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BRCA1 and *BRCA2* Gene Variants and Nonsyndromic Cleft Lip/Palate

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Abstract

Background—Nonsyndromic cleft lip with or without cleft palate is a debilitating condition that not only affects the individual, but the entire family. The purpose of this study was to investigate the association of *BRCA1* and *BRCA2* genes with NSCL/P.

Methods—Twelve polymorphisms in/nearby *BRCA1* and *BRCA2* were genotyped using Taqman chemistry. Our data set consisted of 3,473 individuals including 2,191 nonHispanic white (NHW) individuals (from 151 multiplex and 348 simplex families) and 1,282 Hispanic individuals (from 92 multiplex and 216 simplex families). Data analysis was performed using Family-Based Association Test (FBAT), stratified by ethnicity and family history of NSCL/P.

Results—Nominal associations were found for *BRCA1* in Hispanics and for *BRCA2* in NHW and Hispanics ($P<0.05$). Significant haplotype associations were found for both *BRCA1* and *BRCA2* ($P\leq0.004$).

Conclusions—Our results suggest a modest association between *BRCA1* and *BRCA2* and NSCL/P. Further studies in additional populations and functional studies are needed to elucidate the role of these genes in developmental processes and signaling pathways contributing to NSCL/P.

Keywords

association; family-based; *BRCA1*; *BRCA2*; cleft lip/palate

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Introduction

Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common birth defect with genetic and environmental factors playing etiologic roles. While numerous gene variants have been suggested to confer an increased risk of NSCL/P, it is estimated that only ~20% of the underlying etiology of NSCL/P has been identified, leaving additional causal genes to be identified (Dixon et al. 2011). This is supported by the fact that most of the NSCL/P cases reflect isolated forms, and when familial, the inheritance models are not always clear. Additionally, genetic factors of modest effects may have different contributions depending on the presence of specific environmental factors, thereby complicating the discovery of true etiologic variants. Moreover, in genome-wide association studies of NSCL/P, variants in *IRF6*, and in the chromosome regions *8q24* and *10q25 (VAX1*), were suggested as strongly associated with NSCL/P, however the association signals were seemed to be more population-specific (Dixon et al. 2011).

Studies have demonstrated that the consequences of being born with NSCL/P extend beyond functional and esthetic complications. Individuals with NSCL/P were reported to have shorter lifespan and increased risk for all major causes of death, particularly cancer, when compared with individuals born without clefts (Christensen et al. 2004; Steinwachs et al. 2000; Vieira 2008). Studies in different populations also suggest that families with recurrent NSCL/P have increased susceptibility to cancer (Bille et al. 2005; Menezes et al. 2009; Zhu et al. 2002). A large epidemiological study in Denmark showed an increased occurrence of breast and brain cancer among adult females born with NSCL/P, and of primary lung cancer among adult males with NSCL/P (Bille et al. 2005). While additional studies did not confirm a general increase in the risk of breast cancer among female subjects in NSCL/P families (Dietz et al. 2012; Martelli et al. 2014), a higher frequency in family history of cancer (colon, brain, leukemia, breast, prostate, skin, lung, and liver) was found in NSCL/P families when compared to control families (Menezes et al. 2009). Interestingly, dysregulation of genes in networks associated with DNA double-strand break repair and cell cycle control, including *BRCA1* and *BRCA2*, was observed in dental pulp stem cells of NSCL/P patients, meanwhile the expression of *BRCA1* and *BRCA2* genes was significantly downregulated in NSCL/P dental pulp cells compared to controls (Kobayashi et al. 2013). These observations led us to investigate whether variations in *BRCA1* and/or *BRCA2* genes were associated with NSCL/P, since the association between NSCL/P and cancer raises interesting possibilities to identify risk markers for cancer. In this study, we tested the association of *BRCA1* and *BRCA2* genes with nonsyndromic NSCL/P.

