

The Incidence of $G_s\alpha$ Mutation in Fibro-Osseous Lesions of the Jaws using a PCR-SSCP Method

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Purpose: Fibro-osseous lesions of the jaws are benign lesions that replace bone, generally during childhood or adolescence. Mutation of α subunit of signal-transducing G proteins ($G_s\alpha$) has been implicated in fibrous dysplasia and the Albright-McCune syndrome. In this study, we examined the $G_s\alpha$ mutation in 24 fibro-osseous jaw lesions.

Materials and Methods: The formalin-fixed paraffin embedded samples consisted of eight cases of fibrous dysplasia, 10 cases of cemento-ossifying fibroma, and six cases of focal cemento-osseous dysplasia were selected. Five-micrometer sections of the blocks were prepared and the DNA was then extracted. Subsequently, polymerase chain reaction (nested - PCR) and single-strand conformational polymorphism (SSCP) were carried out to detect the mutations.

Results: This mutation was detected in six fibrous dysplasia, nine cemento-ossifying fibroma and four focal cemento-osseous dysplasia samples studied. Statistical analysis of the data (chi-square test) showed no significant difference between these lesions ($p=0.0506$).

Conclusions: This research showed that a unique mechanism must be responsible for the creation of fibro-osseous lesions, and the possibility of other unknown mutations may be responsible for the differences of these lesions. To confirm our evaluations, DNA sequencing of the fragments is being done.

Key words: $G_s\alpha$ mutation, fibro-osseous lesions, fibrous dysplasia, cemento-ossifying fibroma, focal cemento-osseous dysplasia, PCR-SSCP

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INTRODUCTION

Fibro-osseous lesions of the jaws are a diverse group of processes that produce bone destruction and replace it with fibrous proliferation in the area that contains various amount of immature or woven bone or mature islands of lamellar bone, generally during childhood and adolescence. This group consists of different types of tumoral, developmental or reactive lesions, and three of its most important types are fibrous dysplasia (FD),

cemento-ossifying fibroma (COF) and focal cemento-osseous dysplasia (FCOD) (Neville et al, 2002).

Many diagnostic methods have been introduced for these lesions. The most common way is evaluation of the histological findings, all with clinical examinations and patient radiographs. Sweet et al introduced immuno-histochemical staining of cytokeratin (AE1/AE3 +/Ck-1) as a method to distinguish between osteofibrous dysplasia and FD (Blezikian et al, 2002). Short-term *in situ* culture and Giemsa-band chromosome method has also been

introduced to analyze three COFs in the orbit (Sawyer et al, 1995). Recently, molecular biology has aided the identification of these lesions. Recent investigations confirm a genetic background for this group of lesions. Sakamoto et al (2000) and Marie (2001) traced the mutation in α subunit of G-protein in all FD cases they had analyzed. But further genetic investigations has not been performed on other fibro-osseous lesions, except for Gollin et al (1992) who performed cytogenetic and cariotyping analysis on COF and discovered three translocations responsible for it.

In this research, G-protein mutation, located in chromosome number 13, was investigated to see if this mutation has a diagnostic value for three types of fibro-osseous lesions (FD, COF and FCOD).

MATERIALS AND METHODS

In this retrospective analytical study, 30 formalin-fixed paraffin-embedded fibro-osseous samples, selected among patients referred to the pathology department of Shahid Beheshti School of Dentistry during 1993-2003, were selected using a non-randomized sampling method. The study group consisted of eight fibrous dysplasia (FD), 10 cemento-ossifying fibroma (COF) and six focal cemento-osseous dysplasia (FCOD) samples (six samples were omitted due to improper responses to the tests). 5- μ m sections of the samples were prepared using the Microtom (JUNG, Germany) device and then their DNA was extracted using the phenol-chloroform method (Sambrook and Russel, 2001). To look for mutations in the extracted DNA, nested PCR was performed in two stages, with the procedures shown in Table 1. Single-strand conformational polymorphism (SSCP) analysis was then performed to analyze the PCR products (Sambrook and Russel, 2001) (Figs 1 and 2). The chi-square test was used for statistical analysis.

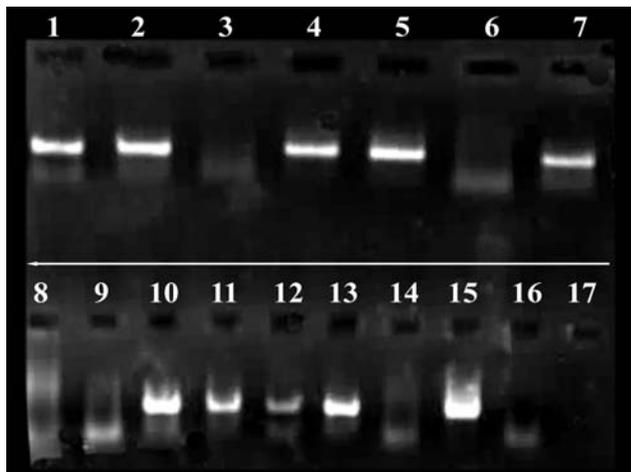


Fig. 1 The electrophoresis results of the gene producing G-Protein α -subunit. Column 4 represents the tissue sample and column 15 represents the blood sample.

