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Evaluation of Proton-Coupled Folate Transporter (*SLC46A1*) Polymorphisms as Risk Factors for Neural Tube Defects and Oral Clefts

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Abstract

Many folate-related genes have been investigated for possible causal roles in neural tube defects (NTDs) and oral clefts. However, no previous reports have examined the major gene responsible for folate uptake, the proton-coupled folate transporter (*SLC46A1*). We tested for association between these birth defects and single nucleotide polymorphisms in the *SLC46A1* gene. The NTD study population included 549 complete and incomplete case-family triads, and 999 controls from Ireland. The oral clefts study population comprised a sample from Utah (495 complete and incomplete case-family triads and 551 controls) and 221 Filipino multiplex cleft families. There was suggestive evidence of increased NTD case risk with the rs17719944 minor allele (odds ratio (OR): 1.29; 95% confidence intervals (CI): [1.00–1.67]), and decreased maternal risk of an NTD pregnancy with the rs4795436 minor allele (OR: 0.62; [0.39–0.99]). In the Utah sample, the rs739439 minor allele was associated with decreased case risk for cleft lip with cleft palate

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(genotype relative risk (GRR): 0.56 [0.32–0.98]). Additionally, the rs2239907 minor allele was associated with decreased case risk for cleft lip with cleft palate in several models, and with cleft palate only in a recessive model (OR: 0.41; [0.20–0.85]). These associations did not remain statistically significant after correcting for multiple hypothesis testing. Nominal associations between *SLC46A1* polymorphisms and both Irish NTDs and oral clefts in the Utah population suggest some role in the etiology of these birth defects, but further investigation in other populations is needed.

Keywords

folate; folic acid; folate transport; proton-coupled folate transporter; neural tube; oral clefts; cleft lip; cleft palate

INTRODUCTION

Periconceptional intake of folic acid is known to reduce the incidence of neural tube defects (NTDs) by up to 70% [MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992; Kirke et al., 1993; Steegers-Theunissen et al., 1994]. Moreover, NTDs are known to have a genetic component. Polymorphisms in genes related to folate metabolism and transport could affect the encoded proteins, making these polymorphisms attractive candidates for genetic risk factors for NTDs. Single nucleotide polymorphisms (SNPs) c.665C>T (p.Ala222Val, “677C→T”, rs1801133) in 5, 10-methylene-tetrahydrofolate reductase (*MTHFR*) [van der Put et al., 1995; Shields et al., 1999; Botto and Yang, 2000; Zhang et al., 2013], and c.1958G>A (p.Arg653Gln, rs2236225) in methylene-tetrahydrofolate trifunctional C1 synthase (*MTHFD1*) [Brody et al., 2002; De Marco et al., 2006; Parle-McDermott et al., 2006] are associated with an increased risk in cases and mothers, respectively.

Oral clefts have also been investigated for the role of folate in their origins (reviewed in [Wehby and Murray, 2010]). Studies of folic acid supplement use, dietary folate, maternal blood levels of folate, and folic acid fortification have produced variable results [Castilla et al., 2003; Czeizel et al., 2004; Canfield et al., 2005; Wilcox et al., 2007; Little et al., 2008; Munger et al., 2011]. Multivitamins have been found to reduce the recurrences of oral clefts in some studies [Shaw et al., 1995; Tolarova and Harris, 1995; Loffredo et al., 2001] but not in others [Shaw et al., 2006]. While these supplements included folic acid, they also contained a wide range of other micronutrients. SNPs in folate metabolizing enzymes, including *MTHFR677*C→T, have also been examined for associations with oral clefts but the results are contradictory [Blanton et al., 2002; Boyles et al., 2008; Mills et al., 2008]. However, because there is suggestive evidence for the involvement of folate in oral clefts, and genetic factors are known to influence cleft risk, there continues to be intense investigation of the effects of folic acid intake and variants in folate-metabolism genes on the risk of oral clefts.

