Transmission analysis of candidate genes for nonsyndromic oral clefts in Brazilian parent-child triads with recurrence

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Abstract

Cleft lip and/or palate (CL/P) is a major congenital defect with complex etiology, including multiple genetic and environmental factors. Approximately two thirds of the cases are not accompanied by other anomalies and are called nonsyndromic (NS). In the present study, we performed transmission distortion analysis of the MSX1-CA, TGFβ3-CA and MTHFR-C677T polymorphisms in 60 parent-child triads, in which the NS-CL/P affected child had at least one affected parent. No association with genes MSX1 or TGFB3 was found, but the results were suggestive of an association of the MTHFR-C677T polymorphism with NS-CL/P.

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Nonsyndromic cleft lip and/or palate (NS-CL/P) affects about 1/700 livebirths, with wide variability concerning geographic distribution, ethnic background and socioeconomic status (Murray, 2002). The etiology is complex, including multiple genetic and environmental factors. The MSX1 and TGFB3 genes have been suggested as candidate genes based on animal models (Satokata and Maas, 1994; Kaartinen et al., 1995; Proetzel et al., 1995) and association studies in humans with genes MSX1, TGFB3 and MTHFR have pointed to the involvement of these genes in NS-CL/P etiology (Maestri et al., 1997; Lidral et al., 1998; Romitti et al., 1999; Mills et al., 1999; Wyszynsky and Diehl, 2000; Beatty et al., 2001). The MSX1 gene maps to chromosome 4p16. Animal models indicate that this gene plays a role in craniofacial anomalies, leading to a clefting phenotype. Van den Boogaard et al. (2000) identified a stop codon in the MSX1 gene in a three-generation Dutch family with tooth agenesis and combinations of CP only and CLP, providing further evidence for the involvement of this gene in orofacial clefting. Jezewski et al. (2003) sequenced the whole MSX1 gene in a large sample of NS-CL/P patients from different geographic regions, and found mutations in about 2% of the cases.

The TGFβ3 gene maps to 14q24. Animal model studies demonstrated that TGFβ3 plays a role in CP (Kaartinen et al., 1995; 1997; Proetzel et al., 1995; Sanford et al., 1997), and Lidral et al. (1998) showed an association between TGFβ3 markers and NS-CP.

The MTHFR gene maps to 1p36.1, and plays a key role in the metabolism of folate by reducing methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary methyl donor for methionine synthesis. The C677T nucleotide variant in the MTHFR gene results in a thermally unstable protein with reduced activity, leading to elevated plasma homocysteine levels. Considerable heterogeneity in the prevalence of the C677T polymorphism throughout the world was reported (Pepe et al., 1998).

The present work aimed to investigate if genes TGFβ3, MSX1 and MTHFR-C677T are involved in the etiology of NS-CL/P, by testing 60 patients, all with an affected parent. We utilized a nuclear family-based approach, to determine for each form of clefting whether these genes were in linkage disequilibrium, as measured by transmission distortion.

A total of 60 triads, each one including an affected child and at least one affected parent, were studied. Of these, 24 triads included affected mothers, and 35 triads included affected fathers. In one triad, both parents were affected, but the mother was not informative for all markers, and the triad was grouped with those of affected fathers.
Families were ascertained at the Hospital for Rehabilitation of Craniofacial Anomalies, in Bauru, SP, Brazil. All triads were examined by a clinical geneticist to confirm that they were nonsyndromic. In addition to the information about family history and personal and medical history, parents provided blood samples. Informed consent was obtained from all adult subjects and legal guardians of underage patients. The study was performed in the years 2001 to 2003, and the participation rate of potentially eligible families was 90%.

DNA was extracted from peripheral blood samples using a Puregene DNA Isolation Kit (Genta Systems, Minneapolis, MN, USA). Subjects were tested for the markers TGFB3-CA (Lidral et al., 1997) and MSXI-CA (Padanilam et al., 1992) by means of the mutation-detection enhancement (MDE) technique. The MTHFR-C677T polymorphism was investigated by Hinf I-restriction enzyme digestion of polymerase chain reaction (PCR) products, followed by 2% agarose gel electrophoresis (Frosst et al., 1995).

