

Genetics and Morphogenesis

Michael J. Dixon

Faculty of Life Sciences and Dental School, University of Manchester, UK

Summary: Facial development involves a complex and highly integrated series of events that are frequently disturbed resulting in a wide variety of craniofacial anomalies. Dissection of the molecular events underlying such conditions has provided insights into the fundamental mechanisms driving normal facial development and how these are disrupted in congenital anomalies. There are numerous craniofacial syndromes each of which provides a unique opportunity to study a particular aspect of morphogenesis. The inheritance patterns that underlie these conditions are variable and include Mendelian and non-Mendelian modes of transmission. The integration of molecular biology, cell biology, experimental embryology, human and mouse genetics has allowed the molecular pathology underlying an increasing number of these conditions to be elucidated. By consideration of Treacher Collins syndrome and some forms of cleft lip and cleft palate this review has its focus on the progress that has been made in recent years within this field. Nevertheless, despite this progress in understanding the molecular pathology of these (and other) disorders of facial development and the consequent increase in our knowledge of the fundamental mechanisms driving facial development, significant challenges remain.

Key words: Treacher Collins syndrome, cleft lip, cleft palate

Oral Biosci Med 2005; 213: 67-73

TREACHER COLLINS SYNDROME

Treacher Collins syndrome (TCS) is an example of a disorder of facial development where an integrated approach has significantly increased our understanding of the condition, but where, nevertheless, significant challenges remain to be met. TCS is an autosomal dominant disorder that affects approximately one in 50,000 live births (Rovin et al, 1964; Fazen et al, 1967). More than 60% of cases do not appear to have a previous family history and are thought to arise as the result of a *de novo* mutation (Jones et al, 1975). TCS is characterised by numerous developmental anomalies which include hypoplasia of the facial bones, particularly the mandible and zygomatic complex; downward slanting palpebral fissures with a coloboma of the lower eyelids and a paucity of lid lashes medial to the defect; and alterations in the shape, size and position of the external ears, which are frequently associated with atresia of the external auditory canals and abnormalities of the middle ear ossicles, leading to conductive hearing loss (Fig 1) (Phelps et al, 1981).

Although the genetic mutations underlying TCS are highly penetrant, the condition is characterised by marked inter- and intra-familial phenotypic variability (Dixon et al, 1994; Marres et al, 1995). Some individuals

are so mildly affected that it can be extremely difficult to establish an unequivocal diagnosis and to provide accurate genetic counselling on clinical grounds alone. Indeed, some patients are so mildly affected that they are only diagnosed after the birth of a more severely affected child. Together with the high rate of *de novo* mutations, this feature can result in significant challenges in providing genetic counselling.

On the basis that the tissues affected in TCS are derived from the first and second pharyngeal arches, which are populated extensively by cranial neural crest cells, several theories have been suggested to explain the cellular basis of this disorder. These include abnormal neural crest cell migration (Poswillo, 1975), improper cellular differentiation during development (Wiley et al, 1983) or an abnormality of the extracellular matrix (Herring et al, 1979). However, until the gene mutated in TCS was identified, there was no direct evidence to support any of these theories.

Gene Mutations

In the absence of a candidate gene or a mouse model for the disorder, the TCS locus (*TCOF1*) was mapped to human chromosome 5 using genetic linkage analysis (Dixon et al, 1991) and the mutated gene isolated using