Folate transporter genes are important candidates to screen for genetic polymorphisms related to NTDs and oral clefts. Decreased transport of folate through the intestine could cause lower levels of maternally circulating folate and put the embryo at increased risk of

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birth defects. It has been shown that women carrying affected fetuses have lower plasma and red cell folate levels than women carrying unaffected fetuses [Kirke et al., 1993; Daly et al., 1995]. In Utah [Munger et al., 2011] and the Philippines [Munger et al., 2004], mothers of children with oral clefts were found to have lower mean levels of plasma folate. Supplemental folic acid could reduce NTD occurrence by overcoming a relatively poor folate transporter. Previous investigations have not shown consistent associations between risk of NTDs and either the reduced folate carrier (solute carrier family 19 [folate transporter], member 1, *SLC19A1*) [Shaw et al., 2002; Morin et al., 2003; O'Leary et al., 2006; Franke et al., 2009] or other folate receptors (*FOLR1, FOLR2, FOLR3*) (O'Leary et al., 2003; Franke et al., 2009; O'Byrne et al., 2010). Other studies have also produced conflicting evidence for an association with oral clefts (Shaw et al., 2003; Scapoli et al., 2005; Pei et al., 2006; Boyles et al., 2009).

Significantly, none of thesestudies have examined the oneprotein responsible for transporting the majority of the folates absorbed from the diet. While *SLC19A1, FOLR1, FOLR2*, and *FOLR3* are known to mediate cellular folate uptake, only the proton-coupled folate transporter (solute carrier family 46 [folate transporter], member 1, *SLC46A1*) is responsible for intestinal absorption of dietary folate. *SLC46A1* optimally functions at the low pH found in the intestine where absorption of dietary folate occurs. Polymorphisms in SLC46A1 have not been evaluated as risk factors for either NTDs or oral clefts. While rare but severe defects in the protein cause hereditary folate malabsorption (HFM; OMIM 229050) [Qiu et al., 2006; Zhao et al., 2007], it is unclear if the gene contains common polymorphisms affecting the function of this transporter. We tested whether common genetic variants in *SLC46A1* are associated with NTDs and oral clefts by performing case-control and family-based tests of association for selected SNPs in or near the *SLC46A1* gene.

MATERIALS AND METHODS

Neural Tube Defects Study Population

Study subjects and controls were recruited and samples collected from throughout the Republic of Ireland as previously described [Swanson et al., 2005; Pangilinan et al., 2010]. The NTD population consists of complete triads, with DNA from both unaffected parents and the NTD-affected case, and incomplete triads where DNA was not available from all three members of the family. The NTD cases were affected with spina bifida (96%) or encephalocele (4%). Control subjects were randomly selected from a bank of pregnant women (n = 56,049) recruited from maternity hospitals in the Dublin area between 1986 and 1990 who did not have an NTD-affected child and had no history of an NTD-affected pregnancy. There were 2,561 samples: 549 cases, 532 NTD mothers, 481 NTD fathers, and 999 controls. All subjects gave consent for the samples. Ethical approval for the recruitment of this study population and the collection of biological samples was obtained from the Research Ethics Committee of the Health Research Board of Ireland and the Institutional Review Board of the National Human Genome Research Institute.

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Oral Clefts Study Population

The Utah oral cleft cases were ascertained via a statewide birth defects registry and controls were randomly selected from all Utah birth certificates as previously described [Munger et al., 2011]. Other phenotypic data (e.g., smoking, multivitamin use, blood folate) was available on a subset of the mothers in this sample [described in Munger et al., 2011]. Medical records of affected children were reviewed by a medical geneticist and classified by type of oral cleft and other associated birth defects or known genetic abnormality. The inclusion criterion for participating cases was a diagnosis of an oral cleft. Complete and incomplete family triads were recruited in Utah (Table I). These were simplex families in which the child was the affected case (cleft case). The Utah study population also included 552 control children who did not have oral clefts. Also recruited were 221 Filipino multiplex clefts families (Table II) in which more than one family member was affected by an oral cleft. All study participants and/or their parents gave informed consent under a protocol approval by the institutional review boards at the Hope Foundation (Bacolod City, Negros-Philippines) or the Utah State University.

DNA Isolation

Genomic DNA was isolated from whole blood samples (Irish NTD triads and controls) using Qiagen Blood Mini kits (Qiagen, Valencia, CA). Genomic DNA was isolated from whole blood (Filipino samples and Utah cleft mothers) or buccal swabs (Utah cases, Utah controls and Utah cleft fathers) using standard protocols.

SNP Selection

To select a minimum set of SNPs that covered genetic variation within *SLC46A1* (Fig. 1), the SNPs within the gene and 5 kb on either side of the gene were evaluated in the CEU population of the Hapmap dataset (Version 3, Release R2; [The International Hapmap Consortium, 2003]). Haploview Tagger [Barrett et al., 2005] was used to reduce a set of nine Hapmap SNPs in this region to four tagSNPs (MAF > 0.05, r^2 threshold of 0.8): rs9894260, rs739439, rs2239907 and rs4795436. With the exception of rs4795436 (a singleton SNP not found to have significant LD with other SNPs), these SNPs were typed in the oral cleft samples. All SNPs were typed in the NTD samples with additional singleton SNPs in *SLC46A1* and its 10 kb flanking regions from a previous Hapmap release (Release 28) (rs6505079, rs17719944, rs8076949, and rs11080058).

