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Molecular Screening of *VAX1* Gene Polymorphisms Uncovered the Genetic Heterogeneity of Nonsyndromic Orofacial Cleft Among Saudi Arabian Patients

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Objective: Nonsyndromic orofacial cleft (NSOFC) including cleft lip with or without cleft palate (CL±P) and cleft palate (CP) are multifactorial developmental disorders with both genetic and environmental etiological factors. In this study we investigated the association between CL±P and CP, and two polymorphisms previously determined using genome-wide association studies, as well as the association between consanguinity and CL±P and CP.
Methods: DNA was extracted from saliva specimens from 171 triads consisting of affected individuals and their parents, as well as 189 control triads (matched for age, gender, and location) that were recruited from 11 referral hospitals in Saudi Arabia. Two polymorphisms, rs4752028 and rs7078160, located in the *VAX1* gene were genotyped using real-time polymerase chain reaction. A transmission disequilibrium test was carried out using the Family-Based Association Test and PLINK (genetic tool-set) to measure the parent-of-origin effect.
Results: Significant differences were found between affected individuals and the control group. In the case of the rs4752028 risk allele in cleft, the phenotypes were: CL±P (fathers: odds ratio [OR] 2.16 [95% CI 1.38–3.4]; mothers: OR 2.39 [95% CI 1.53–3.71]; and infants: OR 2.77 [95% CI 1.77–4.34]) and CP (fathers: OR 2.24 [95% CI 1.15–4.36] and infants: OR 2.43 [95% CI 1.25–4.7]). For CL±P and the rs7078160 risk allele, the phenotypes were: (fathers: OR 1.7 [95% CI 1.05–2.86]; mothers: OR 2.43 [95% CI 1.49–3.97]; and infants: OR 2.34 [95% CI 1.44–3.81]). In terms of consanguinity, we found significant association between consanguinity and the rs4752028 polymorphism minor allele among CL±P compared with controls ($p = 0.001$).
Conclusion: This is the first study to find a relationship between these two loci on 10q25 (rs4752028 and rs7078160) and NSOFC in a population with high levels of consanguinity.

Keywords: cleft lip, cleft palate, VAX1, consanguinity, etiology

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Introduction

THE ETIOLOGY OF NONSYNDROMIC orofacial cleft (NSOFC) includes cleft lip with or without cleft palate (CL±P) and isolated cleft palate (CP), which is complex. A combination of risk factors contributes to its etiology: genetics, environmental, and gene–environmental interaction (Mossey *et al.*, 2009). Genome-wide association studies (GWAS) identified 10q25 as a risk locus for CL±P (Beaty *et al.*, 2010; Mangold *et al.*, 2010). *Ventral anterior homeobox 1 (VAX1)*, a gene that codes for a protein that plays a role in the regulation of the body's developmental and morphogenesis processes, was reported to be associated with infants (product of consanguinity) affected by multiple craniofacial defects (Slavotinek *et al.*, 2012). In Saudi Arabia, a meta-analysis conducted in 2014 revealed that consanguinity is a risk factor for NSOFC (Sabbagh *et al.*, 2014). Therefore, two single nucleotide polymorphisms (SNPs) (rs4752028 and rs7078160) were considered plausible candidates for investigation of NSOFC in a community with a high prevalence of parental consanguinity in Saudi Arabia (el-Hazmi *et al.*, 1995).

The aim of this case triad–control triad study was to investigate the association between infant–parental rs4752028 and rs7078160 SNPs and both CL±P and CP in a Saudi population. We also investigated their relationships with the risk of NSOFC phenotypes in the presence of parental consanguinity.

Materials and Methods

Recruitment of clinical subjects

This article is part of a series of studies on the prevalence of NSOFC (Abdulhameed *et al.*, 2014) and the etiology of CL±P and CP in Saudi Arabia. Participants were recruited from three main cities: Riyadh (the capital city), Jeddah (the second largest city in Saudi Arabia), and Medina (one of the main cities in Saudi Arabia). Cases were recruited from neonatal units, the plastic surgery departments, and/or orthodontic clinics; all cases were examined in the Department of Genetic Medicine. Criteria for subject selection included: 18 months or younger infants recruited from participating hospitals between January 1, 2010, and December 31, 2011. The total case–control study sample included 171 case triads and 189 control triads. The age, gender, and recruiting hospitals were matched in both the cases and control groups. The controls were healthy noncleft infants who were selected at random from the neonatal or vaccination units. The control group, including parents and infants, were not affected by clefting of the lip and/or palate.