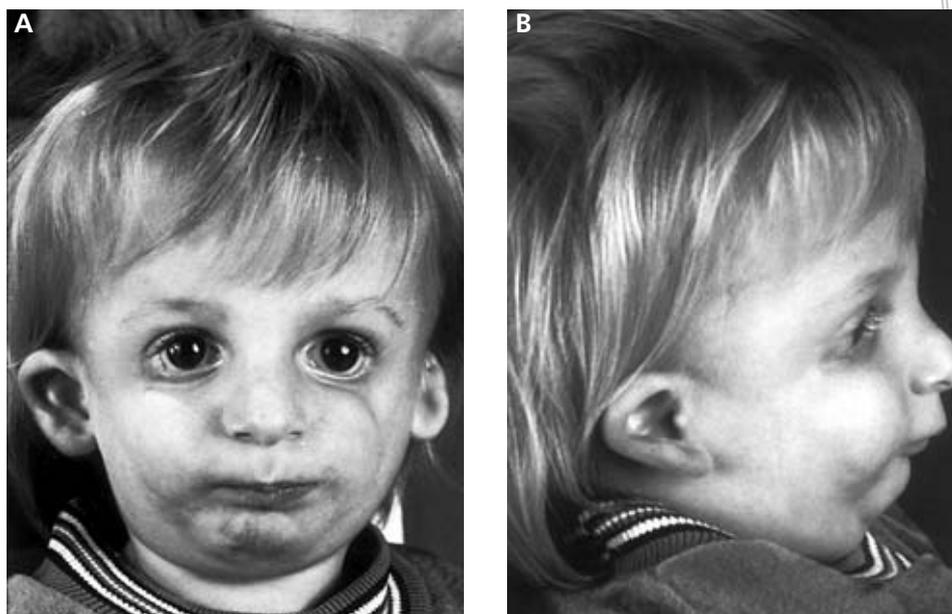


Fig 1 A patient with Treacher Collins syndrome. **(a)** A frontal view of the patient illustrates the colobomas and paucity of eyelid lashes on the medial side of the abnormality. Flattening of the zygomatic complex is apparent. **(b)** A lateral view of the same individual illustrates the mandibular hypoplasia and the abnormal external ear.

a positional cloning strategy (Treacher Collins Syndrome Collaborative Group, 1996). Subsequent analysis of *TCOF1* has resulted in the identification of over 120 mutations that are spread throughout the gene (Treacher Collins Syndrome Collaborative Group, 1996; Gladwin et al, 1996; Edwards et al, 1997; Wise et al, 1997; Splendore et al, 2000). While the mutations identified include splicing mutations, insertions and non-sense mutations, by far the majority are deletions, which range in size from one to 40 nucleotides. In nearly every case, the result of these mutations is to introduce a premature termination codon into the encoded protein, which has been named Treacle. These observations suggest that the mechanism underlying TCS is haploinsufficiency. Although the majority of mutations tend to be family-specific, the recurrent mutation nt4135 del(GAAAA), which occurs in exon 24 of *TCOF1*, accounts for 18.6% of mutations.

Diagnosis

Before molecular studies of TCS were commenced, pre-natal diagnosis was only possible using fetoscopy or ultrasound imaging (Nicolaidis et al, 1984; Meizner et al, 1991; Milligan et al, 1994; Cohen et al, 1995). While the quality of ultrasound imaging has improved markedly in recent years, allowing non-invasive prenatal diagnosis to be performed, it is still difficult to make a positive diagnosis, particularly where the fetus is mildly affected. Given these circumstances, the procedure is usually not diagnostic for apparently unaffected fetuses. Moreover, prenatal diagnosis using either fetoscopy or

ultrasound imaging is not possible until the second trimester of pregnancy. Conversely, with the identification of the *TCOF1* locus, molecular diagnosis in the first trimester of pregnancy became possible. However, genetic counselling of families with TCS is complicated by the fact that, in general, mutations between families are different. In addition, even if the fetus is found to carry the disease-causing mutation, no conclusions can be drawn about the severity with which the child will be affected. Therefore, parents may opt to delay any decision-making until ultrasound scanning can be used to provide further information on the extent of the abnormalities. However, for those cases in which the fetus does not carry the mutation, parents can be reassured that their child will be unaffected. Molecular analysis has also been utilised in the area of post-natal diagnosis of TCS, particularly in confirming the clinical diagnosis in mildly affected individuals and also in accurately counselling apparently unaffected parents of a child in which TCS has supposedly arisen as the result of a *de novo* mutation (Dixon et al, 2004).

Molecular Pathology

Identification of the TCS locus has not only impacted on patient management, but has also allowed us to learn more about the molecular pathology of the disorder. Using a combination of bio-informatics analyses, sub-cellular localisation studies and biochemical approaches, Treacle has been shown to be a nucleolar phosphoprotein. Immunocytochemical analysis with anti-Treacle antibodies revealed bright punctate staining in the dense fib-

rillar component of the nucleolus (Marsh et al, 1998; Winokur et al, 1998; Isaac et al, 2000). Similarly, western blotting indicated that anti-Treacle antibodies recognise a single protein band in cell lysates. Treatment of *in vitro* translated protein with alkaline phosphatase in the presence or absence of phosphatase inhibitors further demonstrated that the discrepancy between the theoretical molecular weight of Treacle (~140 kDa) and that observed by SDS-PAGE (~220 kDa) is due to a high degree of phosphorylation by casein kinase II (Isaac et al, 2000).

