitals where cleft-repair surgeries are centralized in Norway (Wilcox et al. 2007). Controls were recruited from a random sample of all Norwegian births in the same period.

The second Norwegian sample was obtained from the Norway National Mother and Child Cohort Study (MoBa), a population-level study of nearly 100,000 pregnancies enrolled between 1999 and 2009 (Magnus et al. 2006). Children with OFC enrolled in MoBa were identified from the Norwegian Medical Birth Registry (Kubon et al. 2007). Because the MoBa sample does not include all children with clefts in a given year, but only a small sample of these children, the overlap with the NCL cases for the 2000-2001 birth years is minimal (less than 15 cases were enrolled in MoBa before 2002). Thus, it was unlikely that families participated in both studies. The overlap, if any, could not be verified due to confidentiality restrictions. Controls born without OFC were randomly selected from MoBa. The total Norway sample includes 589 isolated cases, 158 non-isolated cases, and 1139 controls.

Utah

The Utah sample came from a case-control study of OFC enumerated from a state-wide birth defect surveillance registry; it included OFC cases born in 1995 through June 2004

and children without a major birth defect randomly sampled as controls from all birth certificates in Utah (Munger et al. 2011). Beginning July 2004, children born with OFC and controls were enrolled into the NBDPS described below. The Utah sample included 319 isolated cases, 92 non-isolated cases, and 470 controls.

Iowa

The Iowa sample was from a case-control study of children born with oral clefts in 1987-1991 and identified by the state-wide surveillance program for congenital and inherited disorders, and children without a major birth defect randomly selected from Iowa birth certificates as controls (Munger et al. 1996). Beginning in 1997, children with OFC and controls were enrolled in the NBDPS described below. The Iowa sample included 151 isolated cases, 96 non-isolated cases, and 254 controls.

U.S. National Birth Defects Prevention Study (NBDPS)

The NBDPS (http://www.nbdps.org/index.html) is a multi-site, case-control study to assess genetic and non-genetic risk factors of birth defects (Reefhuis et al. 2015). The sample included children with OFC and controls (liveborn infants without major birth defects) who had estimated dates of delivery (EDD) between October 1997 and December 2009 ascertained from Arkansas, California, Georgia, Iowa, Massachusetts, New York, North Carolina, Texas and Utah. Note that all cases and controls from Utah in NBDPS had EDD after July 1, 2004, so there was no overlap with the Utah cases and controls described above. In each site, children with OFC were identified through population-based surveillance programs and controls born without major birth defects were randomly selected annually at each study site from either birth hospital records or birth certificates. Cases with known syndromes were not enrolled. The total sample from the NBDPS included 816 isolated cases, 113 non-isolated cases, and 1,886 controls.

Genotyping

31 SNPs in 17 loci supported in previous GWAS or high quality candidate gene studies were genotyped in this consortium of population-based studies (Supplementary Table 1). For genes/loci with more robust evidence in GWAS, we focused on the lead SNPs reported in the GWAS. For some of the genes/loci with less robust evidence such as *FOXE1* and *FGFR2*, we included more SNPs for greater coverage of these loci. A Dynamic Array was used to generate genotypes using competitive allele specific PCR KASPar chemistry (KBioscience Ltd., Hoddesdon, UK) on a Fluidigm (Fluidigm Corp., South San Francisco, CA, USA) nanofluidic platform (Wang et al. 2009). For genotype calling, default settings of the Fluidigm SNP genotyping software, version 4.1.2, were utilized, including a non-template control normalization method, and a K-means clustering method. Genotype quality was assessed first by setting the genotype calling algorithm to a confidence threshold of 65% followed by visual inspection and manual calling of all genotyping plots. Evaluated SNPs had high call rates (most exceeding 98%) and a minimum minor allele frequency $\geq$2% and passed the Hardy-Weinberg disequilibrium (HWE) test at $P < 10^{-4}$ in the total sample as well as study-specific samples (Supplementary Table 1).

Discussion of Findings on Specific Loci

Our study is the first to report that SNP rs560426 within the *ABCA4-ARHGAP29* genomic interval appears to be specifically related to CLP but not CLO or CPO. Most previous examinations of this SNP grouped CLP and CLO together and the few CPO studies did not find associations between rs560426 and CPO. This SNP was originally reported in a GWAS of Caucasian and Asian populations (Beaty et al. 2010) and was observed to have the highest association with CL/P among Asians, although the association was replicated in several ancestries, including Northern Europeans (Beaty et al. 2013; Fontoura et al. 2012; Yuan et al. 2011). Sequencing this interval revealed multiple rare (<5%) nonsynonymous variants within *ARHGAP29* for CL/P, particularly CLO, suggesting that rare variants may still play a role for CLO (Leslie et al. 2012). Recently, loss of function variants in this locus were also associated with risk of CL/P (Savastano et al., 2017). Moreover, expression studies

indicated specific expression in facial and palatal prominences as well as decreased expression levels in Irf6 null mice. SNP rs560426 maps to the intron of *ABCA4,* but also is within a transcriptional craniofacial enhancer for *ARHGAP29* (Attanasio et al. 2013). Therefore, *ARHGAP29* (a RhoA GTPase activating protein) is likely the cleft candidate gene in this interval, possibly functioning downstream of the *IRF6* gene regulatory network, which is known to play a key role in lip and palate morphogenesis (Leslie et al. 2012). Although Rho signaling is implicated in cellular processes critical to lip and palate formation (Kardassis et al. 2009; Kutys and Yamada 2015; Schlessinger et al. 2009), and *ARHGAP29* expression is detected in both the nasal prominences and the palatal shelves (Leslie et al. 2012), the lack of association to CLO or CPO in our large sample suggests that it is involved in a separate etiological mechanism for CLP in humans, but not for the other two cleft types. Future well-powered studies of variants near *ARHGAP29* will likely continue to shed light on the specific role of this locus in OFC phenotypes.