Triads with missing information or those who failed to give saliva samples were excluded from the analysis. In addition, infants with syndromes in case or control groups were excluded from the study.

A questionnaire using yes/no questions was distributed to participants to collect personal information and consanguinity information; the details are shown in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/gtmb). Interviews were conducted with parents to understand the type of consanguinity.

Clinical sampling

Saliva samples were collected from infants and parents in both groups (case and control). Oragene kits were used for both samples; for the parents, we used OG-500; however, for the infants, we used OG-575. A consent form for both groups was signed by one of the parents.

Genetic analysis

DNA was extracted by using QIAamp DNA Mini Kit (Catalogue #51306). Quality and quantity measurements were evaluated using Qubit® 2.0 Fluorometer. Amplification of the two polymorphism, rs4752028 and rs7078160, was performed using 7500 FAST Real-Time PCR (Applied Biosystem International, Inc.) by TaqMan® Genotyping assay and TaqMan Genotyping master mix (Applied Biosystem International, Inc). Samples were analyzed by TaqMan® Genotyper Software (Applied Biosystem International, Inc.) for scatter plot analysis. Supplementary Table S2 shows the characteristics of the two polymorphisms.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) tests were carried out using an online program (www.dr-petrek.eu/documents/HWE.xls) to look for indications of inbreeding, population stratification, and problems in genotyping. This was carried out using chi-squared goodness-of-fit test with p-values of 0.05 to compare differences between the observed and expected values of the included homozygous and heterozygous genotype frequencies (Wigginton *et al.*, 2005). A transmission disequilibrium test (TDT) was carried out using the Family-Based Association Test (FBAT) and PLINK (genetic tool-set) (Purcell *et al.*, 2007), which were also used to measure the parent-of-origin effect. Polymorphism frequencies among CL±P and CP cases compared with controls were analyzed using chi-square test.

In addition, to detect which of the three types of polymorphisms provided the significant relationship, and to acknowledge the burden on type-1 error rate, the threshold for declaring statistical significance based on Bonferroni correction was determined to be $p = 0.00056$ using SPSS version 16.0 (SPSS, Inc., Chicago, IL).

The degree of association between allele frequency with CL±P and CP and also with parental consanguinity, compared with controls, was estimated by measuring the odds ratio (OR) and 95% confidence intervals (95% CI) using an online program (www.quantpsy.org/chisq/chisq.htm). The OR and 95% CI were also used to measure the degree of association between rs4752028 and rs7078160 SNPs variants and parental consanguinity among oral cleft infants compared with controls. In addition, multinomial logistic regression was carried out to measure the interaction between consanguinity and genotype variant among NSOFC compared with control.

Results

Of the 171 NSOFC case–parental triads, 10 cases could not be grouped to a cleft phenotype because of missing information, resulting in 161 NSOFC (127 CL±P and 34 CP) cases for our analysis. In addition, 16 fathers of the 189 control parental triads did not provide a saliva sample. Of the total parental consanguinity in these triads, first cousin marriages

Table 1. Transmission Disequilibrium Test Results for rs4752028 and rs7078160 Variants Among Nonsyndromic Orofacial Cleft Infant–Parental Triads and Its Phenotypes (CL±P and CP) Using Family-Based Association Test Analysis

Type of NSOFC	*Allele*	*afreq*	*fam#*	p	*OR (95% CI)*
rs4752028					
CL±P	C	0.233	53	0.651	1.1 (0.71–1.71)
CP	C	0.221	14	1.00	1 (0.4–2.3)
rs7078160					
CL±P	A	0.128	45	0.327	0.76 (0.44–1.32)

The TDT for rs4752028 and rs7078160 using FBAT and PLINK test.
afreq, estimating allele frequencies; CL±P, cleft lip with or without cleft palate; CI, confidence interval; CP, cleft palate; NSOFC, nonsyndromic orofacial cleft; OR, odds ratio; TDT, transmission disequilibrium test.

accounted for 55/86 (64%) of the NSOFC and 60/92 (65.2%) of the controls.