Genotyping

All SNPs in the NTD samples were genotyped at the National Human Genome Research Institute (Bethesda, MD) using matrix assisted laser desorption/ionization—time-of-flight (MALDITOF) mass spectrometry (Sequenom, San Diego, CA) of allele-specific extension products (primers sequences and assay conditions available upon request). The genotyping call rates for each SNP among NTD cases, NTD mothers, NTD fathers and controls ranged from 93% to 99%; the overall call rate was 96% for all SNPs in all samples. At least 10% of samples were re-genotyped for each SNP to evaluate genotyping consistency. Concordance for these subsamples ranged from 98.3% to 100% (average of 99.1%). NTD triads exhibiting non-Mendelian inheritance were excluded from analysis of that SNP, as were any discordant

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genotypes. Hardy–Weinberg equilibrium (HWE) for each SNP was evaluated separately in NTD cases, NTD mothers, NTD fathers and controls to maximize the ability to detect genotyping bias—the most likely reason for violation of HWE. None of the eight SNPs were out of HWE ($P < 0.01$) in any subgroup.

All SNPs in the oral cleft samples were genotyped at the University of Iowa (Iowa City, IA) using Taqman assays from Applied Biosystems (Foster City, CA). The genotyping call rates for each SNP in the Utah cleft cases, cleft mothers, cleft fathers and controls ranged from 79% to 94%, and the overall call rate was 87% for all SNPs in the Utah samples. The genotyping call rates for each SNP in Filipino cases and unaffected parents ranged from 88% to 90%, and the overall call rate was 89% for all SNPs in the Filipino samples. Significant sample failure occurred in both cleft populations; no genotype was obtained for any of the tested SNPs in 4.7% (90/1925) of Utah samples and 7.4% (147/1989) of Filipino samples. For each of the three SNPs examined, Haploview software [Barrett et al., 2005] was used to test for HWE separately in Utah case families, Utah controls, and Filipino multiplex families to maximize ability to detect potential genotyping bias. With the exception of rs2239907 in the Utah cleft mothers ($P = 0.0003$) there was no violation of HWE ($P < 0.01$) for any SNP in any subgroup. As all other groups (Utah cleft cases, Utah cleft fathers, Utah controls and all Filipino subjects) genotyped by the same method were consistent with HWE, genotypes for rs2239907 in the Utah cleft mothers were retained for analysis.

Statistical Methods

We used both case-control and family-based tests of association to assess the eight selected SNPs for NTD risk. HWE predicts consistent genotype distributions within each generation of a population, allowing the use of a single control group whether evaluating case or maternal effects. Logistic regression analyses were therefore performed with an unaffected pregnant mother control group for the Irish NTD analyses and an unaffected child control group for the Utah oral cleft analyses. Using a multiplicative model, case-control associations were tested in logistic regression analyses that generated odds ratio (OR) estimates and 95% confidence intervals (CI). If a statistically significant result was found, dominant and recessive models were also tested. Mother-control associations were evaluated using similar logistical models. Interactions between other phenotypes and SNP genotypes were carried out using PLINK v1.07.

NTD triads were used to test for association using two log-linear models. Case effects were tested using the transmission disequilibrium test (TDT) [Spielman et al., 1993]. This test uses a single parameter for the number of risk alleles observed in the case genotype, giving a one degree of freedom test of allelic association. Additionally, a log-linear model allowing for direct maternal effect was used. This test uses two parameters each for the case and maternal genotypes, which allows a separate effect for one or two copies of the risk allele [Weinberg et al., 1998]. Case genotype effects are tested using a two degree of freedom test based on the case parameters. Direct maternal effects are tested from the same log-linear model by using a two degree of freedom test based on the maternal genotype.

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The statistical methods used to assess NTD risk were also applied to the Utah cleft triads and controls to assess the risk of oral clefts. The Family-based Association Tests (FBAT) software [Horvath et al., 2001] was used to test the composite null hypothesis of no linkage and no association between risk and the selected SNPs with oral clefts in Filipino multiplex families. Nominal *P*-values are presented for all tests. Final evaluation of these *P*-values included Bonferroni correction for multiple hypothesis testing.