Our study identified differential associations with GWAS-identified SNP rs227731 (Mangold et al. 2010) near *NOG1* by cleft type. Our observation of an increased risk of isolated CL/P associated with this variant has been reported in several studies (Beaty et al. 2013; Figueiredo et al. 2014; Ludwig et al. 2012; Sun et al. 2015). To our knowledge, no prior study has reported this variant to be significantly associated with a reduction in isolated CPO risk. Recenty, Yu et al. (2017) found no significant association of this variant with isolated CPO in a Chinese sample, suggesting potential heterogneity in risk by ancesry. We also provide the first evidence that the elevated risk for isolated CLO with this variant is larger than the risk for isolated CLP, suggesting varying importance in etiological mechanisms between these two OFC types. *NOG1* is an extracellular antagonist of various *BMP* family members, which are expressed in the palatal shelves during development. Loss of Noggin leads to augmented *BMP* signal in the palatal epithelium causing increased cell death and palatal epithelium disturbance that leads to abnormal fusion of the palatal shelves to the mandible and a subsequent failure to elevate resulting in complete cleft palate (He et al. 2010). Previous targeted sequencing of the *NOG1* locus in about 1500 trios identified

SNP rs227727, which is in complete linkage disequilibrium (LD) with the GWAS-identified SNP rs227731. SNP rs227727 disrupts enhancer activity in human fetal oral epithelial cells and appears to be the causal variant within the 17q22 loci (Leslie et al. 2015). The protective effect of the rare allele of rs227731 on CPO once again demonstrates evidence supporting distinct mechanisms in the etiology between cleft types. The decrease in enhancer activity caused by the minor allele of the rs227727 SNP (i.e. the functional SNP in LD with rs227731), while deleterious for CL/P, is protective for CPO, and may indicate different effects in the sequence of events and in the cellular processes that lead to primary and secondary palatogenesis.

Similar to *NOG1,* we observed a larger association of SNP rs1873147 near *TPM1* with isolated CLO than CLP. Interestingly, we also observed a trend towards reduction in CPO risk with the minor allele of this same SNP, although non-significant (*P*=0.11). The increased risk for isolated CL/P with this variant has been reported in several studies across various ancestries (Ludwig et al. 2012; Ludwig et al. 2014; Mangold et al. 2010; Pan et al. 2013; Qian et al. 2016), but differential effects by cleft type have not been suggested. *TPM1* encodes for α-tropomyosin which regulates muscle contraction and cytoskeleton function important in cell migration, cell proliferation and apoptosis (Lin et al. 2008). *TPM1* mutations have been associated with cardiomyopathy (Redwood and Robinson 2013), yet its specific role in lip and palate morphogenesis is unclear. The study by Quian et al., (Qian et al. 2016) also noticed distinct risk associations by cleft types for another *TPM1* SNP (rs1972041) located within an intron of *TPM1*, with a reduction in risk for the minor allele of this SNP with CLP and CL/P. In addition, they found that *TPM1* expression is decreased in pulp-derived stem cells from cleft cases compared to controls (Qian et al. 2016), rendering support to future studies addressing the function of *TPM1* in cleft and craniofacial anomalies.

Among all examined loci, we found that 8q24 (rs987525) has the strongest effect on isolated CLO and CLP, with risk increasing by four-fold with a double minor allele dose and two-fold with a single allele dose for both cleft types. Our study not only further confirms the importance of this variant for isolated CL/P, which has been strongly supported in several

studies (Birnbaum et al. 2009; Cura et al. 2016; de Souza et al. 2016; Grant et al. 2009; Leslie et al. 2016), but provides strong evidence that this variant is similarly associated with both isolated CLO and CLP. Our study is also the first to show that this variant has an identical effect on non-isolated forms of CL/P. However, the identity of the etiological variant within this gene desert locus remains unknown. Uslu et al.(Uslu et al. 2014) identified a remote (>1 Mb of DNA) cis-enhancer that maps to the 8q24 interval containing rs987525 that controls *Myc* expression on the future upper lip. The oncogene *MYC*, the closest known gene to SNP rs987525, is activated by *TGFB* family members and promotes cell proliferation in the human mesenchymal palatal tissue prior to elevation and fusion of the palatal shelves (Zhu et al. 2012). Also, specific deletions within the enhancer region decreased expression of *Myc* in the nasal prominences and some were associated with clefts of the lip and the palate in mice (Uslu et al. 2014). This evidence indicates that this region contains multiple regulatory elements that are important for normal craniofacial development and therefore could harbor variants etiological to OFC.

The association of 8q21.3 rs12543318 near the *DCAF4L2* gene has been reported in several ancestries (Beaty et al. 2010; Ludwig et al. 2012; Pan et al. 2013). Our study further confirms this association, but suggests a more prominent effect on CLO, especially when the allele originates from the mother. A similar PoO effect was reported previously in trios from European and Asian descent (Garg et al. 2014). Although little is known about the function of the 8q21.3 locus in craniofacial development, a few human chromosomal imbalances within this region have been found in patients with facial dysmorphology, including cleft lip.

Appendix Table 1. Call Rates, Hardy-Weinberg Equilibrium (HWE) Tests, and Minor Allele Frequency (MAF) for Controls

