Genetic diagnosis of the craniosynostosis in Spain
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1 Introduction

The craniosynostosis consists of an anomalous fusion of cranial bones that originates problems in the normal growth of the cranium and involves dramatic alterations in the shape of the head and the face, and in some cases severe mental delay.

The craniosynostosis is classified under several syndromes: Apert, Crouzon, Pfeiffer, Beckwith-Wiedemann, Saethre-Chotzen, Muenke and Jackson-Weiss. Each one has a particular pattern of inheritance and a few clinical specific characteristics, but in some cases they are difficult to distinguish by the external aspect, specially when prenatal diagnosis is done by means of the ultrasound scans. The genetic diagnosis allows to detect the craniosynostosis in the first stages of the pregnancy and to confirm the clinical observations in the adults patients.

The most important genes associated to these disorders are FGFR1, FGFR2, FGFR3 and TWIST. The genetic study of the craniosynostosis is complex, and may include the four genes involved in the pathology.

In this communication we show the results obtained in the study of these syndromes in Spain.

2 Materials and Methods

Patients
We analyzed several clinical samples taken from adults patients, children and fetus (prenatal diagnosis). The samples were sent to our laboratory from hospitals of Spain and Portugal.

PCR Amplification and Sequencing of Genomic DNA
Genomic DNA was extracted from peripheral blood samples according to a standard protocol using the QiAamp DNA Blood Mini Kit (Qiagen) following the manufacturer’s protocol.

PCR for amplification of certain coding exons as well as adjacent intronic regions were performed using specific primers. The PCR reactions were done in a final volume of 50 microl; in a GeneAmp PCR System 2700 (Applied Biosystems) with the appropriate parameters. After amplification, the single bands generated by the PCR reactions were purified and sequenced, using the PCR amplification primers, in an ABI Prism® 310 Genetic Analyzer (Applied Biosystems). All the products were sequenced in both forward and reverse directions.

4 Conclusions

We studied 20 people and 72 genes involved in craniosynostosis. No previous molecular diagnosis data were reported in any case, so that we had to do the molecular study without other information than the clinical reports. We found 8 different mutations in FGFR3 and Twist genes. All mutations were sporadic, except for the family F4.

The 4 genes involved in craniosynostosis have a total of 52 coding regions. The complete analysis of all these coding regions by sequencing would be so much large and expensive. Our approach was to perform a molecular study that could detect the most common mutations involved in these syndromes, in order to reduce the delivery time of the results and the cost of the study.

Our results show that the analysis of only 6 coding regions of 52 possible allowed us to diagnose a significant number of cases. In the four people not diagnosed, we did not analyse the 6 target regions.

Our conclusion is that it is advisable to do a previous screening of the craniosynostosis using only 6 regions of the genes FGFR1, FGFR2, FGFR3 and Twist. The molecular analysis could be extended to other regions only in the case of a negative result.

5 References