Table 1 Procedures of PCR performed in two stages

Stage	Step	Name	Temp. (°C)	Time (Sec)	Cycle
Stage I	1	Pre-denaturation	94	300	1
	2-a	Denaturation	94	30	30
	2-b	Annealing	67	30	30
	2-c	Extension	72	30	30
	3	Final extension	72	300	1
Stage II	1	Pre-denaturation	94	300	1
	2-a	Denaturation	94	30	34
	2-b	Annealing	56	30	34
	2-c	Extension	72	30	34
	3	Final extension	72	300	1

RESULTS

The G_s α mutation was detected in six FD (75%), nine COF (90%) and four FCOD (66.6%) samples. Statistical analysis of the data showed no significant difference (P=0.506), meaning the mutation is present in all types of fibro-osseous lesions analyzed and cannot be used as a differentiating factor among these lesions.

DISCUSSION

Fibro-osseous lesions form an important group of jaw lesions that show similarities in clinical, radiographic and histological features (Neville et al, 2002); thus, they are all named as fibro-osseous lesions and the results of this study emphasize this fact.

For the first time, this study showed that G-protein mutation is seen in all three lesions of FD, COF and FCOD. This finding confirms the results of investigations

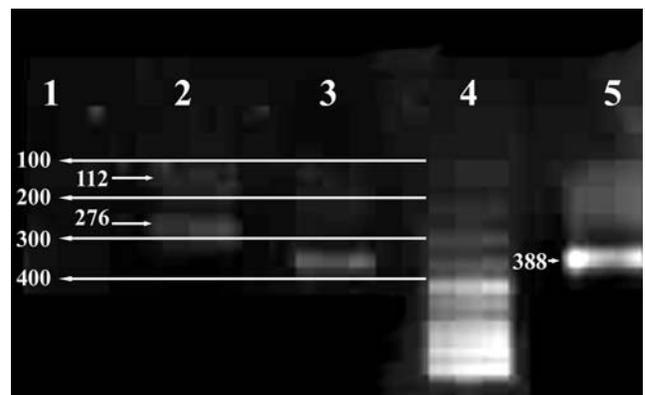
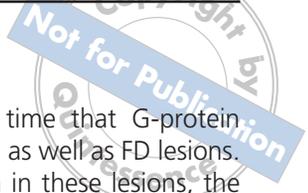


Fig. 2 Confirming the results of PCR using the RsaI enzyme. Column 1 represents the gene fragment after PCR; column 2 represents the standard marker; Column 3 represents the fragments after the enzyme effect.



performed by Marie (2001), Sakamoto et al (2000), Pollandt et al (2001) and Riminucci et al (1999) who have reported the presence of G-protein mutation in fibrous dysplasia. This investigation also confirmed the study performed by Voytek et al (1995) which evaluated the diagnostic value of histological findings in classification of fibro-osseous lesions. They reported that histopathologic findings are not a proper method for spot diagnosis of these lesions and also a common etiology is responsible for production of this group of lesions. Our study also confirmed the results of research performed by Slootweg (2001) and Voytek et al (1995), which showed diagnostic overlaps in this group of lesions. Although in most cases patient's history and clinical, histological and radiographic features lead the pathologist to a proper diagnosis of the members of this group of lesions, diagnostical difficulties still remain in some cases.

Immature woven bone is one of the results of this mutation seen in the area of the lesion and is known as a characteristic landmark for FD lesions; but this finding is also reported to be found in both COF and FCOD lesions. It must be noted that, according to Slootweg (2001), large areas of mineralization like immature woven bone, lamellar bone and cementoma-like calcification can be seen in these lesions and immature Woven bone is not only present in FD lesions.

In order to interpret the findings of this research, two facts should be considered:

- The group of fibro-osseous lesions seems to be more similar both genetically and in the way they are created than previously thought to be. As G-protein mutation is present in all of the members of this group of lesions, it cannot be used as a method for differentiation of fibro-osseous lesions.
- Clinical and histopathologic features of fibro-osseous lesions are still insufficient for proper diagnosis in some cases; thus, error in diagnosis and differentiation among the members of this group of lesions is still one of the major controversies in pathology, which may be considered as an interfering factor in this study.

Although G-protein mutation is found to be one of the factors responsible for creation of fibro-osseous lesions, the small differences among different types of this group of lesions can be a result of other unknown mutations occurred later on. Thus, for locating these mutations, a study is being conducted by DNA sequencing that the results will be published soon.

CONCLUSION

This study showed for the first time that G-protein mutation is seen in COF and FCOD as well as FD lesions. By tracing the G-protein mutation in these lesions, the common etiology of these lesions is thought to be true and the concept of placing these lesions in a unique group is more emphasized.

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