		Minor	Call rate (%)*		Total sample		Norway		Utah		Iowa		NBDPS	
Genes/Locus	SNP	allele	Mother	child	HWE†	MAF	HWE†	MAF	HWE†	MAF	HWE†	MAF	HWE†	MAF
PAX7	rs742071	t	99.7	99.7	0.47	0.39	0.23	0.38	0.33	0.31	0.03	0.38	0.41	0.41
ABCA4-ARHGAP29	rs560426	g	98.9	99.4	0.68	0.45	0.78	0.44	0.82	0.46	0.18	0.42	0.71	0.45
IRF6	rs2235371	t	99.6	99.9	0.32	0.02	0.53	0.02	0.80	0.01	0.80	0.02	0.49	0.02
IRF6	rs642961	a	98.1	99.5	0.21	0.21	0.22	0.23	0.38	0.19	0.24	0.19	0.05	0.21
THADA	rs7590268	g	99.4	99.5	0.45	0.24	0.38	0.25	0.15	0.24	0.56	0.26	0.51	0.23
MSX1	rs3111689	g	99.5	99.5	0.85	0.23	0.51	0.23	0.39	0.21	0.78	0.26	0.69	0.24
8q21.3	rs12543318	c	98.4	99.2	0.19	0.33	0.33	0.31	0.36	0.32	0.09	0.36	0.97	0.33
8q24	rs987525	a	98.8	99.4	0.32	0.22	0.39	0.19	0.71	0.26	0.73	0.25	0.12	0.22
FOXE1	rs7864322	c	99.6	99.9	0.46	0.32	0.68	0.32	0.89	0.30	0.88	0.35	0.51	0.32
FOXE1	rs10818094	a	99.7	93.5	0.08	0.23	0.86	0.22	0.47	0.24	0.49	0.23	0.03	0.24
FOXE1	rs1443433	g	99.6	99.6	0.55	0.15	0.66	0.13	0.83	0.17	0.82	0.16	0.55	0.15
FOXE1	rs74934500	g	99.9	99.8	0.0003	0.03	0.25	0.02	0.69	0.03	0.69	0.04	0.0001	0.03
FOXE1	rs3758249	t	99.6	99.9	0.15	0.38	0.15	0.36	0.94	0.37	0.20	0.44	0.75	0.39
FOXE1	rs10984103	a	99.6	99.7	0.13	0.35	0.07	0.33	0.66	0.36	0.70	0.39	0.52	0.36
KIAA1598-VAX1	rs7078160	a	99.4	99.8	0.44	0.18	0.45	0.17	0.64	0.19	0.60	0.24	0.65	0.17
KIAA1598-VAX1	rs4752028	c	99.3	99.6	0.21	0.18	0.48	0.18	0.80	0.18	0.50	0.25	0.47	0.18
FGFR2	rs4752566	t	99.6	99.7	0.31	0.44	0.79	0.42	0.05	0.45	0.52	0.46	0.82	0.45
FGFR2	rs2912760	t	99.6	99.7	0.76	0.27	0.16	0.28	0.55	0.29	1.00	0.24	0.65	0.26
FGFR2	rs3135761	a	99.7	99.8	0.24	0.18	0.78	0.20	0.99	0.16	0.43	0.19	0.25	0.16
FGFR2	rs2912771	g	97.3	98.7	0.57	0.26	0.22	0.26	0.64	0.28	0.17	0.20	0.68	0.27
FGFR2	rs2981428	t	99.4	99.6	0.26	0.45	0.37	0.43	0.02	0.41	0.17	0.44	0.25	0.47
FGFR2	rs3750817	t	99.1	98.8	0.74	0.39	0.18	0.41	0.01	0.45	0.23	0.38	0.91	0.38
SPRY2	rs8001641	g	99.5	99.7	0.82	0.50	0.14	0.54	0.35	0.49	0.67	0.48	0.64	0.48
TPM1	rs1873147	g	99.0	99.5	0.32	0.27	0.61	0.27	0.21	0.22	0.81	0.24	0.26	0.27
CRISPLD2	rs1546124	g	98.7	98.8	0.21	0.31	0.62	0.30	0.58	0.29	0.09	0.37	0.64	0.32
NTN1	rs4791331	t	91.8	99.4	0.55	0.14	0.94	0.13	0.45	0.10	0.67	0.15	0.80	0.16
NTN1	rs8069536	t	99.7	99.7	0.33	0.46	0.25	0.39	0.75	0.46	0.42	0.44	0.14	0.50
NOG1	rs227731	g	98.7	99.5	0.0007	0.45	0.02	0.47	0.02	0.48	0.62	0.42	0.04	0.44
MAFB	rs13041247	c	99.5	99.5	0.72	0.40	0.49	0.41	0.97	0.40	0.53	0.43	0.86	0.38
MYH9	rs3752462	t	97.7	96.3	0.08	0.32	0.08	0.33	0.45	0.32	0.98	0.35	0.40	0.31
MYH9	rs1002246	a	99.1	99.5	0.68	0.32	0.60	0.33	0.34	0.40	0.95	0.36	0.93	0.31

Notes: * Call rates for 5824 mothers and 4315 children (numbers in Table S2). † p-value for test of deviation from HWE; HWE was only based on Controls.
Norway includes both the Norway Facial Cleft Study and the Moba Study. NBDPS=National Birth Defects Prevention Study

Appendix Table 2. Sample Distribution by Case-Control Status, Cleft Type, and Genetic Data Availability

Case/Control Status		Genetic Data	Utah	Norway	Iowa	NBDPS	Total
Isolated cases	CLO	Complete dyads	36	126	29	175	366
		Only child	2	8	3	4	17
		Only mother	47	14	8	66	135
		Total families	**85**	**148**	**40**	**245**	**518**
	CLP	Complete dyads	44	228	53	228	553
		Only child	8	9	2	9	28
		Only mother	85	36	6	86	213
		Total families	**137**	**273**	**61**	**323**	**794**
	CPO	Complete dyads	41	139	41	173	394
		Only child	4	7	3	8	22
		Only mother	52	22	6	67	147
		Total families	**97**	**168**	**50**	**248**	**563**
	All cases	Complete dyads	121	491	123	576	1311
		Only child	14	23	8	21	66
		Only mother	184	71	20	219	494
		Total families	**319**	**589**	**151**	**816**	**1875**
Non-isolated cases	CLO	Complete dyads	3	19	8	7	37
		Only child	0	0	0	0	0
		Only mother	10	1	3	9	23
		Total families	**13**	**20**	**11**	**16**	**60**
	CLP	Complete dyads	7	44	18	24	93
		Only child	2	2	1	0	5
		Only mother	24	6	16	24	70
		Total families	**33**	**52**	**35**	**48**	**168**
	CPO	Complete dyads	9	76	26	26	137
		Only child	2	2	2	1	7
		Only mother	35	8	22	22	87
		Total families	**46**	**86**	**50**	**49**	**231**
	All cases	Complete dyads	19	139	52	57	267
		Only child	4	4	3	1	12
		Only mother	69	15	41	55	180
		Total families	**92**	**158**	**96**	**113**	**459**
Controls		Complete dyads	180	743	182	1,377	2482
		Only child	21	57	19	80	177
		Only mother	269	339	53	429	1090
		Total families	**470**	**1139**	**254**	**1886**	**3749**

Notes: CLO=Cleft lip only; CLP=Cleft lip with palate; CPO=Cleft palate only

Appendix Table 3. Sensitivity Analysis of Fetal SNP Effects on Isolated Cleft Lip Only Using Complete Mother-Child Dyads Only