Study population

This study was approved by the University of Texas Health Science Center Committee for Protection of Human Subjects. Our study population consisted of a total of 3,473 individuals including 2,191 nonHispanic white (NHW) individuals (from 151 multiplex and 348 simplex families) and 1,282 Hispanic individuals (from 92 multiplex and 216 simplex families). Families were recruited at the University of Texas Health Science Center Craniofacial Clinics. Families were ascertained through probands, and additional relatives

were recruited. Multiplex families are families with 2 or more individuals affected with NSCL/P. Simplex families (trios) are families in which the only the proband is affected with NSCL/P and parents are unaffected. Individuals presenting with syndromic clefts, cleft palate only, or unknown cleft types were excluded. Complete demographic and medical history, and DNA samples from peripheral blood were available for all individuals in the study.

Selection of single nucleotide polymorphisms and genotyping

Twelve single-nucleotide polymorphisms (SNPs) in/nearby *BRCA1* and *BRCA2* genes (Table 1) were selected for genotyping using a tag-SNP approach, considering heterozygosity values, gene structure, and the linkage disequilibrium (LD) block surrounding each gene, as previously described (Carlson et al. 2004) (Supplementary Figure 1). This approach allows for selection of a minimum number of SNPs ensuring maximum coverage within a LD block. Genotyping was performed using Taqman chemistry (Ranade et al. 2001) in a ViiA7 RealTime PCR System (Applied Biosystems, Foster City, CA).

Statistical analyses

Family-based single SNP association tests were performed using Family-Based Association Test (FBAT), with the '–e' option for extended families. Analyses were performed for all families stratified by ethnicity (NHW or Hispanic) and family history of NSCL/P (multiplex and simplex). Pairwise haplotype analyses were performed using the 'hbat' function in FBAT. Bonferroni correction was used to adjust for multiple testing and P-values ≤0.004, considering the number of SNPs tested (0.05/12), were considered statistically significant.

In Silico Prediction of SNP Function

In silico analysis of SNP function was performed using miR*dSNP* v.11.03 (Bruno et al. 2012) for *BRCA1* rs8176318, and using PATCH v.1.0 (Wingender et al. 2000), and PROMO v.3.0 (Messeguer et al. 2002) for *BRCA2* rs206115.

Results

No SNPs met the Bonferroni correction, however, nominal associations (p≤0.05) were found between NSCL/P and individual SNPs in *BRCA1* and *BRCA2*. While SNPs in *BRCA2* showed nominal associations in NHW and Hispanic datasets, SNPs in *BRCA1* were associated with NSCL/P in Hispanics only. *BRCA1* SNPs rs16941 and rs8176318, a missense variant and a 3' UTR variant, respectively, showed modest association in Hispanics (p=0.02 and p=0.04, respectively) (Table 2). For *BRCA2*, the promoter variant rs206115 was also modestly associated with NSCL/P in both NHW and Hispanic multiplex families (p=0.01). Additional *BRCA2* SNPs (rs144848 and rs9534342) showed borderline associations with NSCL/P (p ≤ 0.05) (Table 2).

Haplotype analyses showed a few significant associations in 2-, 3- and 4-SNP haplotype windows for both *BRCA1* and *BRCA2*, and suggest that the combination of specific alleles

in an additive model may contribute to NSCL/P in those families ($p \leq 0.004$) (Supplementary Table 1).

Of note, although the individual SNP associations did not reach the strict Bonferroni criteria significance threshold, the nominal associations and the specific haplotype associations found for *BRCA1* and *BRCA2* variants in our NSCL/P families warrant discussions of the potential biological implications of these variants in NSCL/P phenotypes. For example, *BRCA1* rs8176318 and *BRCA2* rs206115 are located in gene regulatory regions and harbor allele-specific microRNA or transcription factor binding sites, respectively, that may have an impact on gene expression (data not shown). Moreover, *BRCA1* rs8176318 is predicted to bind to hsa-miR-205, known to be involved in epithelial to mesenchymal transformation (EMT) and tumor invasion. During palatogenesis, EMT is an important event contributing to the disappearance of the medial edge epithelia (MEE) and continuous mesenchymal confluence required for palatal fusion (Warner et al. 2015). Interestingly, the expression of miR-205 was found to be significantly downregulated (6-fold) in isolated MEE from wild type murine fetuses on gestational day 13.5 to 14.5 (prior to and during epithelial fusion of the palatal processes, respectively) (Warner et al. 2015). While these are intriguing findings, additional studies elucidating the biological roles of *BRCA1* and *BRCA2* during embryonic development might provide valuable insights into the underlying etiology of birth defects including NSCL/P.

