The Genetic Basis of Human Craniosynostosis syndromes.

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SUMMARY

Craniosynostosis is the premature fusion of one or more sutures of the skull. This can result in the distortion of the shape of the head and face, and affects an estimated 0.25 to 1.6 children per 1,000 live births. The aims of this project were to identify genes involved in craniosynostosis and to characterise mutations of these genes.

To this end, linkage mapping was undertaken in a craniosynostosis pedigree and a gene was localised to chromosomal region 4p16, distal to the marker D4S394. This interval contained two plausible candidate genes, *MSX1* and *FGFR3*. The localisation to 4p16 was the first evidence for the presence of a craniosynostosis syndrome in this region of the genome.

These candidate genes were screened for mutations using single strand confirmation analysis, heteroduplex analysis and sequencing. Three polymorphisms were identified in *MSX1*; however, no mutations were detected. Two polymorphisms were identified in *FGFR3* before a candidate mutation in *FGFR3* was found in a number of craniosynostosis patients, by researchers in North America. This mutation, C749G in *FGFR3* (Pro250Arg) was then demonstrated to segregate with the affected members of the craniosynostosis pedigree localised to chromosomal region 4p16. The mutation was found in a further large pedigree in which autosomal deafness was the major feature, suggesting that the Pro250Arg mutation may account for a proportion of autosomal dominant deafness in the population. The Pro250Arg mutation of FGFR3 was found in five additional unrelated patients and is now recognised as a relatively common recurrent mutation among patients presenting to craniofacial clinics.

Determination of the molecular defect responsible for this craniosynostosis syndrome, led to an investigation of the effect of the mutation on the protein and how it results in craniosynostosis. Antisera to part of the extracellular region of the FGFR3 protein were used in flow cytometry experiments on skin fibroblast cells from an affected member of the craniosynostosis pedigree and from normal controls. The hypothesis was that the receptors

from affected and unaffected cells would be distinguishable in this way, to allow further investigation. However, it was not possible to reliably detect a difference.

In parallel to the FGFR3 craniosynostosis study, patients with a clinical diagnosis consistent with FGFR2 craniosynostosis syndromes were examined for molecular defects. *FGFR2* mutations were found in 12 unrelated Apert patients, 8 unrelated Crouzon patients, 3 unrelated Pfeiffer patients and 6 unrelated patients with uncertain diagnoses. Three of the mutations found were novel; T875A, T797C and G(-1)C (a splice site mutation). Characterisation of *FGFR2* mutations will identify which regions of the gene are functionally important and will form the basis for the study of the genotype-phenotype relations in craniosynostosis disorders.

The genes causing Apert, Crouzon, Jackson Weiss, Pfeiffer and Saethre Chotzen syndromes were identified, by others, while this project was proceeding. Work carried out by the candidate and others during the course of this project elucidated the genetic basis for Craniosynostosis Adelaide Type (FGFR3 craniosynostosis). In addition, the candidate characterised FGFR2 craniosynostosis mutations to further elucidate the molecular genetic basis for that group of disorders.