Genes/Locus	SNP	RR*	95% CI		p-value†	p-site‡
PAX7	rs742071	1.56	1.34 -	1.82	1.68E-08	0.96
ABCA4-ARHGAP29	rs560426	1.10	0.95 -	1.28	0.21	0.39
IRF6	rs2235371	0.83	0.46 -	1.49	0.54	0.97
IRF6	rs642961	1.60	1.35 -	1.89	7.22E-08	0.57
THADA	rs7590268	1.16	0.98 -	1.38	0.09	0.78
MSX1	rs3111689	1.14	0.96 -	1.35	0.15	0.11
8q21.3	rs12543318	1.50	1.29 -	1.75	3.10E-07	0.94
8q24	rs987525	1.83	1.56 -	2.15	9.88E-13	0.62
FOXE1	rs7864322	0.90	0.76 -	1.06	0.21	0.61
FOXE1	rs10818094	0.94	0.78 -	1.13	0.51	0.86
FOXE1	rs1443433	0.91	0.73 -	1.13	0.39	0.74
FOXE1	rs74934500	0.79	0.48 -	1.30	0.36	0.92
FOXE1	rs3758249	0.82	0.70 -	0.97	0.02	0.93
FOXE1	rs10984103	0.84	0.71 -	0.98	0.03	0.89
KIAA1598-VAX1	rs7078160	1.26	1.05 -	1.52	0.01	0.36
KIAA1598-VAX1	rs4752028	1.30	1.08 -	1.56	0.005	0.41
FGFR2	rs4752566	0.90	0.77 -	1.05	0.19	0.29
FGFR2	rs2912760	1.09	0.92 -	1.28	0.34	0.01
FGFR2	rs3135761	1.12	0.92 -	1.35	0.26	0.20
FGFR2	rs2912771	0.77	0.64 -	0.92	0.005	0.18
FGFR2	rs2981428	1.13	0.97 -	1.31	0.12	0.42
FGFR2	rs3750817	0.97	0.83 -	1.13	0.67	0.45
SPRY2	rs8001641	0.83	0.71 -	0.97	0.02	0.67
TPM1	rs1873147	1.31	1.12 -	1.54	0.0010	0.48
CRISPLD2	rs1546124	0.98	0.83 -	1.15	0.77	0.46
NTN1	rs4791331	1.14	0.93 -	1.40	0.22	0.27
NTN1	rs8069536	1.16	1.00 -	1.35	0.06	0.41
NOG1	rs227731	1.38	1.19 -	1.61	3.02E-05	0.71
MAFB	rs13041247	0.71	0.60 -	0.83	3.10E-05	0.79
MYH9	rs3752462	1.06	0.91 -	1.25	0.45	0.13
MYH9	rs1002246	1.08	0.92 -	1.26	0.34	0.93

* Relative risk for heterozygotes. Relative risk for homozygotes is estimated as the square.

† Effect of child's allele in multiplicative model. Significance w. Bonferroni adj. if p<.0016

‡ Test of heterogeneity of estimates across sites

Appendix Table 4. Sensitivity Analysis of Fetal SNP Effects on Isolated Cleft Lip with Palate Using Complete Mother-Child Dyads Only

Genes/Locus	SNP	RR*	95% CI		p-value†	p-site‡
PAX7	rs742071	1.37	1.21 -	1.55	1.69E-06	0.43
ABCA4-ARHGAP29	rs560426	1.22	1.07 -	1.38	0.0021	0.68
IRF6	rs2235371	0.66	0.38 -	1.15	0.14	0.78
IRF6	rs642961	1.32	1.14 -	1.53	1.84E-04	0.33
THADA	rs7590268	1.28	1.11 -	1.47	6.91E-04	0.92
MSX1	rs3111689	1.04	0.90 -	1.21	0.60	0.28
8q21.3	rs12543318	1.22	1.07 -	1.38	0.003	0.08
8q24	rs987525	1.86	1.62 -	2.13	6.80E-18	0.10
FOXE1	rs7864322	0.86	0.75 -	0.99	0.03	0.22
FOXE1	rs10818094	1.07	0.93 -	1.24	0.36	0.93
FOXE1	rs1443433	1.03	0.86 -	1.22	0.78	0.58
FOXE1	rs74934500	0.69	0.44 -	1.08	0.10	0.70
FOXE1	rs3758249	0.79	0.69 -	0.90	4.93E-04	0.85
FOXE1	rs10984103	0.82	0.71 -	0.94	0.004	0.76
KIAA1598-VAX1	rs7078160	1.35	1.16 -	1.57	1.14E-04	0.65
KIAA1598-VAX1	rs4752028	1.38	1.19 -	1.60	3.47E-05	0.54
FGFR2	rs4752566	0.98	0.86 -	1.11	0.77	0.70
FGFR2	rs2912760	0.97	0.85 -	1.12	0.73	0.40
FGFR2	rs3135761	0.94	0.80 -	1.11	0.46	0.58
FGFR2	rs2912771	0.94	0.81 -	1.08	0.39	0.39
FGFR2	rs2981428	1.01	0.89 -	1.15	0.90	0.93
FGFR2	rs3750817	0.99	0.87 -	1.13	0.93	0.54
SPRY2	rs8001641	0.78	0.69 -	0.89	1.57E-04	0.75
TPM1	rs1873147	1.25	1.09 -	1.43	0.0011	0.70
CRISPLD2	rs1546124	1.03	0.90 -	1.18	0.68	0.84
NTN1	rs4791331	1.19	1.00 -	1.41	0.04	0.31
NTN1	rs8069536	1.11	0.98 -	1.26	0.11	0.38
NOG1	rs227731	1.16	1.03 -	1.32	0.02	0.12
MAFB	rs13041247	0.68	0.59 -	0.77	2.65E-08	0.29
MYH9	rs3752462	0.99	0.87 -	1.14	0.92	0.28
MYH9	rs1002246	1.08	0.95 -	1.24	0.23	0.49

* Relative risk for heterozygotes. Relative risk for homozygotes is estimated as the square.

