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SCIENTIFIC SECTION

Mutation testing in Treacher Collins Syndrome

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Abstract

Objective: To report on a study where 97 subjects were screened for mutations in the Treacher Collins syndrome (TCS) gene *TCOF1*.

Method: Ninety-seven subjects with a clinical diagnosis of TCS were screened for potential mutations in *TCOF1*, by means of single strand conformation polymorphism (SSCP) analysis. In those subjects where potential mutations were detected, sequence analysis was performed to determine the site and type of mutation present.

Results: Thirty-six TCS-specific mutations are reported including 27 deletions, six-point mutations, two splice junction mutations, and one insertion/deletion. This brings the total number of mutations reported to date to 105.

Conclusion: The importance of detection of these mutations is mainly in postnatal diagnosis and genetic counselling. Knowledge of the family specific mutation may also be used in prenatal diagnosis to confirm whether the foetus is affected or not, and give the parents the choice of whether to continue with the pregnancy.

Index words:
Mutation detection in TCS.

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Introduction

Treacher Collins syndrome (TCS) can be of interest to orthodontists for several reasons. The first is that the orthodontist may be involved in the management of the patient with TCS, either through orthodontic camouflage or orthognathic surgery. From a broader viewpoint, subjects with TCS and other craniofacial syndromes provide an opportunity to study major developmental disturbances of the face. By discovering what has gone wrong in such cases, it may be possible to gain a better understanding of the mechanisms involved in normal facial development.

Features

The main features of TCS include:

- abnormalities of the external and middle ear, leading to conductive deafness;
- hypoplasia of the mandible and zygomatic bones;
- downward slanting of the eyes and colobomas (absence of tissue, or 'notching') of the outer third of the lower eyelid and cleft palate.

Features that are of particular interest to the orthodontist include the unusually shaped mandible, which is often small and has a particularly broad concave curvature of the inferior border. The second is the temporomandibular joint, which in severe cases of TCS may be almost absent. The third is cleft palate that has been reported as between 28[1] and 35 per cent of subjects with TCS.[2–4] Macrostomia is observed in approximately 15 per cent of cases. Most commonly, TCS patients have a Class II skeletal pattern, where the lower jaw is smaller than the upper jaw, with variable degrees of severity. The Class II antero-posterior discrepancy is often accompanied by a vertical skeletal discrepancy that manifests itself as an anterior open bite.

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The TCS gene (*TCOF1*) and treacle

The gene mutated in TCS has been named *TCOF1* (The Treacher Collins Syndrome Collaborative Group, 1996).[5] *TCOF1* encodes a protein that is 1411 amino acids in length and has been named treacle.[6,7] The function of treacle is as yet unknown, but has been proposed to be involved in the shuttling of proteins between the nucleolus and cytoplasm, although there is currently no experimental evidence to support this hypothesis.[8,9]

Mutations are changes in the coding sequence of a gene that will affect the protein derived from that gene. Mutations in the TCS gene have previously been reported by several authors,[5,7,10–12] and can be predicted to have an affect on the structure and function of treacle. [5,7,10,11,12]

Patients and methods

This study investigated 97 patients with a history of TCS for mutations. Subjects were eligible for inclusion if they displayed two or more clinical features of TCS as determined by a clinician. DNA samples were received from clinicians worldwide including: UK, USA, Brazil, Holland, France, Germany, Sweden, Denmark, and Ireland. The DNA was amplified using the polymerase chain reaction (PCR) then screened using single strand conformation polymorphism analysis (SSCP), following the methodology described by Edwards and coworkers.[11] Once a potential mutation was identified in this way, sequence analysis was carried out to determine the mutation type and location. Where possible family members were also screened.

Results

SSCP screening revealed potential pathogenic mutations in 52 of the 97 patients in this study. The exons where the SSCP shifts occurred were subsequently subjected to sequence analysis to identify the type and location of the mutation. This process led to the detection of 36 mutations distributed throughout the 25 exons of *TCOF1* (Table 1). These mutations range from a single base change, for example, the nonsense mutation Q252X in exon 7 to a 40-base pair deletion in exon 20. The remaining 16 patients who had SSCP shifts were found to have neutral polymorphisms, i.e. single base substitutions that did not change the amino acid encoded.

The most commonly found mutations were deletions, 27 of which were detected in this study. An example of a single base pair deletion is nt41 del(T), which was found in exon 1. Here the deletion of T in codon 13 causes the wild type sequence of CTG to change to CGA. This results in a premature termination codon being introduced into the protein at codon 30. This is likely to lead to an alteration of function of the truncated protein.

Two unusually large mutations were found in this study. The first was the deletion of 29 base pairs in exon 10, which resulted in the removal of nine amino acid residues and the introduction of a termination codon after a further 18 residues. The largest mutation occurred in exon 20, where 40 nucleotides were deleted. This mutation removes a total of 13 amino acid residues and introduces a premature termination codon in exon 22.

Table 1 Mutations found in this study.