More recently, Treacle has been shown to be involved in ribosomal DNA transcription via an interaction with RNA polymerase I transcription factor upstream binding factor, UBF (Valdez et al, 2004). Subsequently, Treacle has been demonstrated to play a role in the 2'-O-methylation of pre-rRNA. The function of Treacle in pre-rRNA methylation is most likely mediated by its direct physical interaction with NOP56, a component of the ribonucleoprotein methylation complex. While Treacle co-localises with UBF throughout mitosis, it co-localises with NOP56 and fibrillarin, a putative methyl transferase, only during telophase when rDNA gene transcription and pre-rRNA methylation are known to commence (Gonzales et al, 2005). These observations suggest that Treacle might link RNA polymerase I-catalyzed transcription and post-transcriptional modification of pre-rRNA (Gonzales et al, 2005). It is therefore possible that haploinsufficiency of Treacle in TCS patients results in inhibition of production of properly modified mature rRNA in addition to inhibition of rDNA gene transcription, which consequently affects proliferation and proper differentiation of specific embryonic cells during development.

Developmental Aspects

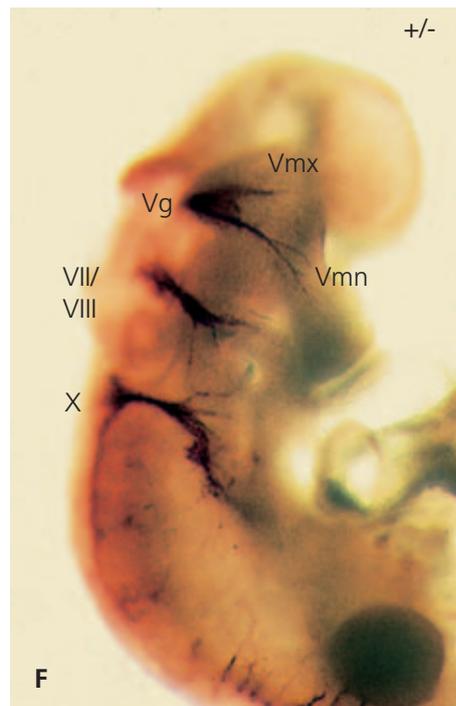
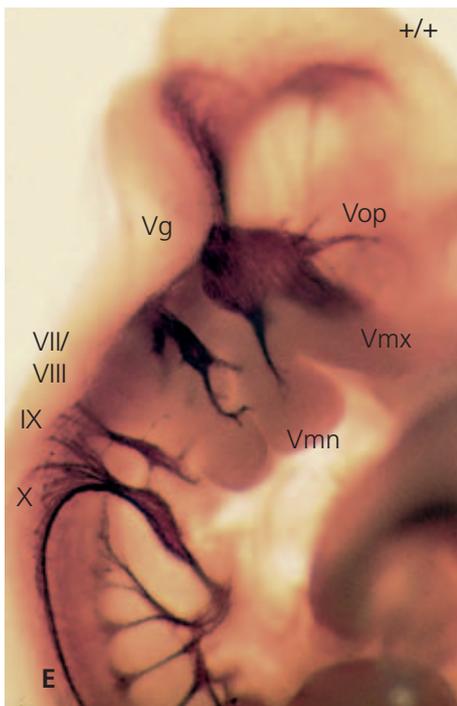
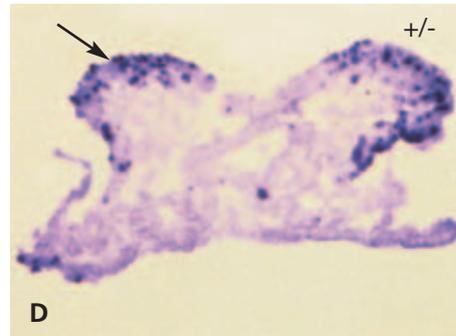
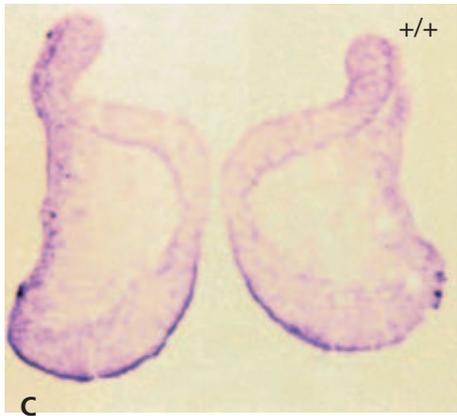
To investigate the developmental basis of TCS, research has focussed largely on the mouse. Expression analyses initially indicated that, although the gene is widely expressed, the highest expression levels are observed in the neural folds immediately prior to fusion, and in the developing first pharyngeal arch (Dixon et al, 1997). These studies laid the foundations for the use of gene targeting technology to study the function of the protein *in vivo*. To ensure complete functional inactivation of *Tcof1*, Dixon and co-workers replaced exon 1, which contains the translation initiation codon, with a neomycin resistance cassette via homologous recombination in embryonic stem cells (Dixon et al, 2000). The chimaeric animals generated using these cells were inter-crossed with C57BL/6 female mice. On a mixed 129-C57BL/6 background, confirmed *Tcof1* heterozygous (*Tcof1*^{+/-}) mice died shortly after birth as a result of severe craniofacial anomalies that included agenesis of

