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MSX1 Polymorphism in an Eastern Nepalese Non Syndromic cleft lip/palate patient population

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Abstract

This study was carried out to evaluate the role of MSX1 799 G >T gene polymorphism with non Syndromic cleft lip/palate in Eastern Nepalese patient population. For the study, whole blood samples (2 ml) were obtained from 40 subjects and controls. Genomic DNA was extracted from the blood of the subjects by using ethanol, chloroform treatment. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used to check for the presence of polymorphism. The results indicated that a patient has MSX1 799 G>T variant. The patient was a male aged 24 years was a complete unilateral left sided cleft lip/palate involving alveolus, hard and soft palate. He had normal development and no associated anomaly. There was no family history of cleft lip/palate and no history of any teratogenic exposure during embryonic life as revealed by his mother. This may be a case of sporadic polymorphism. It may be concluded that ,although we detected the presence of a MSX1 799 G>T polymorphism in one patient, a further investigation with large sample size, including many SNP's on families must be performed to get conclusive results.

Key words: Non Syndromic cleft lip/palate, MSX1 gene, Polymorphism, Nepalese.

Introduction:

Non-syndromal cleft lip/palate is one of the most common congenital anomalies seen in mankind.[1]Various etiological factors have been proposed including environmental and genetic ones. Environmental factors range from teratogenic effects of various agents affecting the development of craniofacial complex during early embryonic life. Various agents like viral infections, drugs, cigarette smoking, alcohol and retinoid etc have been associated with cleft lip/palate. On the other hand various genetic studies have supported complex genetic mechanisms and association with MSX1, TGFB1, TGFB3, IRF6, BCLX3, PAX9 etc. MSX1 is one of the homeobox genes and has been showed to be specifically associated with Non syndromal cleft lip/palate in various populations.[2]

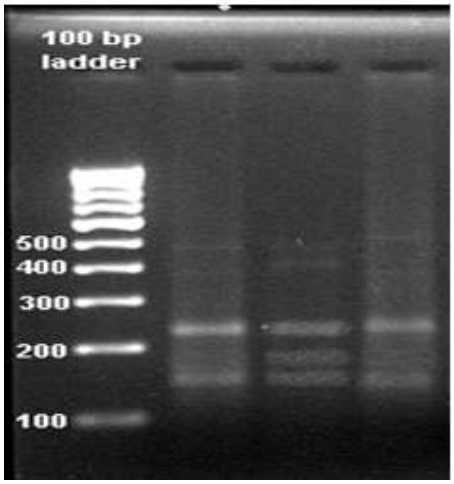
This study is undertaken considering the paucity of any genetic studies regarding Non syndromal cleft lip/palate in Nepalese population and aims to assess the role MSX1 799 G>T polymorphism with non Syndromic cleft lip/palate.

Material and Methods:

The sample consisted of 40 subjects with non syndromal cleft lip/palate reporting to the outpatient department of B.P. Koirala Institute of Health Sciences, Dharan, Nepal. Similar numbers of controls were recruited. The study was carried out after approval from institutional ethical committee and written informed consents were obtained from all subjects. Patients having cleft lip/palate associated with any history of developmental disabilities, including learning disabilities and attention deficits, hearing impairment, and speech deficits or abnormalities were excluded from the study. Whole blood samples (2 ml) were obtained from both subjects and controls. Genomic DNA was extracted from the blood of the subjects by using ethanol, chloroform treatment. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used to check for the presence of polymorphism. The following primers used for MSX1 were procured from new England laboratories, USA with the followingPCR 5'–3' sequence CAGGAAACAGCTATGACCCTGGAAGGGGCCAGAGGCTC having product size of 448 Bp and annealing temperature of 60. The amplified PCR products were digested with the specific restriction enzymes Dde1. We used a protocol already described elsewhere.[3]

Results:

The results indicated that for one of the patient DdeI creates two restriction sites in the 448-bp product digested into 181- and 39-bp products, present with the 220-bp product of the normal allele. Whereas for other cases and controls into 220-, 150-bp, and other smaller products (Figure 1).



The results indicated that this patient has MSX1 799 G>T variant. The patient was a male aged 24 years was a complete unilateral left sided cleft lip/palate involving alveolus, hard and soft palate. He had normal development and no associated anomaly. There was no family history of cleft lip/palate and no history of any teratogenic exposure during embryonic life as revealed by his mother. This may be a case of sporadic polymorphism. None of other samples including cases or controls showed complete digestion with the restriction enzyme indicating the absence of this polymorphism.

Discussion:

Non syndromal cleft lip/palate has been recently associated with many genes and it is thought that the problem is polygenic in nature, several authors have suggested various genes in various population based studies. Homeobox genes play a very important role in craniofacial development and are studied in detail regarding their role in non Syndromic cleft lip/palate.[2] There are various single nucleotide polymorphisms (SNP) which are reported for MSX1. We selected MSX1 799 G>T as this SNP has been reported in Thai[3] and Indian population[4], both being Asian groups, so it was prudent for us considering the geographical similarity to check for this SNP in our population.[3,4]

The patient which showed the presence of SNP, a male aged 24 years has heterozygous alleles, further his phenotype was a complete unilateral left sided cleft lip/palate involving alveolus, hard and soft palate. There was no family history of cleft lip/palate and no history of any teratogenic exposure during embryonic life as revealed by his mother. This may a case of sporadic polymorphism. Similar results were reported by Singh VP[4] et al in a study on Indian patients and Tongkobpetch S[3] in Thai population.

This was a pilot project being the first of its kind in Nepal and there were limitations. The lack of generous funding limited us to test only a single SNP, if we could have tested for more, results could have been more conclusive. Further, modern technologies like next generation sequencing although costly can give more detailed and conclusive results. Further, a large sample size is often needed to derive useful conclusion on a population basis. More useful information regarding transmission can be derived by undertaking family based studies, involving two or three generations.

Conclusion

Although we detected the presence of a MSX1 799 G>T polymorphism in one patient, a further investigation with large sample size, including many SNP's on families must be performed to get conclusive results.

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