† Effect of child's allele in multiplicative model. Significance w. Bonferroni adj. if p<.0016

‡ Test of heterogeneity of estimates across sites

Appendix Table 5. Effects of Major Allele Genotypes Using Homozygotes for the Minor Allele as Reference for SNPs with Higher Risk Associated with the Major Allele

			Single dose (heterozygotes)		Double dose (homozygotes)	
Cleft type	Gene/Locus	SNP	RR	95% CI	RR	95% CI
CLO	FOXE1	rs3758249	1.00	0.73 - 1.38	1.42	1.03 - 1.95
	SPRY2	rs8001641	1.32	1.01 - 1.73	1.52	1.13 - 2.06
	MAFB	rs13041247	1.63	1.14 - 2.35	2.15	1.49 - 3.10
CLP	FOXE1	rs3758249	1.57	1.17 - 2.11	1.84	1.36 - 2.50
	SPRY2	rs8001641	1.36	1.08 - 1.72	1.62	1.26 - 2.09
	MAFB	rs13041247	1.26	0.95 - 1.66	2.02	1.53 - 2.68
CPO	FOXE1	rs3758249	1.14	0.84 - 1.55	1.32	0.97 - 1.81
	SPRY2	rs8001641	1.00	0.79 - 1.27	0.85	0.64 - 1.13
	MAFB	rs13041247	0.84	0.64 - 1.10	1.05	0.79 - 1.39

Appendix Table 6. Maternal SNP Effects* on Isolated Cleft Lip Only

Gene	SNP	RR	95% CI		p-value
PAX7	rs742071	1.15	0.99 -	1.35	0.07
ABCA4-ARHGAP29	rs560426	0.98	0.84 -	1.15	0.82
IRF6	rs2235371	0.74	0.36 -	1.52	0.42
IRF6	rs642961	0.92	0.76 -	1.11	0.39
THADA	rs7590268	1.05	0.88 -	1.26	0.59
MSX1	rs3111689	1.10	0.92 -	1.32	0.29
8q21.3	rs12543318	1.15	0.98 -	1.35	0.08
8q24	rs987525	1.04	0.87 -	1.24	0.68
FOXE1	rs7864322	0.91	0.77 -	1.08	0.29
FOXE1	rs10818094	1.13	0.95 -	1.36	0.17
FOXE1	rs1443433	0.96	0.77 -	1.19	0.71
FOXE1	rs74934500	1.18	0.74 -	1.88	0.49
FOXE1	rs3758249	1.00	0.85 -	1.17	0.99
FOXE1	rs10984103	0.97	0.82 -	1.14	0.69
KIAA1598-VAX1	rs7078160	1.01	0.83 -	1.23	0.94
KIAA1598-VAX1	rs4752028	0.99	0.82 -	1.21	0.96
FGFR2	rs4752566	0.95	0.81 -	1.11	0.51
FGFR2	rs2912760	1.03	0.87 -	1.23	0.72
FGFR2	rs3135761	1.08	0.88 -	1.31	0.48
FGFR2	rs2912771	1.12	0.93 -	1.33	0.23
FGFR2	rs2981428	0.86	0.74 -	1.01	0.06
FGFR2	rs3750817	1.05	0.90 -	1.23	0.54
SPRY2	rs8001641	1.06	0.91 -	1.23	0.50
TPM1	rs1873147	0.98	0.82 -	1.16	0.80
CRISPLD2	rs1546124	1.12	0.95 -	1.32	0.19
NTN1	rs4791331	0.91	0.73 -	1.14	0.40
NTN1	rs8069536	0.95	0.81 -	1.12	0.54
NOG1	rs227731	1.06	0.91 -	1.24	0.48
MAFB	rs13041247	0.98	0.83 -	1.14	0.77
MYH9	rs3752462	1.04	0.88 -	1.23	0.63
MYH9	rs1002246	1.06	0.90 -	1.25	0.47

* Effects were estimated in a model of multiplicative maternal SNP effects that also controlled for a multiplicative fetal genetic effect in all SNPs where maternal genotype was available

Appendix Table 7. Maternal SNP Effects* on Isolated Cleft Lip with Palate

Gene	SNP	RR	95% CI		p-value
PAX7	rs742071	0.97	0.85 -	1.10	0.67
ABCA4-ARHGAP29	rs560426	1.03	0.91 -	1.17	0.67
IRF6	rs2235371	1.67	1.10 -	2.52	0.02
IRF6	rs642961	1.03	0.88 -	1.20	0.71
THADA	rs7590268	0.96	0.83 -	1.12	0.64
MSX1	rs3111689	1.06	0.91 -	1.23	0.46
8q21.3	rs12543318	1.07	0.94 -	1.22	0.33
8q24	rs987525	0.88	0.76 -	1.03	0.11
FOXE1	rs7864322	1.03	0.90 -	1.18	0.72
FOXE1	rs10818094	0.89	0.76 -	1.03	0.12
FOXE1	rs1443433	1.00	0.83 -	1.19	0.96
FOXE1	rs74934500	0.95	0.63 -	1.42	0.80
FOXE1	rs3758249	1.02	0.89 -	1.16	0.80
FOXE1	rs10984103	1.03	0.90 -	1.17	0.72
KIAA1598-VAX1	rs7078160	1.04	0.89 -	1.22	0.60
KIAA1598-VAX1	rs4752028	1.02	0.87 -	1.20	0.79
FGFR2	rs4752566	1.04	0.91 -	1.18	0.59
FGFR2	rs2912760	0.96	0.83 -	1.10	0.55
FGFR2	rs3135761	1.17	0.99 -	1.38	0.06
FGFR2	rs2912771	0.93	0.80 -	1.07	0.32
FGFR2	rs2981428	0.96	0.84 -	1.09	0.52
FGFR2	rs3750817	1.06	0.93 -	1.20	0.42
SPRY2	rs8001641	1.07	0.95 -	1.22	0.27
TPM1	rs1873147	1.00	0.87 -	1.15	0.99
CRISPLD2	rs1546124	0.81	0.71 -	0.93	0.003
NTN1	rs4791331	0.99	0.82 -	1.18	0.88
NTN1	rs8069536	0.96	0.84 -	1.09	0.54
NOG1	rs227731	0.89	0.78 -	1.01	0.08
MAFB	rs13041247	0.98	0.86 -	1.11	0.73
MYH9	rs3752462	0.96	0.84 -	1.11	0.62
MYH9	rs1002246	1.06	0.93 -	1.21	0.40

* Effects were estimated in a model of multiplicative maternal SNP effects that also controlled for a multiplicative fetal genetic effect in all SNPs where maternal genotype was available