NSCL/P is a complex disorder in which genes and environmental factors may have individual and interactive roles (Dixon et al. 2011). Disruptions of early developmental processes such as cell migration, proliferation, transdifferentiation and apoptosis due to variations in developmental genes have been shown to be closely related to the occurrence of NSCL/P (Greene and Pisano 2010) and cancer as well (Vieira 2008). Furthermore, variations in genes with critical roles in DNA damage repair, including *BRCA1*, have also been assessed and implicated in increased susceptibility to NSCLP and reinforced the hypothesis of etiological overlap between this common birth defect and cancer (Kobayashi et al. 2013; Mostowska et al. 2014). It is intriguing, however, that the available GWAS on NSCL/P (Birnbaum et al. 2009; Grant et al. 2009; Mangold et al. 2010) have not identified variants in *BRCA1* or *BRCA2* meanwhile numerous association studies in independent populations have reported the association of cancer-related genes with NSCL/P (Han et al. 2014; Letra et al. 2012; Letra et al. 2009; Menezes et al. 2009; Suzuki et al. 2009; Vieira 2008). Discrepancies among the results of previous studies may reflect genetic/allelic heterogeneity as well as differences in study design (family-based or case-control), sample sizes, and selection of SNPs to be studied (or specific genotyping arrays used in GWAS). A major GWAS finding is that the majority of risk variants for complex traits map to noncoding regions. For NSCLP, GWAS have identified around 40 risk loci, the majority of which are located in noncoding regions (Beaty et al. 2010; Birnbaum et al. 2009; Grant et al. 2009; Ludwig et al. 2012; Mangold et al. 2010; Yu et al. 2017), and for which the functional effects of on NSCLP phenotypes remain to be elucidated.

Recently, studies using whole exome sequencing of NSCLP families have also identified rare mutations in developmental and cancer-related genes as likely contributing to the cleft phenotypes (Fu et al. 2017; Liu et al. 2015). A frameshift variant caused by a 2bp insertion

mutation (c.819_820dupCC; p.Gln274fs) in *TP63*, was found in a father-proband duo with NSCLP. Further, mRNA studies demonstrated that the mutant allele underwent nonsense-mediated-mRNA decay (NMD) as only the wild-type allele observed in mRNA obtained from the patient's lymphocytes and lymphoblasts (Basha et al. 2018). *TP63* encodes a transcription factor and a master regulator of epithelial lineage commitment during embryonic development, and was previously implicated in syndromic and NSCLP (Leslie et al. 2017; Scapoli et al. 2008). Interestingly, exome sequencing has also identified variations in *TP63* in patients and tumor tissues of oral squamous cell carcinoma (Al-Hebshi et al. 2016; Stransky et al. 2011). Additional large-scale next-generation sequencing studies have the ability to reveal fundamental mechanisms underlying developmental and tumorigenic events and potential overlaps in disease pathways. These studies also have the potential to elucidate the full spectrum of genetic variation contributing to NSCLP. Corroborating previous findings, our study highlights the need to conduct a thorough medical and family history questionnaire, to identify families showing both NSCLP and cancer appearing together, and for which a same genetic variant may be contributing to the phenotype.

In summary, our results suggest a modest role for *BRCA1* and *BRCA2* in NSCL/P susceptibility. While the findings of our study may represent private mutations within private families, they continue to support the notion that genes involved at early stages of embryonic development may also have roles in cancer development later in life. Additional studies in other populations and functional studies are necessary to elucidate the role of *BRCA1* and *BRCA2* genes in the developmental processes and signaling pathways contributing to NSCL/P. Nonetheless it is possible that these genes may act through more than one signaling pathway to elicit numerous cellular responses that are cell- and tissue-dependent to contribute to birth defects and cancer later in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Details of SNPs genotyped in *BRCA1* and *BRCA2* genes.