RESULTS

Neural Tube Defects

NTD cases, their parents and controls were genotyped for the candidate risk SNPs in order to perform both case-control and family-based tests of association. Genotype counts, genotype frequencies and allele frequencies are reported in Table III. A total of ninelog-linear, case-control, and mother-control tests of association were performed (Table IV, Supporting Table SI). Logistic regression using a multiplicative model revealed evidence of association between case inheritance of the *SLC461A* rs17719944 minor C allele and increased NTD risk (OR: 1.29; 95% CI: [1.00–1.67]; $P = 0.0$). This SNP also exhibited association under a dominant model (OR: 1.39 [1.05–1.84]; $P = 0.02$). Logistic regression using a multiplicative model revealed an association between maternal carriage of the *SLC46A1* rs4795436 minor G allele and reduced risk of an NTD pregnancy (OR: 0.62 [0.39–0.99]; $P = 0.04$). Follow-up tests using dominant and recessive models were not significant, although the dominant model trended toward significance (OR: 0.64 [0.40–1.03]; $P = 0.06$). These nominal associations failed to survive Bonferroni correction for multiple tests (8 SNPs × 9 tests = 72 tests; Bonferroni *P*-value = 0.05/72 0.007). No association between any *SLC46A1* SNP and case or maternal NTD risk was observed in family-based TDT or log-linear analyses (Supporting Table SI).

Oral Clefts

In the Utah study population, cases were categorized as having cleft lip, cleft lip with cleft palate, or cleft palate only (Table I). Cases were also categorized for clefts occurring in isolation, or in the context of multiple birth defects, or in the context of other syndromes or chromosomal abnormalities. There were insufficient numbers to perform separate analyses for clefts that occurred in conjunction with multiple defects or in a syndromic context. Therefore, analyses focused on searching for genetic effects in: (i) each isolated cleft type; and 2) all cases of each cleft type. Oral cleft cases, their parents and controls were genotyped and subject to the same association tests performed in the NTD analyses. Genotype counts, genotype frequencies and allele frequencies were calculated for the Utah subjects (Table V). Association testing showed *SLC46A1* rs739439 and *SLC46A1* rs2239907 were nominally associated with oral clefts (Table IV).

SLC46A1 rs739439 was nominally associated with isolated cleft clip and palate by the transmission disequilibrium test (TDT; genotype relative risk, GRR: 0.56 [0.32–0.98]; $P = 0.04$). Log-linear analyses also showed that *SLC46A1* rs2239907 is associated with isolated cleft lip with cleft palate cases. A protective effect in isolated cleft lip with cleft palate cases was observed in the presence of two copies (GRR: 0.25 [0.08–0.85]; $P = 0.03$) of the

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rs2239907 minor A allele in the child. The two degree-of-freedom test for overall effect of alleles in cases was also significant ($P = 0.04$). Lastly, a maternal protective effect was observed for the presence of one copy of the A allele in mothers of children with isolated cleft lip with cleft palate (GRR: 0.37 [0.16–0.86]; $P = 0.02$). Nearly identical results were observed when these tests and models were applied to all, rather than isolated, cleft lip and cleft palate cases (Table IV).

SLC46A1 rs2239907 was also associated with isolated cleft palate only cases by logistic regression using a multiplicative model (OR: 0.66 [0.48–0.92]; $P = 0.01$). Dominant and recessive models were applied in follow-up testing. Although the dominant model was not significant ($P = 0.06$), the recessive model showed a significant protective effect of the minor A allele (OR: 0.41 [0.20–0.85]; $P = 0.02$). Similar results were observed when all cleft palate cases were tested; the minor A allele of *SLC46A1* rs2239907 showed a protective effect in tests of logistic regression using a multiplicative (OR: 0.73 [0.54–0.97]; $P = 0.03$) and recessive model (OR = 0.41[0.22–0.78]; $P = 0.01$). The observed associations did not remain statistically significant after adjustment by Bonferroni correction (3 SNPs × 6 cleft types × 9 tests = 162 tests; Bonferroni P-value = 0.05/162 = 0.003; Supporting Table SII).

Filipino multiplex cleft families were similarly classified for cleft types, taking into account that different cleft types could occur within a pedigree (Table II). In the absence of available controls, association was restricted to the TDT. Genotype counts, genotype frequencies and allele frequencies were calculated for the Filipino subjects (Table VI). None of the three SNPs tested was associated with oral clefts in Filipino multiplex families (Supporting Table SIII).