the nasal passages, abnormal development of the maxilla, exencephaly and anophthalmia (Dixon et al, 2000).

Developmental analysis showed that abnormalities were first detected in *Tcof1*^{+/-} mice at embryonic day 8 (E8). At E8.5, wild-type embryos displayed well-developed head folds and the first signs of optic development, whereas *Tcof1*^{+/-} embryos exhibited smaller, more rounded neural folds and an absence of the optic evagination. By E10.5, the formation of the olfactory pit in wild-type mice divided the frontonasal mass into medial and lateral nasal processes. In contrast, *Tcof1*^{+/-} embryos failed to develop either of these processes, and exhibited exencephaly with neuroepithelium protruding through the rostral neuropore. By E14.5, mutant mice displayed a lack of nasal passages, severe under-development of the middle third of the face and mandibular hypoplasia (Fig 2a, b). Skeletal analysis of E18 *Tcof1*^{+/-} mice revealed an absence of the frontal, parietal and inter-parietal bones of the vault of the skull. The nasal capsule and the maxilla were grossly malformed. The zygomatic arch, the tympanic ring and the middle ear ossicles were also hypoplastic and misshapen in *Tcof1*^{+/-} mice (Dixon et al, 2000).

As the phenotype suggested an abnormality of neural crest cell development, whole-mount TUNEL analysis, a technique that identifies cells undergoing apoptosis, was used by Dixon and co-workers (Dixon et al, 2000). This technique revealed that the number of apoptotic cells was markedly elevated in the *Tcof1* heterozygous embryos; in particular, the neuroepithelium of the cranial neural folds and the neural tube displayed a profusion of apoptotic cells (Fig 2c, d). The high levels of cell death in the neuroepithelium, together with the gross morphological phenotype, strongly suggested that a proportion of the premigratory neural crest cells were depleted. In addition, immunohistochemistry of E10.5 mice with an anti-neurofilament antibody indicated that the neural crest cell-derived cranial ganglia were severely hypoplastic, the ophthalmic branch of the trigeminal nerve and the glossopharyngeal ganglia/nerves were absent and the dorsal root ganglia were markedly disorganised (Fig 2e, f). These results suggest that the TCS phenotype results, at least in part, from a massive increase in apoptosis in the pre-fusion neural folds.

Subsequent studies focussed on breeding the *Tcof1* mutation onto different genetic backgrounds. The resulting offspring exhibited markedly variable strain-dependent phenotypes which ranged from extremely severe and lethal in a mixed CBA/Ca x 129 background, to apparently normal and viable in a mixed BALB/c x 129 background (Dixon and Dixon, 2004). It is therefore clear that the genetic background on which the *Tcof1* mutation resides is placed is a key determinant in the penetrance and severity of the resulting phenotype (Fig 2g, h). Intriguingly, analysis of RNA isolated from wild-