The case and control parental homozygous and heterozygous polymorphism frequencies in rs4752028 were aligned to HWE except for paternal control (0.039). However, there were significant differences between the observed and expected values for both parental cases and controls at rs7078160 locus with $p < 0.05$ (Supplementary Table S3).

The TDT results for rs4752028 and rs7078160 using FBAT and PLINK test are shown in Tables 1 and 2, respectively. No statistically significant over-transmission of the minor allele (C in rs4752028 and A in rs7078160) was found in CL±P or CP families. In addition, PLINK test found no parent-of-origin relationship (Supplementary Table S4). For CP, the number of heterozygous alleles was insufficient to produce a *p*-value in FBAT analysis, and the data were not included in Table 1.

Comparison between case and control rs4752028 and rs7078160 genotypes and alleles

Table 3 shows the distribution of rs4752028 and rs7078160 genotypes in case and control infant–parental triads. There were statistically significant differences between cases and controls in rs4752028 and rs7078160 genotypes in infant–parental triads for CL±P and CP cases.

After chi-square adjustment using Bonferroni correction in infant–parent triads for rs4752028 SNP, in fathers, the homozygous TT common allele genotype was detected significantly more often in controls compared with cases for CL±P and CP ($p < 0.05$). Furthermore, the heterozygous CT genotype was significantly more prevalent in cases compared with controls for the different cleft phenotypes ($p < 0.05$). For mothers and infants, the homozygous CC minor allele genotype was significantly associated with CL±P cases compared with controls; the homozygous TT common allele genotype was detected significantly more often in controls compared with CL±P; and the heterozygous CT genotype was present significantly more often in CL±P and CP except in mothers of CP infants.

For rs7078160 SNP frequencies after chi-square adjustment using Bonferroni correction, the homozygous AA minor allele genotype was significantly more frequent in CL±P infants compared with controls ($p < 0.0056$). The heterozygous AG genotype was significantly more frequent in control infants compared with CL±P infants ($p < 0.0056$).

The frequency of the rs4752028 and rs7078160 minor alleles in case and control infant–parental triads CL±P and CP was compared. Significant differences between cases versus controls for rs4752028 risk allele in cleft phenotypes were: CL±P (fathers: OR 2.16 [95% CI 1.38–3.4]; mothers: OR 2.39 [95% CI 1.53–3.71]; and infants: OR 2.77 [95% CI 1.77–4.34]) and CP (fathers: OR 2.24 [95% CI 1.15–4.36] and infants: OR 2.43 [95% CI 1.25–4.7]). For CL±P and rs7078160, these were as follows: fathers: OR 1.7 (95% CI 1.05–2.86); mothers: OR 2.43 (95% CI 1.49–3.97); and infants: OR 2.34 (95% CI 1.44–3.81). Consanguinity was significantly related to the rs4752028 polymorphism minor allele among CL±P compared with controls ($p = 0.001$, OR 2.97 [95% CI 1.54–5.76]) (Supplementary Table S5).

For rs7078160 SNP, there were statistically significant differences between CL±P cases and controls; fathers had significantly greater frequency of the minor A allele in CL±P cases compared with controls ($p < 0.05$). However, this relationship was not statistically significant for CP.

Table 2. Testing rs4752028 and rs7078160 for Transmission Disequilibrium Using PLINK Analysis for Nonsyndromic Orofacial Cleft Infant–Parental Triads and Cleft Phenotypes (CL±P and CP)

NSOFC	*Transmitted/untransmitted minor alleles*	p	*OR*	*A:U_PAR*	p	*Combined statistics* p
rs4752028						
CL±P	41/37	0.651	1.11	01:01	1	0.655
CP	11/11	1	1	00:00	NA	1
rs7078160						
CL±P	22/29	0.327	0.759	02:01	0.564	0.414
CP	2/5	0.257	0.4	00:00	NA	0.257

The TDT for rs4752028 and rs7078160 using FBAT and PLINK test.
A:U_PAR, parental discordance counts by counting the number of alleles in affected versus unaffected parents.