Appendix Table 8. Maternal SNP Effects* on Isolated Cleft Palate Only

Gene	SNP	RR	95% CI		p-value
PAX7	rs742071	1.08	0.93 -	1.25	0.34
ABCA4-ARHGAP29	rs560426	1.04	0.89 -	1.21	0.62
IRF6	rs2235371	1.46	0.90 -	2.37	0.13
IRF6	rs642961	0.97	0.80 -	1.17	0.75
THADA	rs7590268	1.15	0.96 -	1.36	0.12
MSX1	rs3111689	0.98	0.82 -	1.17	0.84
8q21.3	rs12543318	1.04	0.89 -	1.22	0.61
8q24	rs987525	1.00	0.83 -	1.20	0.97
FOXE1	rs7864322	1.09	0.93 -	1.28	0.28
FOXE1	rs10818094	0.91	0.76 -	1.09	0.30
FOXE1	rs1443433	1.16	0.94 -	1.42	0.16
FOXE1	rs74934500	0.50	0.27 -	0.90	0.02
FOXE1	rs3758249	1.03	0.88 -	1.20	0.71
FOXE1	rs10984103	1.05	0.90 -	1.23	0.53
KIAA1598-VAX1	rs7078160	0.82	0.67 -	1.01	0.06
KIAA1598-VAX1	rs4752028	0.80	0.65 -	0.98	0.03
FGFR2	rs4752566	1.09	0.94 -	1.27	0.25
FGFR2	rs2912760	0.90	0.76 -	1.07	0.22
FGFR2	rs3135761	0.94	0.77 -	1.14	0.54
FGFR2	rs2912771	1.01	0.85 -	1.20	0.93
FGFR2	rs2981428	0.91	0.78 -	1.06	0.23
FGFR2	rs3750817	1.20	1.03 -	1.40	0.02
SPRY2	rs8001641	1.02	0.88 -	1.19	0.76
TPM1	rs1873147	0.98	0.82 -	1.16	0.80
CRISPLD2	rs1546124	0.85	0.72 -	1.00	0.05
NTN1	rs4791331	0.89	0.71 -	1.11	0.30
NTN1	rs8069536	0.91	0.78 -	1.06	0.23
NOG1	rs227731	0.98	0.84 -	1.15	0.85
MAFB	rs13041247	0.91	0.78 -	1.07	0.25
MYH9	rs3752462	0.93	0.79 -	1.10	0.42
MYH9	rs1002246	1.02	0.87 -	1.20	0.82

* Effects were estimated in a model of multiplicative maternal SNP effects that also controlled for a multiplicative fetal genetic effect in all SNPs where maternal genotype was available

Appendix Table 9. Effects of Maternal and Paternal Alleles on Isolated CLO in Heterozygote Offspring and Test of Parent-of-Origin (PoO) Effect

		Maternal allele		Paternal allele		
Gene/Locus	SNP	RR	95% CI	RR	95% CI	*P*-difference
PAX7	rs742071	1.83	1.38 - 2.41	1.48	1.06 - 2.07	0.23
ABCA4-ARHGAP29	rs560426	1.03	0.75 - 1.43	1.36	1.02 - 1.82	0.18
IRF6	rs642961	1.75	1.33 - 2.31	2.32	1.80 - 3.01	0.17
THADA	rs7590268	1.25	0.96 - 1.62	1.17	0.87 - 1.57	0.75
8q21.3	rs1254331	1.57	1.21 - 2.03	1.14	0.83 - 1.56	0.07
8q24	rs987525	2.10	1.62 - 2.73	1.86	1.40 - 2.45	0.56
FOXE1	rs3758249	0.74	0.56 - 0.97	0.73	0.54 - 0.99	0.89
VAX1	rs7078160	1.39	1.06 - 1.82	1.21	0.90 - 1.63	0.47
KIAA1598	rs4752028	1.43	1.08 - 1.88	1.30	0.97 - 1.74	0.59
SPRY2	rs8001641	0.86	0.65 - 1.14	0.93	0.69 - 1.26	0.87
TPM1	rs1873147	1.29	0.98 - 1.69	1.35	1.02 - 1.78	0.67
NOG1	rs227731	1.71	1.27 - 2.29	1.39	0.98 - 1.96	0.25
MAFB	rs1304124	0.73	0.55 - 0.97	0.87	0.66 - 1.15	0.42

Notes: P-difference is the p-value of the difference in effects between maternal and paternal alleles.

Appendix Table 10. Effects of Maternal and Paternal Alleles on Isolated CLP in Heterozygote Offspring and Test of Parent-of-Origin (PoO) Effect

		Maternal allele		Paternal allele		
Gene/Locus	SNP	RR	95% CI	RR	95% CI	*P*-difference
PAX7	rs742071	1.21	0.94 - 1.56	1.34	1.06 - 1.71	0.58
ABCA4-ARHGAP29	rs560426	1.21	0.95 - 1.55	1.11	0.86 - 1.44	0.66
IRF6	rs642961	1.41	1.14 - 1.76	1.39	1.10 - 1.76	0.84
THADA	rs7590268	1.34	1.07 - 1.68	1.43	1.14 - 1.79	0.76
8q21.3	rs12543318	1.32	1.07 - 1.63	1.07	0.83 - 1.37	0.20
8q24	rs987525	1.65	1.30 - 2.10	2.09	1.68 - 2.61	0.09
FOXE1	rs3758249	0.88	0.71 - 1.09	0.90	0.71 - 1.13	0.89
VAX1	rs7078160	1.33	1.05 - 1.69	1.48	1.17 - 1.87	0.28
KIAA1598	rs4752028	1.27	0.99 - 1.62	1.55	1.23 - 1.95	0.15
SPRY2	rs8001641	0.94	0.76 - 1.17	0.78	0.61 - 1.00	0.21
TPM1	rs1873147	1.28	1.03 - 1.59	1.20	0.94 - 1.53	0.52
NOG1	rs227731	1.12	0.87 - 1.44	1.32	1.03 - 1.70	0.35
MAFB	rs13041247	0.59	0.46 - 0.75	0.68	0.53 - 0.86	0.62