DISCUSSION

The proton-coupled folate transporter is the intestinal, high-affinity folate transport protein (encoded by the *SLC46A1* gene) responsible for moving folate from the intestine into the circulation. It is highly specific for folates and anti-folates [Qiu et al., 2006]. Rare mutations in *SLC46A1* that interfere with folate transport cause acute folate malabsorption (OMIM 229050). Because of these findings and the importance of folate in the prevention of NTDs, we examined common variants in *SLC46A1* to determine whether there were associations with NTDs. We also examined associations with oral clefts because previous reports suggest that folate could influence the occurrence and recurrence of these birth defects. We observed several nominally significant associations between *SLC46A1* variants and NTD and oral clefts. The SNPs that showed nominally significant association with NTDs (rs17719944 and rs4795436) are intronic and do not appear to be associated with any other phenotypes in the literature. In contrast, the SNPs showing nominally significant association with oral clefts (rs739439 and rs2239907) are located in the 3′-untranslated region (UTR). As part of the transcribed mRNA, these 3′-UTR SNPs may influence its expression or stability, although variation in *SLC46A1* is negative for association with its gene expression in the Genotype-Tissue Expression Project (GTEx, http://www.gtexportal.org). Interestingly, a single study reported significant association but opposite directions of allelic effect for rs739439 with measures of plasma high-density lipoprotein levels in two populations [Clifford et al., 2013], while rs2239907 has been examined for association with plasma homo-cysteine with

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inconsistent results [Huang et al., 2011]. Further study is required to determine whether these SNPs influence gene function, and to further establish their potential role in risk for NTDs and oral clefts.

Replication and meta-analyses are essential tools in establishing that genetic associations of small effect are true associations. Because we analyzed several loci each with multiple models of inheritance, we chose to employ a highly conservative correction for multiple hypothesis testing. Although this rendered these results nominally significant, this study did identify *SLC461A* candidate SNPs for replication studies. Additionally, the reported genotype counts will be of value for future meta-analyses (Table III, Table V, Supporting Table SIV).

Our study had a number of strengths. It is the first study, to our knowledge, that has examined variants in the *SLC46A1* gene for association with NTDs and oral clefts. The Irish NTD sample represents a large and relatively homogeneous population with very little exposure to supplemental folic acid, as Ireland does not require fortification of any food products and periconceptional supplement use was uncommon during the time interval when the affected individuals were in utero. The sample sizes of oral cleft cases in the Utah and Filipino study populations were robust and the numbers of affected individuals in some oral cleft categories were large enough to permit separate analyses to be conducted for these sub-categories of oral clefts. Studying both the Utah and Filipino oral cleft populations also allowed evaluation of *SLC46A1* variation in different ethnic backgrounds; a recent genome-wide study of oral clefts has shown differing strengths of association for genetic risk factors in Filipino versus Caucasian populations [Beaty et al., 2010].

Despite these strengths, it is possible that the Irish study population we examined was simply not large enough to detect small effects. In addition, some samples failed genotyping in the Filipino and Utah samples: almost 8% of Filipino samples and 4% of Utah samples could not be genotyped by any method we employed. These sample failures are likely due to the age and condition of the DNA. HWE analyses showed no skewing of allele detection. Therefore this sample failure is likely to be random, may not have introduced bias, and is problematic mainly by reducing the number of observable genotypes. Also, we cannot exclude the possibility that the associations seen here between *SLC46A1* SNPs and these two common birth defects were due to chance alone because they were no longer statistically significant after Bonferroni adjustment for multiple comparisons. The top six associations identified in a recent genome-wide meta-analysis of four oral cleft studies were unrelated to the folate pathway [Ludwig et al., 2012]. Lastly, depending on the population or subgroup, we had limited or no information on risk factors (maternal folic acid intake, medication use, alcohol use, and smoking) that could influence NTDs and oral clefts and could potentially interact with genetic variants. The limited information we have is restricted to the Utah cleft mothers [Munger et al., 2011]. No significant associations were observed when these risk factors were considered jointly with the genotypic information. It should be noted that this dataset is underpowered to test for association between genotype and these additional factors.