Table 3. Distribution of rs4752028 and rs7078160 Infant–Parental Triad Genotypes According to Nonsyndromic Orofacial Cleft Phenotypes (CL±P and CP) and Compared with Controls

Genotype	*CL±P*	*CP*	*Control*
rs4752028[a]			
Paternal genotype, frequency (%)			
Total	122	33	168
TT[b]	73 (59.8)	18 (54.5)	134 (79.8)
CT	44 (36.1)	15 (45.5)	29 (17.3)
CC	5 (4.1)	0	5 (2.9)
p	0.001*	0.001*	
Maternal genotype, frequency (%)			
Total	126	34	187
TT[b]	80 (63.5)	23 (67.6)	151 (80.7)
CT	36 (28.6)	9 (26.5)	32 (17.1)
CC	10 (7.9)	2 (5.9)	4 (2.1)
p	0.001*	0.180	
Infant genotype, frequency (%)			
Total	120	35	188
TT[b]	72 (60)	21 (60)	153 (81.4)
CT	39 (32.5)	13 (37.1)	32 (17)
CC	9 (7.5)	1 (2.9)	3 (1.6)
p	<0.001*	0.020*	
rs7078160[c]			
Paternal genotype, frequency (%)			
Total	119	34	167
GG[b]	86 (72.3)	26 (76.5)	141 (84.4)
AG	28 (23.5)	5 (14.7)	19 (11.4)
AA	5 (4.2)	3 (8.8)	7 (4.2)
p	0.150	0.320	
Maternal genotype, frequency (%)			
GG[b]	90 (71.4)	29 (85.3)	164 (86.8)
AG	27 (21.4)	3 (8.8)	19 (10.1)
AA	9 (7.1)	2 (5.9)	6 (3.2)
Total	126	34	189
p	0.004*	0.730	
Infant genotype, frequency (%)			
Total	122	35	186
GG[b]	90 (73.8)	32 (91.4)	157 (84.4)
AG	20 (16.4)	3 (8.6)	26 (14)
AA	12 (9.8)	0	3 (1.6)
p	0.003*	0.490	

The table shows the distribution of rs4752028 and rs7078160 genotypes in case and control infant–parental triads. There were statistically significant differences between cases and controls in rs4752028 and rs7078160 genotypes in infant–parental triads for CL±P and CP cases.

[a]Eleven (six cases and five controls) paternal samples, five (three cases and two controls) maternal samples, and seven (six cases and one control) infant samples did not produce genotyping values for rs4752028. The phenotype diagnoses for 10 NSOFC cases are missing.

[b]The homozygous common allele genotype.

[c]Fourteen (eight cases and six controls) paternal samples, one maternal sample, and seven (four cases and three controls) infant samples did not produce genotyping values for rs7078160. The phenotype diagnoses for 10 NSOFC cases are missing.

*The *p*-value is significant at the 0.05 level.

Paternal consanguinity and infant rs4752028 and rs7078160 genotype variants as risk factors for CL±P and CP

CL±P and CP cases and controls were distributed according to parental consanguinity and then were compared according to rs4752028 and rs7078160 infant–parental triad genotype variance. There were no statistically significant differences found in either analysis ($p > 0.05$) (Supplementary Tables S6 and S7). In addition, multinomial logistic regression with NSOFC as an outcome variable, consanguinity as main effect, and phenotype as main effect with interaction term of the last two variables was carried out. It indicated significant main effect of genotype ($p = 0.0001$ for rs4752028 and $p = 0.05$ for rs7078160) with no significant effect of consanguinity or interaction between them ($p = 0.5$ for rs4752028 and $p = 0.2$ for rs7078160).

Table 4. Distribution of Infant rs4752028 Alleles in Cases and Controls with Consanguineous Parents

Allele type	*CL±P*	*CP*	*Control*
rs4752028 ($\chi^2 = 28.28$, df = 2, $p < 0.0001$*)			
Total	130	43	182
T[a]	101 (77.7)	34 (81.4)	166 (91.2)
C	29 (22.3)	8 (18.6)	16 (8.8)
p	0.001*	0.059	
OR (95% CI)	2.97 (1.54–5.76)	2.44 (0.97–6.16)	
rs7078160 ($\chi^2 = 6.11$, df = 2, $p = 0.047$*)			
Total	134	42	182
G[a]	112 (86.6)	41 (97.7)	168 (92.3)
A	18 (13.4)	1 (2.3)	14 (7.7)
p	0.081	0.290	
OR (95% CI)	1.93 (0.92–4.04)	0.29 (0.04–2.29)	

[a]Common allele.

