Genetic craniofacial aberrations

Sinikka Pirinen
Institute of Dentistry, Department of Pedodontics and Orthodontics, University of Helsinki, Helsinki, Finland

Pirinen S. Genetic craniofacial aberrations. Acta Odontol Scand 1998;56:356–359. Oslo. ISSN 0001-6357.

Many craniofacial and dental anomalies have a genetic background. Much research related to the molecular pathology of genetic conditions is being carried out, and new information related to mapping of disease genes, gene identification, and mutations in these genes is accumulating with incredible speed. It is important to be well informed of the molecular background of the conditions that we treat at anomaly clinics. This article reviews the most recent molecular findings related to Turner syndrome, Beckwith–Wiedemann syndrome, Marfan syndrome, Treacher Collins syndrome, cleidocranial dysplasia, and cleft lip and palate. □ *Craniofacial abnormalities; genetics; genetics, biochemical*

Sinikka Pirinen, Department of Pedodontics and Orthodontics, Institute of Dentistry, University of Helsinki, P.O. Box 41, FIN-00014 Helsinki, Finland

Craniofacial and dental anomalies often have a genetic background. Recent advances in molecular genetics have revealed a genetic explanation for conditions that do not even appear to be genetic in origin. At the moment, molecular and clinical genetics are advancing at incredible speed. Understanding the molecular background of the conditions that we meet and treat at centers for craniofacial anomalies is essential. Patients and families are nowadays well informed of the nature of their abnormalities. Knowledge of the molecular bases of these conditions and of the possible effects of the mutations sometimes helps in understanding the clinical picture.

The human genome consists of 46 chromosomes: 22 pairs of autosomes plus the X and Y chromosomes. In these we have between 50,000 and 100,000 individual genes. Chromosomes can be separated, stained, and seen under a high-power microscope; their staining and identification has been possible for a long time. Modern techniques with the fluorescence microscope, the FISH techniques (fluorescence in situ), are now also used in visualizing the chromosomes and even certain genes in them (1). Pathologic conditions may arise from variation in the number of chromosomes. Well-known examples of numeric chromosomal aberrations are Down syndrome (trisomy 21) and Turner syndrome (45, X). Structural variations in chromosomes are also known: deletions, inversions, translocations, and/or ring chromosomes.

Because each chromosome is paired, there is a maternal and a paternal contribution to each gene. Genes consist of a series of base pairs, and their sequence is a template for the messenger RNA produced. This is used in turn to synthesize proteins, which are the products of protein-coding genes. Genes that code for proteins are called structural genes. Control genes regulate the expression of a structural gene. Genes vary widely in size, from a few hundred to many thousands of base pairs. Between the genes in the chromosomes are base sequences with no known function. Genes consist of sequences that regulate gene expression and of exons and introns; exons encode amino acids, whereas introns are non-functional.

Abnormalities of gene structure may be deletions of one or more base pairs, or mutations, which may disturb or completely prevent the protein synthesis. Because each gene exists in a paternal and a maternal copy, the ultimate product of the mutated gene is a combination of the normal and abnormal. If an abnormal gene is inherited from both mother and father, the product is of course entirely of the mutant type. Disease may, however, manifest when there is a mutation in one allele only, because the presence of the mutant product sometimes interferes with the function of the normal gene product (dominant negative action). Disease or an anomaly may also become manifest if the other allele is inactivated owing to a deletion or mutation (haploinsufficiency). Moreover, some genes are imprinted. Imprinting is a non-Mendelian form of gene regulation resulting in differential somatic expression of the two parental alleles in a gene pair. The allele derived from one gene pair is silenced, not by a mutation, but by an epigenetic factor. Beckwith–Wiedemann, Prader–Willi, and Angelman syndromes are examples of disturbed imprinting in human disease. Uniparental disomy (UPD) is still another anomaly in the genome that can cause disturbances in gene expression and protein production. In UPD there are two alleles of one parent and none of the other. The maternal or paternal origin of an allele can be detected with restriction-fragment-length polymorphism (RFLP) analysis.