Statistical tests were performed within the standard transmission disequilibrium test framework (TDT). The computer program FBAT (“Family Based Association Tests”) was used for overall TDT calculations, where the biallelic TDT compares each allele to all others and the multiallelic TDT simultaneously tests the distribution of all alleles. For parent-specific TDT calculations, the S.A.G.E. TDT program was used. The entire dataset was analyzed, as well as two subsets of the data, as follows: (1) subset in which the mothers were affected; (2) subset in which the fathers were affected. In contrast to S.A.G.E., FBAT cannot separate maternal and paternal transmission calculations, but it utilizes all parental genotype information. A large portion of the data (21 families) had missing information on one or both of the parents, rendering those families uninformative for standard TDT analyses. Therefore, we also applied the likelihood ratio test (LRT) of Weinberg (1999), under the assumption that the distribution of paternal alleles is the same as the maternal. The LRT method includes the information from families with missing parental data. Since there were no significant findings, the LRT results are not reported here. Because multiple loci were evaluated in this study, a modified significance level was necessary for the interpretation of the results. We used the conservative Bonferroni adjustment for determining the appropriate significance level under multiple testing, i.e., p-values ≤ 0.017 were considered statistically significant, and p-values ≤ 0.03 were considered suggestive. Nominal significant results (i.e., p-values ≤ 0.05) were also reported for thoroughness and for comparison to other published studies.

For MSXI, there were no statistically significant results considering parental transmission patterns in the entire dataset (without regard to parental affection status, FBAT), or in the data subset of triads with affected mothers. Some previous studies showed association or not between MSXI variants and NS-CL/P (Lidral et al., 1997; Romitti et al., 1999; Beaty et al., 2001; Mitchell et al., 2001). Blanco et al. (2001) presented evidence of a sex-dependent association between MSXI and NS-CL/P.

For TGFB3, in the subset with affected fathers, the overall parental transmission distortion of allele 3 was suggestive (p-value = 0.03, Table 2), but with only five informative triads. There was no significant transmission disequilibrium in the subset of triads with affected mothers. Several studies using this same marker in either case-control, family-based or both approaches combined did not find an association with NS-CL/P in populations as diverse as those in the Philippines, North America, and Japan (Lidral et al., 1997, 1998; Tanabe et al., 2000; Beaty et al., 2001). However, in a case-control study in Iowa, Romitti et al. (1999) reported an increased risk for NS-CL/P associated with the rare allele 3 of TGFB3-CA, when analyzed under a dominant model. One of us (LAR, unpublished data) found a positive association between allele 1 of the TGFB3-CA marker and NS-CL/P in Brazilian patients.

The results were suggestive of an association between NS-CL/P and the MTHFR-C677T polymorphism. When considering parental transmission without regard to parental affection status in the entire dataset, there were 13 informative triads showing significant over-transmission of the C allele (p-value = 0.02 with S.A.G.E., and p-value = 0.01 with FBAT, Tables 1 and 2). In the subset with affected fathers, there was suggestive over-transmission of the C allele (p = 0.04 with S.A.G.E., and 0.02 with FBAT) (Tables 1 and 2). In the two subsets with an affected parent, there was no maternal or paternal over-transmission of either the C or T allele (Table 1). The number of informative families was too small to draw conclusions in the “affected mother” and “affected father” subsets. It should be noted that there were no genotypic frequency differences between affected and unaffected mothers, or between affected and unaffected fathers.

The majority of affected individuals (children and parents) had the CC genotype, regardless of gender: 53.85% of the males, 46.15% of the females. There was a possible gender difference (p = 0.075) with females having more CC genotypes than expected (48 observed, 45 expected). Eight out of nine T carriers were males (88.89%) and only one (11.1%) was a female. This allele frequency difference between genders was found in another study of our group (unpublished data). The T allele frequency of 3.9% did not differ from the frequency in the general Brazilian population (Arruda et al., 1998). Previous studies on the association between C677T polymorphism and NS-CL/P produced contradictory results (Shaw et al., 1995, 1996, 1998; Tolarova and Cervenka, 1998; Gaspar et al., 1999; Mills et al., 1999; Blanton et al., 2000, 2002; Wyszynski and Diehl, 2000; Martinelli et al., 2001; Prescott et al., 2002; van Rooij et al., 2003).
In conclusion, our data suggest that there is an association between MTHFR, but not MSX1 or TGFB3, and NS-CL/P. The sample, however, is small and does not allow definitive conclusions. While these results are in agreement with some previous studies, the literature in general is controversial. This might reflect differences in study designs or in the investigated populations.

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