*The *p*-value is significant at the 0.05 level.

Finally, when infants rs4752028 and rs7078160 minor allele frequencies in CL±P and CP cases were compared with controls, there were more CL±P and CP cases with consanguineous parents and the minor C allele at rs4752028, but this was statistically significant for CL±P only ($p = 0.001$, OR 2.97 [95% CI 1.54–5.76]). However, for rs7078160, although the minor A allele prevalence was higher in CL±P (13.4%) compared with controls (7.7%), the difference was not statistically significant ($p = 0.081$, OR 1.93 [95% CI 0.92–4.04]) (Table 4).

Discussion

Our study showed statistically significant differences in the genotype variance and allele frequencies between CL±P and CP cases compared with control infant–parental triads. However, the FBAT and PLINK analyses did not show significant over-transmission of the rs4752028 and rs7078160 SNP alleles and parent-of-origin effect in NSOFC cases.

In this study, we selected cases and controls from the same hospitals; however, it was not possible to match ethnicity. Saudis, especially in the Western Region, have been of mixed ethnicity for hundreds of years. People from all over the world, of different ethnic origins, have traveled to Mecca and Medina on pilgrimage, then settled and mixed races through marriage. Additionally, Saudi Arabia has a unique geographic location between the three continents, Asia, Africa, and Europe; as a result of this, it can be difficult to group people according to their ethnicity in Saudi Arabia, although they are generally considered Caucasian (Risch *et al.*, 2002).

Moreover, Lewonin (2006) reported that every population has a separate geographic race and that they differ genetically to some degree from every other population. This emphasizes the need to carry out genetic research for each population.

Our sample met the HWE in rs4752028 SNP, suggesting that our sample resembled expected population genotype frequencies of Pritchard and Korf (2008) study. However, both cases and controls at rs7078160 had significant differences between the observed and expected values of the included homozygous and heterozygous genotype frequencies suggesting the absence of random mating. This could be explained by the high prevalence of paternal consanguinity in the target population (el-Hazmi *et al.*, 1995).

The infant case–control results do not represent independent replication of the results from parental groups. Moreover, due to consanguineous mating, the maternal and paternal case–controls results are not fully independent of each other. Therefore, this study examined and explained two methods of transmission of the alleles in question, the TDT and case–control analysis; the TDT remains robust for linkage in the presence of consanguineous populations. Autozygosity mapping might have been another consideration, which assumes the identical-by-descent cotransmission of mutations (Oliveira *et al.*, 2017), and this (also called consanguinity mapping) has not been applied to NSOFC, in part because parental consanguinity is uncommon in places where research efforts have historically been carried out. This would assume no genetic heterogeneity and tight linkage of a disease gene with DNA markers.

An association between 10q25 locus and CL±P was supported by Leslie *et al.* (2017) who reported in their genome-wide meta-analyses of NSOFCs that SNPs on 10q25 approached genome-wide significance in NSOFC and CL±P groups among Asians.

Our rs4752028 and rs7078160 SNPs association finding was further supported by the results of Butali *et al.* (2013) (15) in their replication of GWAS signals on 651 case–parental triads (Asian [494 infant–parent triads] and European [157 infant–parent triads] populations). FBAT analysis revealed a statistically significant strong association in the transmission of the rs7078160 SNP among the Asian population ($p < 0.001$) but found no significant association in the European population, similar to our findings. However, their comparison of cases with controls in the Asian population showed an increased frequency of the common G allele compared with controls, which differed from our findings. Such differences could indicate ethnic and geographic variation between the Saudi population, who are Caucasians, and the Asian population in the genetic etiology of CL±P.

Although the CP sample was small, it was still interesting to study a possible link with the included variants that could give preliminary information for planning future research. The rs4752028 was the only SNP examined that showed association with CP compared with controls ($p = 0.015$ for father, $p = 0.049$ for mothers, and $p = 0.009$ for infants). However, as the sample of CP in this study is considered small (34), this finding could only suggest a trend of association. Furthermore, Butali *et al.* (2013) reported no significant association between rs4752028 and CP. Also, Duan *et al.* (2017) reported parent-of-origin effect and no association between rs7078160 and rs4752028 SNPs and CP. However, their finding was concluded from TDT (FBAT) analysis and not from a case–control design. As *10q25* is a recently discovered locus in terms of risk for CL±P and CP, studies that clarify the relationship between NSOFC and rs7078160 and rs4752028 polymorphisms are still required.