When the molecular pathology of a genetic condition is studied, the mode of inheritance of the disorder is first analyzed. This can be seen when a pedigree of the patient's family is constructed. Monogenic conditions and polygenic conditions can be analyzed by the same principles. The study starts with collection and isolation

of DNA from both healthy and diseased family members, and as an initial step the chromosome on which the mutated gene is situated is identified. The segment of a chromosome where the gene 'maps' is often quite large and may contain dozens of genes. It takes a considerable amount of further work to identify the gene in which a mutation causes the condition studied. When the gene is finally found, the mutations have to be sought. The structure and nature of the protein produced by the gene is analyzed. Discovering in which tissues and when the gene is expressed often requires construction of an animal model.

Here I review current knowledge of the molecular background of several conditions and anomalies commonly seen in facilities where craniofacial anomalies are treated.

Turner syndrome

Turner syndrome is an anomaly based on the number or function of the sex chromosomes. The patients, always girls, have only one X chromosome, while the other is completely or partly deleted. Other variations in this karyotype exist (2). The girls are of normal intelligence, but grow poorly and do not develop secondary sex characters. Birth prevalence of the condition is quite high, estimated at 1 in 2,500 female births (3). Craniofacial and oral aspects include small deciduous and permanent teeth (4–7), early formation of permanent teeth, small mandible, and sharp cranial base (8). Features like small stature, gonadal aplasia, and cognitive and psychosocial problems have remained unexplained until quite recently. Identification of a growth-regulation gene in the X and Y chromosomes, *SHOX*, has offered an explanation of the short stature of Turner girls (9). Because the *SHOX* gene is in the pseudoautosomal parts of the X and Y chromosomes, it escapes X inactivation. In healthy females with two X chromosomes, both alleles produce the growth-promoting protein. Having the product of only one allele thus explains the short stature in Turner syndrome. This is further confirmed by the finding that patients who have deletions of the whole short arm of the X chromosome are short of stature (10). Moreover, *SHOX* mutations have now been detected in patients with dyschondrogenesis, with their short forelegs and forearms (Leri–Weill syndrome) (11, 12). The mouse homolog (og-12a) has been identified (9), and it does not reside in the mouse X chromosome. The autosomal location of the mouse gene and the lack of short stature in XO mice are consistent with *SHOX* having a role in human short stature.

An interesting new feature in the profile of the Turner girls was introduced in summer 1997 when an article in the journal *Nature* offered a genetic explanation for the frequently seen social adjustment problems in these patients (13). Eighty females with Turner syndrome were examined. All had a single X chromosome; the X was maternally derived in 55 subjects (45, X^m) and of paternal origin in 25 (45, X^p). Members of the latter group were found to be significantly better adjusted. The scientists concluded there must be a genetic component for social cognition that is imprinted and not expressed from the maternally derived X chromosome. The locus of the supposed gene obviously also escapes X inactivation. This finding, if correct, is of great importance to applied psychology, because it gives a genetic explanation for psychosocial and cognitive differences between normal males and females.

Beckwith–Wiedemann syndrome

Beckwith–Wiedemann syndrome (BWS) was described by both Beckwith (14) and Wiedemann (15). The condition is characterized by overgrowth, general or regional. The patients have a predisposition to specific embryonal tumors (16, 17). The tongue is very large in infancy, which brings the patients to craniofacial anomaly clinics. Generally, BWS cases appear sporadically, but because familial cases were also seen, the condition was examined as a genetic disorder. The gene locus was mapped to chromosome 11p15 (18). This locus is naturally imprinted in several tissues, so that in healthy individuals only the paternal allele is active (19). At present, six genes have been identified and are known to be imprinted in 11p15, but the locus possibly contains other genes as well. Two major genes are implicated in BWS: the insulin-like growth factor 2 gene (*IGF2*) and $p57^{KIP2}$ (20). Biallelic expression of the *IGF2* gene is behind 50% of BWS cases: the normally repressed maternal allele and the paternal allele. In addition, paternal duplication of the *IGF2* allele has been seen. Mutations in the $p57^{KIP2}$ gene are seen in a minority of BWS cases (21–23). Experimentation with these genes by generation of murine models has demonstrated that increased expression of *IGF2* is what causes many phenotypic features of BWS (24). Interaction of the $p57^{KIP2}$ gene seems to play a role in creating the various forms of *IGF2* overexpression (25).