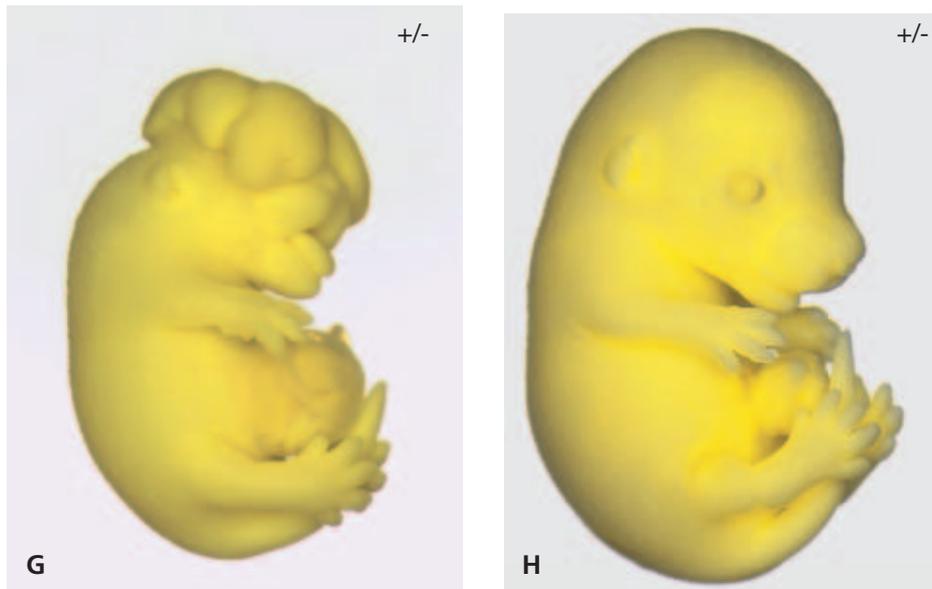


Fig 2 Analysis of wild-type (+/+) and *Tcof1* mutant heterozygous (+/-) mice. **(a, b)** At embryonic day 14.5, mutant mice display severe craniofacial anomalies that include agenesis of the nasal passages, abnormal development of the maxilla, exencephaly, and anophthalmia. **(c, d)** TUNEL analysis suggests that these abnormalities arise as the result of increased levels of apoptosis in the crests of the neural folds (an example of an apoptotic cell is arrowed). **(e, f)** Whole-mount immunohistochemical analysis of E10.5 wild-type and mutant mice using the anti-neurofilament marker, 2H3, reveals that the cranial ganglia, indicated by roman numerals, are poorly developed and disorganised in the mutant mice. In addition, the ninth cranial nerve is absent in *Tcof1*^{+/-} mice. Intriguingly, the background on which the *Tcof1* gene targeted mutation is placed is a key determinant in the penetrance and severity of the resulting phenotype which ranges from **(g)** extremely severe and lethal on a mixed CBA/Ca x 129 background, to **(h)** apparently normal and viable on a mixed BALB/c x 129 background.

type and *Tcof1*^{+/-} heterozygous mouse embryos from strains that exhibit a lethal phenotype showed a significant reduction in 2'-O-methylation of 18S rRNA; however, there was no significant difference in rRNA methylation between wild-type and *Tcof1*^{+/-} heterozygous embryos which have no obvious craniofacial phenotype (Gonzales et al, 2005). Continued investigation of the contribution of the genetic background to phenotypic variation in mice may, in the longer term, help us to understand the basis of the marked inter- and intra-familial variation observed in TCS families.

OROFACIAL CLEFTING

In contrast to TCS, orofacial clefting (OFC) is a common developmental genetic disorder that occurs with a prevalence that has been estimated at one in 500-2,500 live births depending on geographic origin, racial and ethnic variation, and socio-economic status (Vanderas et al, 1987; Murray et al, 2002). OFC is a significant cause of morbidity to affected individuals and their families. Individuals who exhibit OFC may experience problems with eating, speaking and hearing, which can be corrected to varying degrees by surgery, dental treatment, and speech therapy. In addition, the altered facial appearance may lead to psychosocial consequences. Owing to its complex mode of inheritance, non-syn-

dromic OFC has proven to be much less tractable to molecular genetic analysis than syndromic OFC. On the basis of familial clustering of affected individuals and an elevated concordance rate in monozygotic twins (25-40%) compared with their dizygotic counterparts (3-6%), there is compelling evidence for a strong genetic component to non-syndromic OFC (Mitchell and Risch, 1992). Despite these facts, few pedigrees conform to simple Mendelian patterns and many cases appear to be sporadic rather than familial. Moreover, OFC is genetically heterogeneous and thought to be influenced by environmental factors, such as phenytoin, retinoic acid derivatives, cigarette smoking and maternal nutrition, possibly in an interactive manner. An oligogenic model of inheritance is favoured with between two and 10 interacting loci having been proposed, although several theoretical studies have supported the role of a major susceptibility locus. Nevertheless, the relative contribution of the individual susceptibility loci is likely to vary between different populations (Mitchell and Risch, 1992; Farrall and Holder, 1992; Fitzpatrick and Farrall, 1993; Christensen and Mitchell, 1996). These combined factors preclude a classical positional cloning approach for the vast majority of cases. Genetic approaches to non-syndromic OFC have therefore utilised extrapolation from the study of syndromic forms of OFC, parametric-based genetic linkage analysis, non-parametric

affected sib-pair approaches, chromosomal analysis, and candidate gene-based association studies.

