P7-METHYLATION STATUS OF THE RUNX2 P2 PROMOTER IN A FAMILY WITH ECTOPIC MAXILLARY CANINES.

Camilleri S[1]. Scerri C[2]. McDonald F[1].

[1] Kings College London, Dental Institute,Department of Orthodontics, Floor 22 Guy's Tower, St Thomas Str, London SE1 9RT. United Kingdom.
[2]Molecular Genetics, Department of Biochemistry and Phsyiology, University of Malta, Msida Msd 2080. Malta

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Introduction

Genetic factors contribute to the aetiology of the ectopic canine (EC) (OMIM 189490), the inheritance pattern being autosomal dominant with variable expression and incomplete penetrance. However there is also evidence for an epigenetic component [1]. The Runx2 gene is intimately involved in the mechanism of tooth eruption. It controls differentiation and maturation of osteoblasts and is expressed in the dental follicle and alveolar bone at all stages of development [2,3]. Mutations of this gene result in delayed and ectopic eruption of teeth. There is a large CpG island spanning its proximal promoter, first exon and part of the first intron. Similarities between the inheritance patterns of EC and other methylation disorders raises the hypothesis that differential methylation of the Runx2 promoter may contribute to EC.

Materials and methods

DNA from a 21 member, three-generation family, exhibiting 8 cases of EC (Fig.1) was extracted from saliva (Oragene, DNAGenotek) using the phenol-chloroform method and converted with bisulphite (Methyleasy Xceed, Human Genetic Signatures) in order to determine 5mC content. The age of the subjects in the pedigree ranged from 8 years to 74. The possibility of a localised methylation aberration was addressed by investigating dental tissues. The follicles of eighteen ectopically erupting maxillary canines from non syndromic subjects referred to the Oral Surgery Department, Mater Dei Hospital, Malta for extraction or exposure were frozen immediately after surgery. Control tissues were obtained from the pulps and periodontal ligaments of upper first premolars from unaffected subjects as these are derived from the same neural crestal populations [4]. Three primer pairs were designed, one being 2kb upstream of the transcription start site (TSS), one less than 1kb upstream of the TSS, within the promoter region and another in first intron (Table 1.). Polymerase chain reaction was used to amplify the relevant sites and the product directly sequenced using the Applied Biosystems 3130 Genetic Analyser. Analysis was carried out using the BIQ Analyzer program.

Results

No methylation was found in any of the three sites examined in the salivary DNA, irrespective of age or the status of eruption of the canine. Similarly, all samples of ectopic canine follicle or periodontal ligament DNA were entirely unmethylated. The first intron of pulpal tissue was found to be methylated up to 5% in aggregate and is considered hypomethylated.

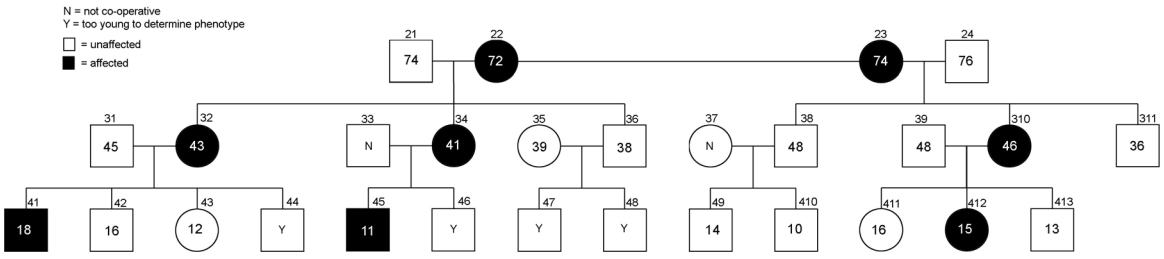

Fig.1 The pedigree used in the study. The position of the subjects in the pedigree is given by the number outside the symbol, the number inside denotes the age.

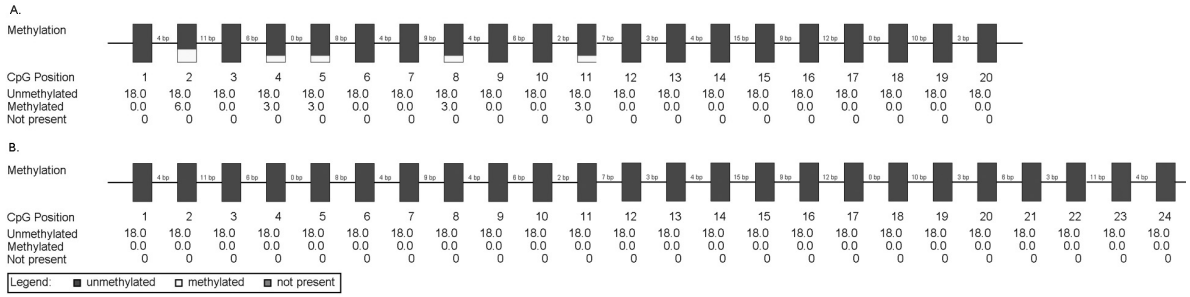

Fig.2. Methylation of dental pulp where methylation of the first intron was 5%.(A) This contrasts with the situation in all other amplicons, where methylation was 0% (B).

Conclusions

The *Runx2* P2 promoter is completely unmethylated both in normal dental tissue, i.e. periodontal ligament and hypomethylated in pulpal tissue. It is also unmethylated in ectopic canine follicle tissue. A similar situation exists in lymphocytes in subjects of a range of ages, providing evidence against differential promoter methylation as a means of control of expression of Runx2 in these subjects. This study provides no evidence that this mechanism may be responsible for the aetiology of EC.

References

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