Marfan syndrome

Marfan syndrome (MS) is another common genetic condition, though its phenotypic features are mild, and it often goes undetected. In Finland 150 cases have been diagnosed, but the presumed number of cases is 350. Because of their slightly peculiar faces and oral structures, these patients often seek orthodontic care; an orthodontist is in a good position to suspect undiagnosed cases because the clinical picture is distinct, if not clearly abnormal. The specific visible features are tallness, long limbs, slender fingers, eye abnormalities, and narrow dental arches, often Class II malocclusions. The inheritance is autosomal dominant, and the molecular pathology is well known (26, 27). The mutated gene, *FBN-1* for fibrillin 1, causes production of abnormal fibrillin, and the phenotype is

related to the severity of the mutation in this huge gene. Mutation in the fibrillin 2 gene, *FBN-2,* causes congenital contractural arachnodactyly (28). A Marfan-like phenotype has been described in relation to mutation in the gene coding for alpha 2(1) collagen and an unknown gene in chromosome 3.8 (29).

The latest news related to the molecular pathology in MS is that germline mosaicism has been demonstrated in a symptomless mother who had two daughters with MS (30). This had been suspected for a long time because of unexplained familial occurrence of the condition.

Cleidocranial dysplasia

Cleidocranial dysplasia (CCD) is a condition in which patients are small of stature, have hypoplasia or aplasia of the clavicles, patent fontanelles, and a major dental problem due to noneruption of permanent teeth and the presence of supernumerary teeth. This condition is inherited in an autosomal-dominant manner. Recently, the molecular pathology of this condition suddenly became very clear. Almost the entire May 1997 issue of the journal *Cell* concentrated on mutations detected in the *CBFA* gene and the findings revealed by the animal model (31, 32). The gene that had been found mutated in CCD patients, *CBFA 1,* was shown to be a transcription factor, essential for osteoblast differentiation and bone formation. This finding explains the general bone dysplasia in the condition, while the supernumerary teeth in CCD remain a mystery.

Treacher Collins syndrome

Treacher Collins syndrome (TCS) is a common craniofacial syndrome with a birth prevalence of 1 in 50,000 (33). The abnormalities include anomalies of the external ears, hypoplasia of the mandible, zygomatic complex, and cleft palate. The condition is genetic with autosomal-dominant inheritance. Expression of the gene has, however, appeared to be variable, making genetic counseling difficult. Sixty percent of cases have been considered to be new mutations (34). The mutated gene causing the condition, *TCOF1*, has been identified on chromosome 5q31–34 (35). The name *treacle* has been given to the protein product of the gene. The function of the protein is still unclear.

A complete genomic organization of the gene has been elucidated (Dixon, unpublished data). Mutations are mostly family-specific (36). The clinical picture shows wide variation and is not directly linked to the mutation. The recent article cited above discusses the possibility that other acrofacial conditions (Nager syndrome, Miller syndrome, hemifacial microsomia) may be allelic with TCS. In the *far* mouse (the first branchial arch mutant), the same mutation may result in a bilateral or unilateral anomaly, depending on the genetic background on which the mutation is placed (37).

Cleft lip and palate

The possible genetic background of nonsyndromic cleft lip and/or palate (CL/P) or cleft palate only (CPO) is a much-discussed issue in craniofacial biology. A family history of clefting has been shown in 25%–35% of CL/P and 10%–20% of CPO patients (38). It has been estimated that 2 to 20 genes interact to cause clefts, including a possible major gene that may contribute to 10%–50% (39). Several genes are already known to be associated with CL/P and CPO and their family members (40). This research group has analyzed further two of the genes, *MSX1* and *TGFB3*, in a group of CL/P and CPO patients with the candidate-gene linkage-disequilibrium (LD) strategy. Several previously suggested candidate genes could not be confirmed with this procedure, but significant LD was found between CL/P and both *MSX1* and *TGFB3,* and between CPO and *MSX1*, suggesting that these genes may be involved in the pathogenesis of clefting. No common mutations were found in the mutation search, but several rare variants of *MSX1* and *TGFB3* were found. On the basis of these findings, the group from Iowa plans to start a search for *MSX1* and *TGFB3* mutations in these patients and extend the search to the noncoding parts of *MSX1*.