Syndromes

Van der Woude syndrome (VWS) is an autosomal dominant disorder that represents the most common form of syndromic OFC, accounting for 2% of all cases (Rintala and Ranta, 1981). Importantly, the clinical features of VWS, which include OFC (both cleft lip and cleft palate), paramedian lower lip pits, and hypodontia, closely mirror those observed in non-syndromic forms of OFC. Recently, the VWS locus, which maps to chromosome 1q32-q41, has been identified as interferon regulatory factor 6 (IRF6) (Kondo et al, 2002). Moreover, popliteal pterygium syndrome (PPS), a disorder with a similar orofacial phenotype plus additional skin and genital anomalies, is allelic with VWS (Kondo et al, 2002).

Members of the IRF family are characterised by a highly conserved N-terminal DNA binding domain (DBD) and a less well conserved protein interaction domain. The signature motif of the DBD is a tryptophan repeat consisting of five residues spaced at 10-18 amino acid intervals. Protein truncation mutations are significantly more common in VWS than in PPS, consistent with the observation that deletions of IRF6 lead to VWS rather than PPS (Bocian and Walker, 1987; Sander et al, 1994; Schutte et al, 1999) and strongly supporting haploinsufficiency as the mechanism underlying VWS. The mis-sense mutations that have been observed in VWS and PPS fall into two distinct categories. Whereas the mis-sense mutations that cause VWS are almost evenly divided between the two domains, most mis-sense mutations that cause PPS are found in the DBD, suggesting that VWS- and PPS-causing mis-sense mutations affect IRF6 function differently. Comparison with the crystal structure of the IRF1 DBD indicated that every amino acid residue mutated in PPS individuals directly contacts DNA whereas only one of eight of the residues mutated in VWS individuals contacts DNA (Kondo et al, 2002). Strikingly, a mis-sense mutation of arginine 84 was observed in seven unrelated PPS families. This residue is one of four that makes critical contacts with the core sequence, GAAA, and is essential for DNA binding. The observed change of this residue to a cysteine or histidine is predicted to cause a complete loss of that essential contact. One possible explanation for this apparent genotype/phenotype relationship is that mis-sense mutations which cause VWS are due to a complete loss of function of the mutated IRF6 protein, both DNA and protein binding, whereas mis-sense mutations causing PPS affect only its ability to bind DNA. The ability of IRF6 to bind to other proteins is unaffected, therefore it can act as a dominant negative molecule. Intriguingly, polymorphic variation within IRF6, at least in a subset of populations, is a significant contributor to

OFC and increases the recurrence risk in families that already have an affected child (Zuccherro et al, 2004).

To provide insights into the potential role of IRF6 during embryogenesis, the expression of this molecule during mouse and chick facial development has been analysed. IRF6 was found to be expressed in the ectoderm covering the facial processes during their fusion to form the upper lip and primary palate in both mouse and chick. Conversely, while *Irf6* was expressed in the medial edge epithelia of the developing secondary palate of the mouse, which fuses as in man, *Irf6* was not expressed in the medial edge epithelia of the naturally cleft chick secondary palate (Dixon, unpublished data). Together these results support a role for IRF6 during the fusion events that occur during development of the lip and palate. Studies to address the role of IRF6 *in vivo* are ongoing.

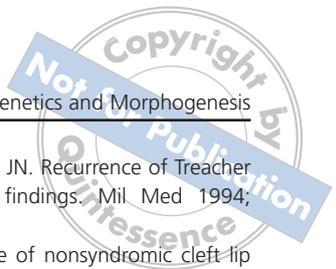
Despite the advances that have been made in understanding some forms of cleft lip and cleft palate, the genetic variation underlying the non-syndromic forms and the environmental influences that contribute to these distressing conditions remain poorly characterised. Nevertheless, the application of modern gene discovery techniques applied to clinically well characterised families, particularly when coupled with innovative approaches for the selection and analysis of candidate genes and a more detailed knowledge of the cascade of molecular events driving development of the lip and palate, is likely to impact significantly on our ability to dissect the molecular pathology of cleft lip and cleft palate.