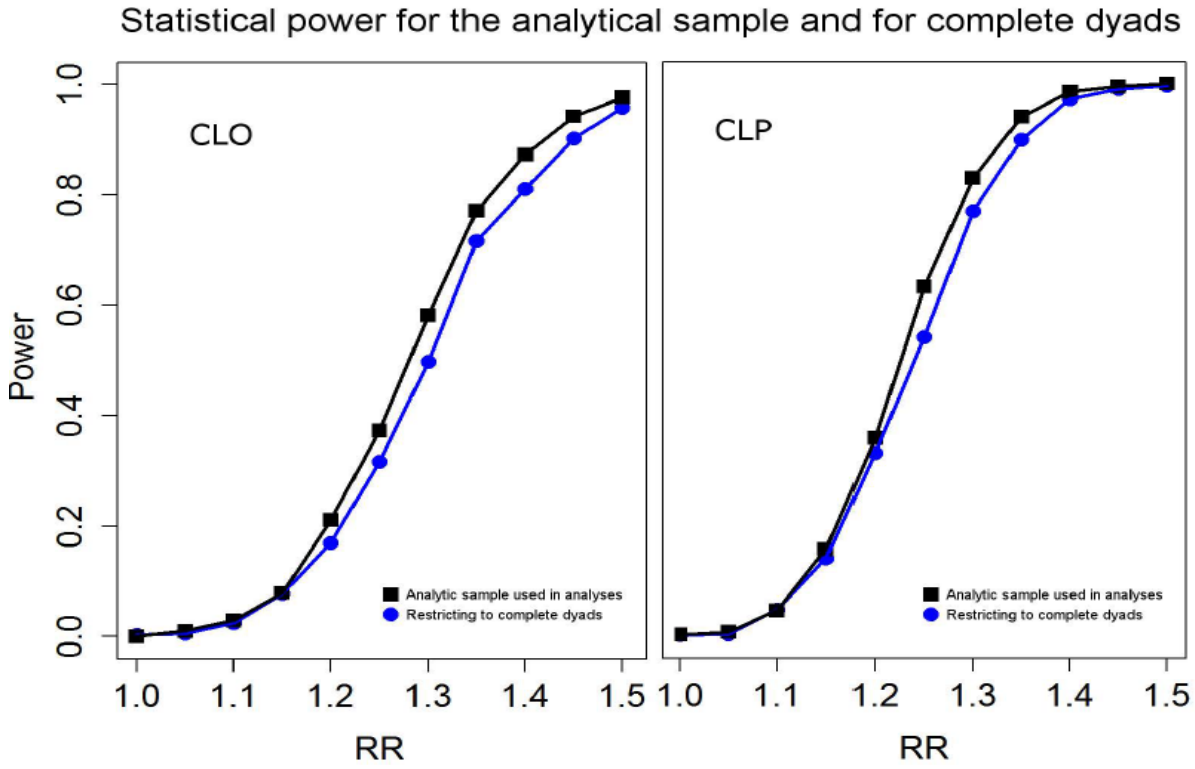
Notes: P-difference is the p-value of the difference in effects between maternal and paternal alleles.

Appendix Table 11. Effects of Maternal and Paternal Alleles on Isolated Cleft Palate Only in Heterozygote Offspring and Test of Parent-of-Origin Effect

		Maternal allele		Paternal allele		
Gene/Locus	SNP	RR	95% CI	RR	95% CI	P-difference*
PAX7	rs742071	1.21	0.92 - 1.59	1.21	0.92 - 1.58	0.98
ABCA4-ARHGAP29	rs560426	0.94	0.71 - 1.25	0.89	0.66 - 1.19	0.75
IRF6	rs642961	0.91	0.68 - 1.22	0.91	0.67 - 1.24	0.86
THADA	rs7590268	1.15	0.88 - 1.51	1.02	0.76 - 1.36	0.53
8q21.3	rs12543318	0.97	0.73 - 1.27	0.94	0.70 - 1.26	0.74
8q24	rs987525	0.97	0.72 - 1.29	0.77	0.55 - 1.07	0.43
FOXE1	rs3758249	0.82	0.62 - 1.09	0.95	0.72 - 1.26	0.46
KIAA1598-VAX1	rs7078160	0.89	0.64 - 1.24	1.36	1.03 - 1.79	0.06
SPRY2	rs8001641	1.27	0.96 - 1.70	1.06	0.77 - 1.45	0.33
TPM1	rs1873147	1.00	0.76 - 1.31	1.03	0.77 - 1.37	0.89
NOG1	rs227731	0.76	0.57 - 1.02	0.92	0.70 - 1.20	0.39
MAFB	rs13041247	0.67	0.48 - 0.92	0.99	0.75 - 1.30	0.13

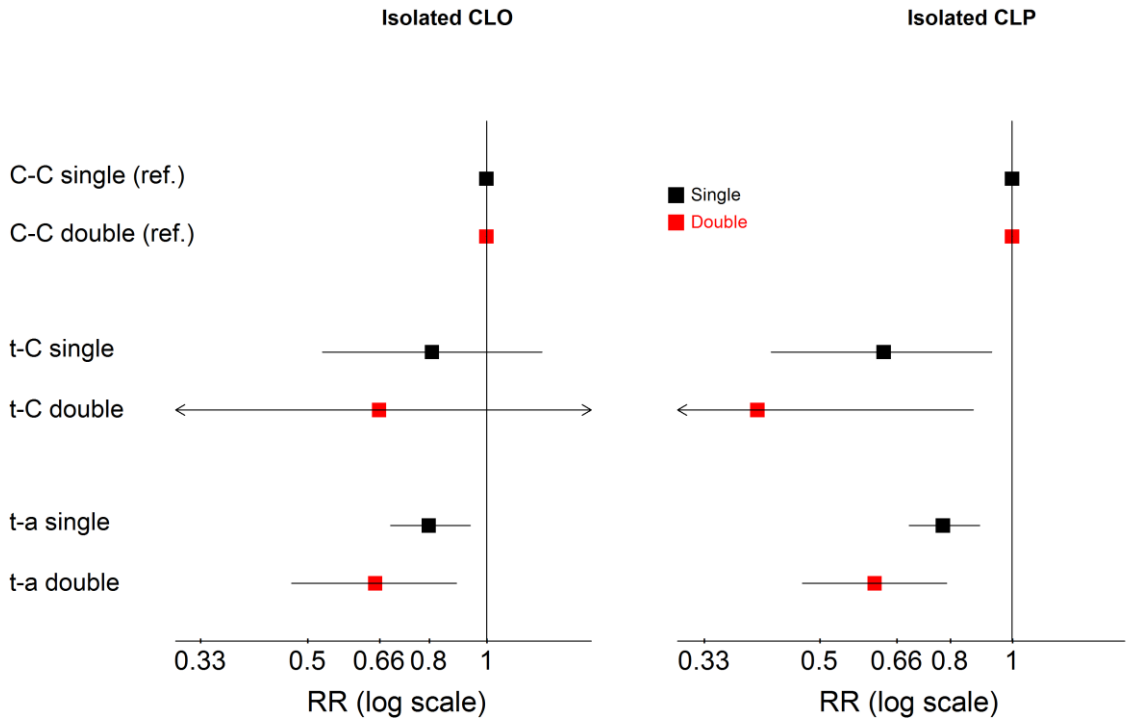
* P-value of the difference in effects between maternal and paternal alleles

Appendix Figure 1.