Conclusion

Recent findings related to the molecular pathology of six common genetic disorders with craniofacial manifestations serve as examples of rapid development in the 'new genetics'. Sometimes the effect of the mutation in a gene with a known function explains the clinical picture. However, quite often new, previously unknown genes are identified, and the function of these genes is obscure. In these instances a considerable amount of work is needed to clarify the normal product and expression pattern of the gene before the effect of the mutations can be studied.

References

1. Heiskanen M, Peltonen L, Palotie A. Visual mapping by high resolution FISH. Trends Genet 1996;12:379–82.
2. De la Chapelle A. Sex chromosome abnormalities. In: Emery E, Rimoin D, editors. Principles and practice of medical genetics. Edinburgh: Churchill Livingstone; 1983. p. 193–215.
3. Lemli L, Smith DW. The XO syndrome. A study of the differentiated phenotype in 25 patients. J Pediatr 1963;63:577–688.
4. Kari M, Alvesalo L, Manninen K. Sizes of deciduous teeth in 45, X females. J Dent Res 1980;59:1382–5.
5. Varrela J, Townsend G, Alvesalo L. Tooth crown size in human females with 45, X/46XX chromosomes. Arch Oral Biol 1988;33:281–4.
6. Midtbo M, Halse A. Skeletal maturity, dental maturity and