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In summary, we observed nominally significant (but not definitive) associations between *SLC46A1* SNPs and NTDs and oral clefts, and this finding warrants follow-up in other populations. The data presented would be a valuable addition to future meta-analyses. We found no associations between *SLC46A1* and oral clefts in our sample of Filipino multiplex clefts families, suggesting genetic risk factors for this birth defect might differ by race/ethnicity. Further research will be needed to define the roles of maternal and placental *SLC46A1* with respect to the folate needs of the human embryo and to investigate whether common variants in the gene impact its function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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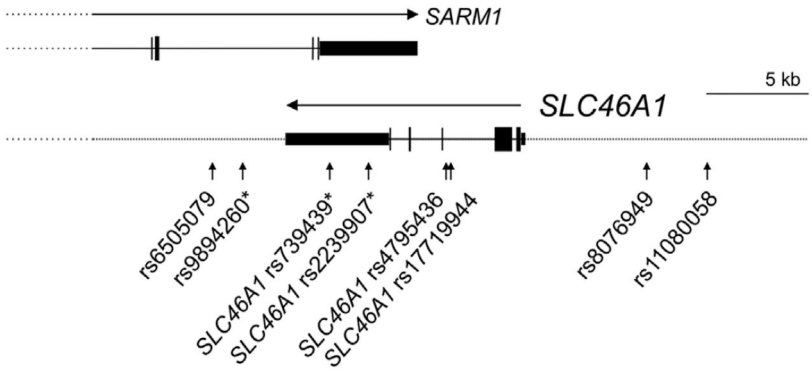

FIG. 1.

Physical map of *SLC46A1* and SNPs selected for study. The genomic region surrounding *SLC46A1* and the relative distances between markers are shown to scale. The 3′ end of the sterile alpha and TIR motif containing 1 (*SARM1*) gene overlaps with the 3′ end of *SLC46A1*. Gene names are included in the SNP name if the variant falls in the gene. All eight SNPs were tested for neural tube defect association, while the starred SNPs were tested for association with cleft phenotypes in the Utah and Filipino samples.

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TABLE I.

Utah Case Family Triads

Cleft type[a]	Cases (N = 451)	Fathers (N = 440)	Mothers (N = 483)	Triads (N = 495)
CL isolated	107	95	108	111
CL multiple	8	11	11	13
CL syndromic	0	3	4	4
CL—all	115	109	123	128
CLP isolated	159	150	158	162
CLP multiple	21	18	22	23
CLP syndromic	9	18	18	19
CLP—all	189	186	198	204
CPO isolated	108	98	108	109
CPO multiple	21	23	27	27
CPO syndromic	18	24	27	27
CPO—all	147	145	162	163

[a]CLP, cleft lip with cleft palate; CL, cleft lip only; CPO, cleft palate only; isolated, sole cleft defect; multiple, in the presence of multiple birth defects; syndromic, in the presence of a recognized syndrome or chromosomal abnormality.

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TABLE II.

Filipino Multiplex Families

Cleft type[a]	Number of pedigrees (nuclear families)	Affected individuals			Unaffected individuals			Total individuals
		Genotyped	Failed genotyping	Total	Genotyped	Failed genotyping	Total	
CL—all	15 (26)	32	3	35	61	1	62	97
CL—non-syndromic	14 (24)	30	3	33	59	1	60	93
CL—questionably syndromic	1 (2)	2	0	2	2	0	2	4
CLP—all	67 (128)	158	7	165	322	12	334	499
CLP—non-syndromic	62 (118)	146	7	153	296	12	308	461
CLP—syndromic	5 (10)	12	0	12	26	0	26	38
CL/CLP—all	118 (300)	344	35	379	724	69	793	1172
CL/CLP—non-syndromic	103 (252)	300	25	325	632	41	673	998
CL/CLP—syndromic	10 (30)	28	6	34	67	16	83	117
CL/CLP—questionably syndromic	5 (18)	16	4	20	25	12	*37*	57
CPO—all	2 (4)	5	1	6	17	0	17	23
CPO—non-syndromic	1 (1)	2	0	2	9	0	9	11
CPO—syndromic	1 (3)	3	1	4	8	0	8	12
Mixed CL/CLP and CPO—all	19 (47)	58	6	64	117	17	134	198
Mixed CL/CLP and CPO—non-syndromic	16 (37)	49	4	53	90	11	101	154
Mixed CL/CLP and CPO—syndromic	2 (8)	*7*	1	8	22	3	25	33
Mixed CL/CLP and CPO—questionably syndromic	1 (2)	2	1	3	5	3	8	11
All families	221 (505)	597	52	649	1241	99	1340	1989

[a]CL, families in which all affected members had cleft lip only; CLP, families in which all affected members had cleft lip with or without cleft palate; CL/CLP, families in which at least one affected member had cleft lip only and at least one affected member had cleft lip with or without cleft palate; CPO, families in which all affected members had cleft palate only; mixed CL/CLP and CPO, families in which at least one affected member had either cleft lip only or cleft lip with or without cleft palate and at least one affected member had cleft palate only.