ACKNOWLEDGEMENTS

I would like to thank the patients and their families who have provided samples and an endless source of inspiration over the past 15 years, and the clinicians who have collected the samples. I would also like to thank the people that have worked alongside me during these projects; in addition to members of my own laboratory, I should particularly like to thank Jeff Murray, Brian Schutte and Benigno Valdez. The financial support for this research has been generously provided by many different funding agencies including the Wellcome Trust, the Medical Research Council, the National Institute for Dental and Craniofacial Research, the European Union and the Birth Defects Foundation.

REFERENCES

- Bocian M, Walker AP. Lip pits and deletion 1q32-q41. *Am J Med Genet* 1987;26:437-443.
- Christensen K, Mitchell LE. Familial recurrence-pattern analysis of non-syndromic isolated cleft palate - a Danish Registry study. *Am J Hum Genet* 1996;58:182-190.
- Cohen J, Ghezzi F, Goncalves L, Fuentes JD, Paulyson KJ, Sherer DM. Prenatal sonographic diagnosis of Treacher Collins syndrome: a case and review of the literature. *Am J Perinatol* 1995;12:416-419.



- Dixon MJ, Read AP, Donnai D, Colley A, Dixon J, Williamson R. The gene for Treacher Collins syndrome maps to the long arm of chromosome 5. *Am J Hum Genet* 1991;49:17-22.
- Dixon MJ, Marres HAM, Edwards SJ, Dixon J, Cremers CWRJ. Treacher Collins syndrome: Correlation between clinical and genetic linkage studies. *Clin Dysmorph* 1994;3:96-103.
- Dixon J, Hovanes K, Shiang R, Dixon MJ. Sequence analysis, identification of evolutionary conserved motifs and expression analysis of murine TCOF1 provide further evidence for a potential function for the gene and its human homologue, *TCOF1*. *Hum Mol Genet* 1997;6:727-737.
- Dixon J, Brakebusch C, Fässler R, Dixon MJ. Increased levels of apoptosis in the pre-fusion neural folds underlie the craniofacial disorder, Treacher Collins syndrome. *Hum Mol Genet* 2000;10:1473-1480.
- Dixon J, Dixon MJ. Genetic background has a major effect on the penetrance and severity of craniofacial defects in mice heterozygous for the gene encoding the nucleolar protein Treacle. *Dev Dyn* 2004;229:907-914.
- Dixon J, Ellis I, Bottani A, Temple K, Dixon MJ. Identification of mutations in TCOF1: Use of molecular analysis in the pre- and postnatal diagnosis of Treacher Collins syndrome. *Am J Med Genet* 2004;127A:244-248.
- Edwards SJ, Gladwin AJ, Dixon MJ. The mutational spectrum in Treacher Collins syndrome reveals a predominance of mutations that create a premature termination codon. *Am J Hum Genet* 1997;60:515-524.
- Fazen LE, Elmore J, Nadler HL. Mandibulo-facial dysostosis (Treacher Collins syndrome). *Am J Dis Child* 1967;113:406-410.
- Farrall M, Holder S. Familial recurrence-pattern analysis of cleft lip with or without cleft palate. *Am J Hum Genet* 1992;50:270-277.
- FitzPatrick D, Farrall M. An estimation of the number of susceptibility loci for isolated cleft palate. *J Craniofac Genet Dev Biol* 1993;13:230-235.
- 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 a termination codon into the gene. *Hum Mol Genet* 1996;5:1533-1538.
- Gonzales B, Henning D, So RB, Dixon J, Dixon MJ, Valdez BC. The Treacher Collins syndrome (TCOF1) gene product is involved in pre-rRNA methylation. *Hum Mol Genet* 2005;14:2035-2043.
- Herring SW, Rowlatt UF, Pruzansky S. Anatomical abnormalities in mandibulofacial dysostosis. *Am J Med Genet* 1979;3:225-259.
- Isaac C, Marsh KL, Dixon J, Paznekas W, Dixon MJ, Jabs EW, et al. Characterization of the nucleolar gene product of Treacher Collins syndrome in patient and control cells. *Mol Biol Cell* 2000; 11:3061-3071.