A systematic review of parental consanguinity and NSOFC revealed a significant association (Sabbagh *et al.*, 2014). At the same time, *VAX1* mutation was previously reported to be associated with birth defects in a sample with consanguineous parental marriages (Slavotinek *et al.*, 2012). The relationship between rs7078160 and rs4752028 and consanguinity in case infants compared with control infants was analyzed. For both SNPs, the minor allele was found more often in CL±P cases with consanguineous parents compared with controls (Table 4). However, it was only statistically significant for rs4752028 ($p = 0.001$, OR 2.97 [95% CI 1.54–5.76]).

Conclusion

This is the first study to describe the relationship between two SNPS, rs4752028 and rs7078160, and NSOFC in a population with a high rate of consanguinity. There is an apparent association between rs4752028 and rs7078160 SNPs and both CL±P and CP in the Saudi population, but larger samples are needed for confirmation and definitive evidence. In addition, further investigation in the Saudi population, as well as other populations, is required to ensure consistency and confirm the limits of the association study. Furthermore, it is not possible to expand this study to include other variants in or near the 10q25 loci or other genes, but due to resources limitations, it will be postponed. Therefore, future genome-wide study, gene–gene interaction, and gene–environmental interaction/epigenetics research are recommended to further clarify the etiology of CL±P and CP. Confirmation of a positive association between consanguinity, NSOFC, and genetics will have a great implication for parental counseling and public health.

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References

Abdulhameed FD, Sabbagh HJ, Hummaida TI, *et al.* (2014) Epidemiology of non-syndromic orofacial cleft (NSOFC) in Medina, Saudi Arabia. Exp Clin Cardiol 20:505–516.

Beaty TH, Murray JC, Marazita ML, *et al.* (2010) A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nat Genet 42:525–529.

Butali A, Suzuki S, Cooper ME, *et al.* (2013) Replication of genome wide association identified candidate genes confirm the role of common and rare variants in PAX7 and VAX1 in the etiology of nonsyndromic CL(P). Am J Med Genet A 161A:965–972.

Duan SJ, Huang N, Zhang BH, *et al.* (2017) New insights from GWAS for the cleft palate among han Chinese population. Med Oral Patol Oral Cir Bucal 22:e219–e227.

el-Hazmi MA, al-Swailem AR, Warsy AS, *et al.* (1995) Consanguinity among the Saudi Arabian population. J Med Genet 32:623–626.

Leslie EJ, Carlson JC, Shaffer JR, *et al.* (2017) Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. Hum Genet 136:275–286.

Lewonin RC (2006) Confusion about human races: race and genomics, social sciences research council. Available at http://raceandgenomics.ssrc.org/Lewontin, accessed May 23, 2015.

Mangold E, Ludwig KU, Birnbaum S, *et al.* (2010) Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nat Genet 42:24–26.

Mossey PA, Little J, Munger RG, *et al.* (2009) Cleft lip and palate. Lancet 374:1773–1785.

Oliveira J, Pereira R, Santos R, *et al.* (2017) Homozygosity mapping using whole-exome sequencing: a valuable approach for pathogenic variant identification in genetic diseases. The 10th International Joint Conference on Biomedical Engineering Systems and Technologies. Porto, Portugal, Bioinformatics. 3:210–216.

Pritchard DJ, Korf BR (2008) Medical Genetics at a Glance. Malden, MA; Oxford: Blackwell Publishing.

Purcell S, Neale B, Todd-Brown K, *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575.

Risch N, Burchard E, Ziv E, *et al.* (2002) Categorization of humans in biomedical research: genes, race and disease. Genome Biol 3:comment2007.

Sabbagh HJ, Hassan MH, Innes NP, *et al.* (2014) Parental consanguinity and nonsyndromic orofacial clefts in children: a systematic review and meta-analyses. Cleft Palate Craniofac J 51:501–513.

Slavotinek AM, Chao R, Vacik T, *et al.* (2012) VAX1 mutation associated with microphthalmia, corpus callosum agenesis, and orofacial clefting: the first description of a VAX1 phenotype in humans. Hum Mutat 33:364–368.

Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 76: 887–893.

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