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TABLE III.

Genotype Distributions and Allele Frequencies in Irish Controls and Neural Tube Defect Triads

Marker	**Controls (N = 999)**	**NTD children (N = 549)**	**NTD mothers (N = 532)**	**NTD fathers (N = 481)**
Genotypes	**N (freq.)**	**N (freq.)**	**N (freq.)**	**N (freq.)**
Alleles	**Freq.**	**Freq.**	**Freq.**	**Freq.**
rs11080058				
CC	492 (0.51)	279 (0.53)	256 (0.50)	239 (0.53)
CT	383 (0.40)	207 (0.39)	204 (0.40)	176 (0.39)
TT	81 (0.08)	39 (0.07)	47 (0.09)	39 (0.09)
C	0.71	0.73	0.71	0.72
T	0.29	0.27	0.29	0.28
rs8076949				
AA	6 (0.01)	3 (0.01)	4 (0.01)	2 (0)
AG	149 (0.16)	79 (0.15)	88 (0.18)	58 (0.13)
GG	786 (0.84)	434 (0.84)	405 (0.81)	386 (0.87)
A	0.09	0.08	0.10	0.07
G	0.91	0.92	0.90	0.93
SLC46A1 rs17719944				
CC	11 (0.01)	5 (0.01)	6 (0.01)	9 (0.02)
CT	128 (0.13)	95 (0.18)	68 (0.13)	73 (0.16)
TT	822 (0.86)	432 (0.81)	434 (0.85)	375 (0.82)
C	0.08	0.10	0.08	0.10
T	0.92	0.90	0.92	0.90
SLC46A1 rs4795436				
AA	883 (0.93)	501 (0.94)	491 (0.95)	436 (0.95)
AG	67 (0.07)	31 (0.06)	24 (0.05)	23 (0.05)
GG	2 (0)	0 (0)	0 (0)	2 (0)
A	0.96	0.97	0.98	0.97
G	0.04	0.03	0.02	0.03
SLC46A1 rs2239907				
AA	166 (0.17)	82 (0.16)	77 (0.15)	86 (0.19)
AG	456 (0.48)	260 (0.50)	244 (0.49)	211 (0.47)

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Marker	Controls (N = 999)	NTD children (N = 549)	NTD mothers (N = 532)	NTD fathers (N = 481)
Genotypes	**N (freq.)**	**N (freq.)**	**N (freq.)**	**N (freq.)**
Alleles	**Freq.**	**Freq.**	**Freq.**	**Freq.**
GG	337 (0.35)	179 (0.34)	177 (0.36)	152 (0.34)
A	0.41	0.41	0.40	0.43
G	0.59	0.59	0.60	0.57
SLC4A11 rs739439				
CC	676 (072)	368 (070)	367 (073)	314 (071)
CT	228 (0.24)	146 (0.28)	124 (0.25)	113 (0.25)
TT	34 (0.04)	9 (0.02)	12 (0.02)	18 (0.04)
C	0.84	0.84	0.85	0.83
T	0.16	0.16	0.15	0.17
rs9894260				
AA	4 (0)	5 (0.01)	4 (0.01)	4 (0.01)
AG	159 (0.17)	95 (0.18)	75 (0.15)	73 (0.16)
GG	796 (0.83)	437 (0.81)	433 (0.85)	380 (0.83)
A	0.09	0.10	0.08	0.09
G	0.91	0.90	0.92	0.91
rs6505079				
AA	281 (0.30)	142 (0.27)	148 (0.29)	122 (0.27)
AT	443 (0.48)	264 (0.51)	256 (0.50)	229 (0.50)
TT	205 (0.22)	114 (0.22)	107 (0.21)	103 (0.23)
A	0.54	0.53	0.54	0.52
T	0.46	0.47	0.46	0.48

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TABLE IV.