Gene	dbSNP Id*	Base position*	Location	Alleles§	MAF NHW	MAF Hisp
BRCA1	rs799906	chr. 17:43126099	5' UTR	C/T	0.34	0.35
	rs799917	chr. 17:43092919	Missense	A/G	0.33	0.32
	rs16941	chr. 17:43092418	Missense	A/G	0.32	0.27
	rs8176194	chr. 17:43079204	Intron	G/T	0.36	0.32
	rs8070179	chr. 17:43050671	Intron	A/G	0.33	0.25
	rs8176318	chr. 17:43045257	3' UTR	G/T	0.32	0.19
BRCA2	rs206115	chr. 13:32314336	5' near gene	A/G	0.42	0.29
	rs144848	chr.13:32332592	Missense	G/T	0.29	0.27
	rs206079	chr. 13:32346481	Intron	A/G	0.46	0.44
	rs9943888	chr. 13:32354065	Intron	A/G	0.28	0.27
	rs9534342	chr. 13:32381260	Intron	C/T	0.48	0.46
	rs11571836	chr. 13:32399302	3' UTR	A/G	0.21	0.21

*According to NCBI dbSNP build 147

§Ancestral allele listed first

MAF, minor allele frequency

Table 2

Results of single SNP association analyses.

***BRCA1* SNPs**	**NHW**					**Hispanic**				
	All (n=499)		**Multiplex (n=151)**		**Simplex (n=348)**	**All (n=308)**		**Multiplex (n=92)**		**Simplex (n=216)**
	FBAT	**FBAT-e**	**FBAT**	**FBAT-e**	**FBAT**	**FBAT**	**FBAT-e**	**FBAT**	**FBAT-e**	**FBAT**
rs799906	0.64	0.65	0.64	0.67	0.32	0.56	0.57	0.09	0.10	0.54
rs799917	0.68	0.70	0.64	0.67	0.38	0.99	0.99	0.18	0.17	0.24
rs16941	0.62	0.64	0.77	0.79	0.38	0.12	0.09	**0.04**	**0.02**	0.73
rs8176194	0.62	0.62	0.26	0.27	0.76	0.26	0.24	0.08	0.06	1.00
rs8070179	0.54	0.54	0.82	0.83	0.53	0.35	0.35	0.05	0.06	0.73
rs8176318	0.68	0.69	0.45	0.49	0.26	0.05	**0.04**	0.07	0.08	0.27
***BRCA2* SNPs**	**NHW**					**Hispanic**				
	All (n=499)		**Multiplex (n=151)**		**Simplex (n=348)**	**All (n=308)**		**Multiplex (n=92)**		**Simplex (n=216)**
	FBAT	**FBAT-e**	**FBAT**	**FBAT-e**	**FBAT**	**FBAT**	**FBAT-e**	**FBAT**	**FBAT-e**	**FBAT**
rs206115	0.11	0.13	**0.01**	**0.02**	0.94	0.15	0.15	**0.01**	**0.02**	0.51
rs144848	0.42	0.44	0.21	0.24	0.05	0.89	0.88	0.21	0.24	0.92
rs206079	0.29	0.32	0.10	0.15	0.94	0.42	0.42	0.10	0.15	0.48
rs9943888	0.78	0.78	0.53	0.52	0.38	0.91	0.91	0.53	0.52	0.97
rs9534342	0.54	0.54	0.05	0.06	0.50	0.60	0.60	0.05	0.06	0.85
rs11571836	0.79	0.80	0.31	0.34	0.21	0.55	0.55	0.31	0.36	0.53

FBAT, Family Based Association Test; FBAT –e, extended pedigree option
NHW, nonHispanic white
All = all families combined (multiplex + simplex)

Significant if P≤0.004.