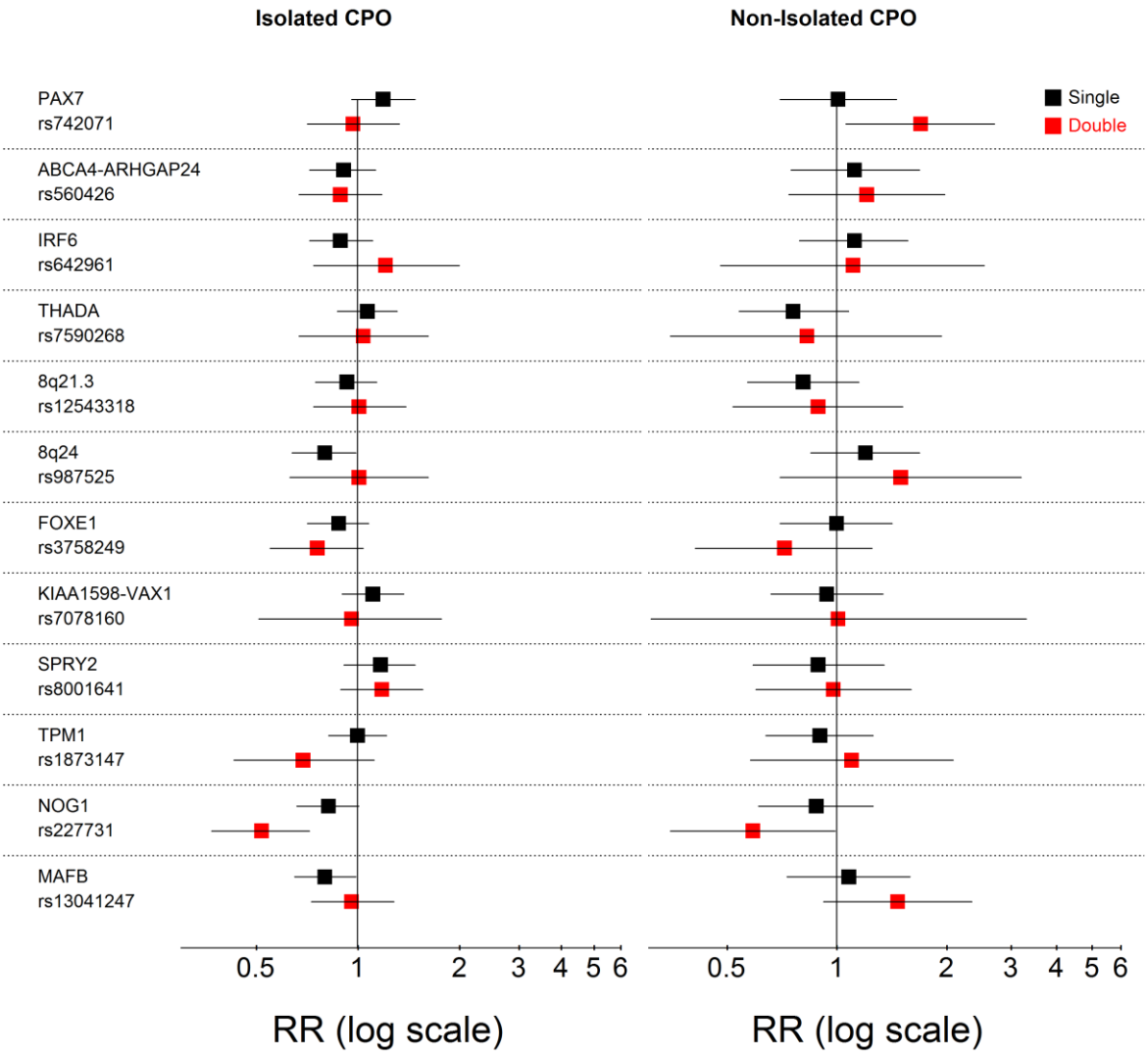
Graphs show statistical power for testing the fetal gene effects shown in Supplementary Tables S3 and S4 with significance level 0.0016 and a minor allele frequency of 0.30 based on an analysis using both complete and incomplete case and control dyads (black squares and lines) and another analysis using only complete case and control dyads (blue circles and lines). For CLO, we calculated power for a sample of 350 mother-child case dyads plus 150 case mothers compared to using only the 350 complete dyads. For CLP, we calculated power for a sample of 550 mother-child case dyads plus 240 case mothers and compared to using only the 550 complete dyads. For both CLO and CLP, the power calculations were based on 2600 mother-child control dyads with or without adding 1100 control mothers.

Appendix Figure 2.



Single and double dose effects measured by relative risks (RR) in a multiplicative model for haplotypes constructed for the two significant SNPs at FOXE1 (rs3758249 and rs10984103) for the two case categories isolated cleft lip only (CLO) and isolated cleft lip and palate (CLP). Black squares represent single dose effect, red squares represent double dose effects and lines are 95% confidence intervals. C and t denotes the major and minor alleles at rs3758249, while C and a denote the major and minor alleles at rs10984103. Note that the C-a haplotype was too rare for estimation.

Appendix Figure 3



Single and double dose effects measured by relative risks (RR) for minor alleles of 15 fetal SNPs for isolated cleft palate only (CPO) (first panel) and for non-isolated cleft palate only (second panel). Black squares represent single dose effects, red squares represent double dose effects and lines are 95% confidence intervals.

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