Nominally Significant Associations Between *SLC46A1* SNPs and Neural Tube Defects in an Irish Population or Oral Cleft in a Utah Population

Case type[a]	SNP	Regression analysis	Effect estimate[b] (95% confidence interval)	*P*-value
NTD	rs17719944	Case-control—multiplicative model	1.29 (1.00, 1.67)	0.05
NTD	rs4795436	Mother-control—multiplicative model	0.62 (0.39, 0.99)	0.04
CLP—isolated	rs739439	Transmission disequilibrium test	0.56 (0.32, 0.98)	0.04
CLP—isolated	rs2239907	Log-linear: 2 alleles in child	0.25 (0.08, 0.85)	0.03
CLP—isolated	rs2239907	2-DF test for overall effect of alleles in child[c]	—	0.04
CLP—isolated	rs2239907	Log-linear: 1 allele in mother	0.37 (0.16, 0.86)	0.02
CLP—all	rs739439	Transmission disequilibrium test	0.55 (0.32, 0.94)	0.03
CLP—all	rs2239907	Log-linear: 2 alleles in child	0.29 (0.10, 0.89)	0.03
CLP—all	rs2239907	2-DF test for overall effect of alleles in child[c]	—	0.02
CLP—all	rs2239907	Log-linear: 1 allele in mother	0.40 (0.19, 0.86)	0.02
CPO—isolated	rs2239907	Case-control—multiplicative model	0.66 (0.48, 0.92)	0.01
CPO—all	rs2239907	Case-control—multiplicative model	0.73 (0.54, 0.97)	0.03

[a]NTD, neural tube defect; CLP, cleft lip with cleft palate; CPO, cleft palate only; isolated, isolated cleft defect; all, includes isolated defects, clefting in the context of multiple birth defects, and clefting in the context of a recognized syndrome or chromosomal abnormalities (see Table I).

[b]For case-control and mother-control logistic regression analyses, the effect estimate is the odds ratio; for the transmission disequilibrium test and log-linear regression analyses the effect estimate is the genotype relative risk.

[c]DF, degrees of freedom; this test generates a *P* value but not effect estimate.

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TABLE V.

Genotype Distributions and Allele Frequencies in Utah Controls and Oral Clefts Triads

Marker	**Controls (N = 551)**	**Oral clefts children (N = 451)**	**Oral clefts mothers (N = 483)**	**Oral clefts fathers (N = 440)**
Genotypes	**N (freq.)**	**N (freq.)**	**N (freq.)**	**N (freq.)**
Alleles	**Freq.**	**Freq.**	**Freq.**	**Freq.**
SLC46A1 rs2239907				
GG	149 (0.32)	120 (0.33)	146 (0.37)	115 (0.30)
GA	222 (0.48)	196 (0.53)	158 (0.40)	191 (0.50)
AA	96 (0.21)	52 (0.14)	90 (0.23)	78 (0.20)
G	0.56	0.59	0.57	0.55
A	0.44	0.41	0.43	0.45
SLC46A1 rs739439				
CC	365 (0.70)	296 (0.72)	278 (0.71)	291 (0.73)
CT	142 (0.27)	109 (0.26)	98 (0.25)	101 (0.25)
TT	11 (0.02)	7 (0.02)	16 (0.04)	9 (0.02)
C	0.84	0.85	0.83	0.85
T	0.16	0.15	0.17	0.15
rs9894260				
CC	413 (0.84)	327 (0.83)	321 (0.84)	336 (0.83)
CT	74 (0.15)	67 (0.17)	53 (0.14)	70 (0.17)
TT	5 (0.01)	2 (0.01)	6 (0.02)	0 (0)
C	0.91	0.91	0.91	0.91
T	0.09	0.09	0.09	0.09

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TABLE VI.

Genotype Distributions and Allele Frequencies in Filipino Multiplex Families with Oral Clefts[a]

Marker	Unaffected individuals (N = 1,241)	Affected individuals (N = 597)
Genotypes	**N (freq.)**	**N (freq.)**
Alleles	**Freq.**	**Freq.**
SLC46A1 rs2239907		
AA	175 (0.15)	73 (0.13)
AG	553 (0.48)	264 (0.47)
GG	431 (0.37)	221 (0.40)
A	0.39	0.37
G	0.61	0.63
SLC46A1 rs739439		
CC	1107 (0.92)	524 (0.91)
CT	90 (0.08)	50 (0.09)
TT	4 (0)	1 (0)
C	0.96	0.95
T	0.04	0.05
rs9894260		
AA	136 (0.12)	59 (0.10)
AG	526 (0.45)	257 (0.45)
GG	510 (0.43)	257 (0.45)
A	0.34	0.33
G	0.66	0.67

[a]Genotype distributions and allele frequencies reported for individuals who were genotyped.