- Jones KL, Smith DW, Harvey MA, Hall BD, Quan L. Older paternal age and fresh gene mutation: data on additional disorders. *J Pediat* 1975; 86:84-88.
- Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, et al. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nat Genet* 2002;32:285-289.
- Marres HAM, Cremers CWRJ, Dixon MJ, Huygen PLM, Joosten FBM. The Treacher Collins syndrome: A clinical, radiological and genetic linkage study on two pedigrees. *Archs Otol* 1995;121:509-514.
- Marsh KL, Dixon J, Dixon MJ. Mutations in the Treacher Collins syndrome gene lead to mislocalisation of the nucleolar protein Treacle. *Hum Mol Genet* 1998;11:1795-1800.
- Meizner I, Carmi R, Katz M. Prenatal ultrasonic diagnosis of mandibulofacial dysostosis [Treacher Collins syndrome]. *J Clin Ultrasound* 1991;19:124-127.
- Milligan DA, Harlass FE, Duff P, Kopelman JN. Recurrence of Treacher Collins syndrome with sonographic findings. *Mil Med* 1994; 159:250-252.
- Mitchell LE, Risch N. Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: a reanalysis. *Am J Hum Genet* 1992;51:323-332.
- Murray JC. Gene/environment causes of cleft lip and/or palate. *Clin Genet* 2002;61:248-256.
- Nicolaides KH, Johansson D, Donnai D, Rodeck CH. Prenatal diagnosis of mandibulofacial dysostosis. *Prenat Diagn* 1984;4:201-205.
- Phelps PD, Poswillo D, Lloyd GAS. The ear deformities in mandibulofacial dysostosis (Treacher Collins syndrome). *Clin Otolaryngol* 1981;6:15-28.
- Poswillo D. The pathogenesis of Treacher Collins syndrome (mandibulofacial dysostosis). *Br J Oral Surg* 1975;13:1-26.
- Rintala AE, Ranta R. Lower lip sinuses. I. Epidemiology, microforms and transverse sulci. *Br J Plast Surg* 1981;34:26-30.
- Rovin S, Dachi SF, Borenstein DB, Cotter WB. Mandibulofacial dysostosis, a familial study of five generations. *J Pediat* 1964;65:215-221.
- Sander A, Schmelzle R, Murray J. Evidence for a microdeletion in 1q32-41 involving the gene responsible for Van der Woude syndrome. *Hum Mol Genet* 1994;3:575-578.
- Schutte BC, Basart AM, Watanabe Y, Laffin JJS, Coppage K, Bjork BC, et al. Microdeletions at chromosome bands 1q32-q41 as a cause of Van der Woude syndrome. *Am J Med Genet* 1999;84:145-150.
- Splendore A, Silva EO, Alonso LG, Richieri-Costa A, Alonso N, Rosa A, et al. High mutation detection rate in TCOF1 among Treacher Collins syndrome patients reveals clustering of mutations and 16 novel pathogenic changes. *Hum Mutation* 2000;16:315-322.
- The Treacher Collins Syndrome Collaborative Group. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. *Nat Genet* 1996;12:130-136.
- Valdez BC, Henning D, So RB, Dixon J, Dixon MJ. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. *Proc Nat Acad Sci USA* 2004;101:10709-10714.
- Vanderas AP. Incidence of cleft lip, cleft palate, and cleft lip and palate among races: a review. *Cleft Palate J* 1987;24:216-225.
- Wiley MJ, Cauwenbergs P, Taylor IM. Effects of retinoic acid on the development of the facial skeleton in hamsters: Early changes involving cranial neural crest cells. *Acta Anat* 1983;116:180-192.
- Winokur ST, Shiang R. The Treacher Collins syndrome (TCOF1) gene product, Treacle, is targeted to the nucleolus by signals in its C-terminus. *Hum Mol Genet* 1998; 7:1947-1952.
- Wise CA, Chiang LC, Paznekas WA, Sharma M, Musy MM, Ashley JA, et al. TCOF1 encodes a putative nucleolar phosphoprotein that exhibits mutations in Treacher Collins Syndrome throughout its coding region. *Proc Natl Acad Sci USA* 1997;94:3110-3115.
- Zuccherro TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *New Eng J Med* 2004;351:769-780.

Reprint requests:

Michael J. Dixon BDS, PhD, FDSRCS (Eng)
 Faculty of Life Sciences and Dental School
 University of Manchester
 Oxford Road
 Manchester M13 9PT, UK
 E-mail: mike.dixon@manchester.ac.uk