eruption in Turner syndrome. Acta Odontol Scand 1992;50: 303–12.
7. Midtbo M, Halse A. Tooth crown size and morphology in Turner syndrome. Acta Odontol Scand 1994;52:7–19.
8. Jensen BL. Craniofacial morphology in Turner syndrome. J Craniofac Genet Dev Biol 1985;5:327–40.
9. Rao E, Weiss B, Fukami M, Rumo A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and a Turner syndrome. Nat Genet 1997;16:54–63.
10. James R, Coppin B, Dalton P, Dennis N, Mitchell C, Sharp A, et al. A study of females with deletions of the short arm of the X chromosome. Hum Genet 1998;102:507–16.
11. Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A, et al. SHOX mutations in dyschondrosteosis (Leri–Weill syndrome). Nat Genet 1998;19:67–9.
12. Shears D, Vassal H, Goodman F, Palmer R, Reardon W, Superti-Furga A, et al. Mutation and deletion of the pseudo-autosomal gene SHOX cause Leri–Weill dyschondrosteosis. Nat Genet 1998;19:70–3.
13. Skuse D, James R, Bishop D, Coppin B, Dalton P, Aamont-Leeper G, et al. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function [letter]. Nature 1997;6634:705–8.
14. Beckwith J. Macroglossia, omphalocele, adrenal cytomegaly, gigantism and hyperplastic visceromegaly. Birth Defects 1968;5: 188–96.
15. Wiedemann HR. Complexe malformatif familial avec hernia ombiliccal et macroglossie-un syndrome nouveau? J Genet Hum 1964;13:223–32.
16. Sotelo-Avila C, Gooch WM. Neoplasms associated with the Beckwith–Wiedemann syndrome. Perspect Pediatr Pathol 1976; 344:255–72.
17. Cohen MM Jr. Beckwith–Wiedemann syndrome, imprinting, *IGF2* and *H19*, implications for hemihyperplasia, associated neoplasms and overgrowth. Am J Med Genet 1994;52:233–4.
18. Koufos A, Grundy P, Morgan K, Aleck K, Hadro T, Lampkin B, et al. Familial Beckwith–Wiedemann syndrome and a second Wilms tumour locus both map to 11p55.5. Am J Hum Genet 1989;44:711–9.
19. Ohlsson R, Hedenborg F, Holmgren L, Walsh C, Ekström T. Overlapping patterns of *IGF2* and *H19* expression during human development: biallelic *IGF2* expression correlates with lack of *H19* expression. Development 1994;120:361–8.
20. Li M, Squire J, Weksberg R. Overgrowth syndromes and genomic imprinting: from mouse to man. Clin Genet 1998; 53:165–70.
21. Brown K, Villar A, Bickmore W, Clayton-Smith J, Catchpoole D, Maher ER, et al. Imprinting mutation in the Beckwith–Wiedemann syndrome leads to biallelic *IGF2* expression through an *H19* independent pathway. Hum Mol Genet 1996;5:2027–32.
22. Hatada I, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, et al. An imprinted gene $p57^{KIP2}$ is mutated in Beckwith–Wiedeman syndrome. Nat Genet 1996;14:171–3.
23. Lee M, Hu R, Johnson L, Feinberg A. Human KVLQT1 gene shows tissue-specific imprinting and encompasses Beckwith–Wiedemann syndrome chromosomal rearrangements. Nat Genet 1997;15:181–5.
24. Sun F-L, Dean W, Kelsey G, Allen N, Reik W. Transactivation of *Igf2* in a mouse model of Beckwith–Wiedemann syndrome. Nature 1997;389:809–15.
25. Zhang P, Liegeois N, Wong C, Feingold M, Hou H, Thompson J, et al. Altered cell differentiation and proliferation in mice lacking $p57^{KIP2}$ indicates a role in Beckwith–Wiedemann syndrome. Nature 1997;387:151–8.
26. Kainulainen K, Sakai L, Child A, Pope F, Puhakka L, Ryhänen, et al. Two mutations in Marfan syndrome resulting in truncated fibrillin polypeptides. Proc Natl Acad Sci U S A 1992;89:17–21.
27. Kainulainen K, Kartunen L, Puhakka L, Sakai L, Peltonen L. Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal Marfan syndrome. Nat Genet 1995;11:64–9.
28. Putnam E, Zhanf H, Ramirez F, Milewich D. Parental somatic and germ-line mosaicism for a FBN2 mutation and analysis of FBN2 transcript levels in dermal fibroblasts. Nat Genet 1995;11:456–8.
29. Burn J, Camm J, Davies M, Peltonen L, Schwartz P, Watkins H. The phenotype-genotype relation and the current status of genetic screening in hyperthrophic cardiomyopathy, Marfan syndrome and the long QT syndrome. A review. Heart 1997;78:110–6.
30. Rantamäki T, Kaitila I, Syvänen A-C, Lukka M, Peltonen L. Recurrence of Marfan syndrome due to maternal germline mosaicism for a FBN1 mutation. Am J Hum Genet. In press 1998.
31. Mundlos S, Otto F, Mundlos C, Mullikien J, Aylsworth S, Albright D, et al. Mutation involving the transcription factor CBFA 1 cause cleidocranial dysplasia. Cell 1997;89:773–9.
32. Otto F, Thornell A, Crompton T, Denzel A, Gilmour K, Risewell I, et al. *Cbfa1*, a candidate gene for Cleidocranial Dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 1997;89:765–71.
33. Connor JM, Ferguson-Smith MA, editors. Essential medical genetics. Oxford: Blackwell Scientific; 1988.
34. Jones KL, Smith D, Harvey MA, Hall B, Quan L. Older paternal age and fresh gene mutation: data on additional disorders. J Pediatr 1975;86:84–8.
35. Gladwin AJ, Dixon J, Loftus SK, Edwards SJ, Wasmuth JJ, Hennekam RCM, et al. Treacher Collins syndrome may result from insertions, deletions or splicing mutations, which introduce an in-frame termination codon into the gene. Hum Mol Genet 1996;5:1533–8.
36. Edwards S, Gladwin A, Dixon M. The mutational spectrum in Treacher Collins syndrome reveals a predominance of mutations that create a premature-termination codon. Am J Hum Genet 1997;60:515–24.
37. Juriloff D, Harris M, Froster-Iskenius U. Hemifacial deficiency induced by a shift in dominance of the mouse mutation far: a possible genetic model for hemifacial microsomia. J Craniofac Genet Dev Biol 1987;7:27–44.
38. Wyszynski DF, Lewanda AF, Beaty TH. Phenotypic discordance in a family with monozygotic twins and non-syndromic cleft lip and palate. Am J Med Genet 1996;66:468–70.
39. Christensen K, Mitchell L. Familial recurrence pattern analysis of nonsyndromic isolated cleft palate—a Danish registry study. Am J Hum Genet 1996;58:182–90.
40. Lidral A, Romitti P, Basart A, Doetschman T, Leysens N, Daack-Hirsh S, et al. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. Am J Hum Genet 1998;63: 557–68.