Location	Mutation
Exon 1	nt28del(C)
Exon 1	nt41del(T)
Exon 1	nt44 del(ACC)
Exon 2	A41V
Exon 2	Q55X
Exon 2	W53C
Exon 5	nt513 del(AG)
Exon 7	nt658 del(A)
Exon 7	nt689(CCAAGGGACCC)→(GGAGGCCT)
Exon 7	Q252X
Exon 7	nt786 del(AG)
Exon 7	nt786 del(AG)
Exon 10	nt1334 del(29bp)
Exon 12	nt1879 del(GAGAA)
Exon 12	S617X
Exon 15	nt2259 del(A)
Exon 15	nt2355 del(AG)
Exon 16	nt2428–2(A→C)
Exon 16	nt2497del(G)
Exon 20	nt3102 del(40bp)
Exon 20	Q1087X
Exon 23	nt3792 del(C)
Exon 23	nt3853 del (GAA)
Exon 23	nt3941 del(AG)
Exon 23	nt4095 del (GAA)
Exon 23	nt4108 del(AA)
Intron 23	nt4111+1 G→T
Exon 24	nt4127–34 del(A)
Exon 24	nt4135 del(GAAAA)
Exon 24	nt4135 del(GAAAA)
Exon 24	nt4135 del(GAAAA)
Exon 24	nt4135 del(GAAAA)
Exon 24	nt4135 del(GAAAA)
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Exon 24	nt4135 del(GAAAA)

The deletion nt4135 del(GAAAA) was one of only two mutations found in more than one individual in this study. This five base pair deletion was found in eight patients and results in a premature stop codon after 13 codons. The truncated protein produced would have nine amino acids less than the normal protein, however, due to the mechanism of nonsense mediated mRNA degradation, where proteins which are incomplete are broken down within the cell, may lead to complete loss of the protein product. The importance of detection of this mutation in so many individuals is of importance in mutation screening and will be discussed further later.

Four nonsense mutations were detected in this study. Nonsense mutations are single nucleotide changes that introduce a premature stop codon and, hence, terminate translation of the protein at the point at which they occur. The location of the nonsense change will affect the size of the protein product, with those occurring in the exons close to the 5′ end of the gene, resulting in shorter proteins for example the nonsense mutation Q55X found in exon 2, whilst those occurring close to the 3′ end resulting in longer, but still truncated proteins, for example, Q1087X found in exon 20. The net effect of these mutations is, however, likely to be the same.

Two mis-sense mutations were detected in this study. A mis-sense change is one where the single base change alters the amino acid encoded. Mis-sense changes can have variable effects on the protein encoded, dependant on the similarity of the amino acids substituted and the site of the change. The mis-sense mutation W53C in exon 2 will result in an amino acid change from tryptophan to cysteine. This is likely to cause a change in the overall structure of the protein due to the differences in structure of the amino acids in question.

Two splice-junction mutations were detected in this study, one splice acceptor mutation and one splice donor mutation. Splicing mutations occur within introns and affect the joining of exons, and hence the protein formed. The first splicing mutation occurred in the splice acceptor site at the 3′ end of exon 16. A mutation at this site can have one of two effects; splicing may not occur at all and therefore the intron sequence would then be included in the final transcript; alternatively, the mutation may result in skipping of the exon downstream from this site.

The second splicing mutation occurred at location nt4111+1, i.e. at the first nucleotide of intron 23. This changes the splice donor sequence. There are two theories as to the effect this may have; the first is that the mutation may cause a failure in recognition of the splice site, leaving the intron as part of the final mRNA product,[13] the second is that the mutation may cause a shift in the splice junction by one base. This would allow splicing, but introduce a frame shift, thus changing the amino acid sequence downstream from the mutation site. Because RNA was not available for study, it was not possible to determine the effect of the mutation in either of the splicing mutations detected.

Several families were examined for mutation in this study and give good examples of the use of mutation testing for genetic counselling. The first family studied contained five members, four of whom were clinically unequivocally affected by TCS. In the fifth family member, however, the clinical diagnosis was uncertain. Analysis of one of the children in the family resulted in the identification of the single nucleotide deletion mutation, nt28del(C), in exon 1. This mutation causes a premature termination codon only three amino acids downstream and also deletes a restriction site for *Alu*I. It was therefore possible to confirm the presence or absence of the mutation in the remaining family members by using SSCP and restriction digestion with this enzyme. These analyses indicated that all five family members shared the same banding pattern and, hence, confirmed that the fifth family member carried the same mutation. This information has important consequences for genetic counselling for this individual.

In a second family of three individuals, the child was clinically diagnosed with TCS; however, both parents showed no clinical features suggesting a *de novo* mutation in this individual. The mutation in the affected child was found to be the two nucleotide deletion, nt786 del(AG), in exon 7. After identifying this mutation it was possible to screen both parents using SSCP and PAGE analysis. This showed no change in banding pattern and, hence, confirmed that the mutation had occurred *de novo*.

Discussion

The 36 mutations in the present study bring the total number of mutations detected so far to 105.[5,7,10–12] The majority of these mutations are deletions (74 out of 105). The deletion mutation nt4135 del(GAAAA) in exon 24 is the most commonly reported mutation in the *TCOF1* gene. This mutation has the effect of introducing a premature stop codon in the subsequent sequence, terminating transcription after 29 amino acids. This mutation represents 20% of *TCOF1* mutations reported

so far. Since most mutations in the TCS gene are family specific, this represents an unusual finding. This has led to this location as being described as a mutational 'hotspot', i.e. a point in the gene where significantly increased numbers of mutations occur. One possible explanation for this phenomenon is that the families in whom this mutation occur are related; however, previous halotype analysis of several of these families has shown this not to be the case.[11] A further explanation for the increased frequency of mutations at this location is that the sequence in this area is repetitive and therefore may be subject to increased errors in transcription. Interestingly, the five nucleotides immediately preceding this deletion are also deleted in a different family.[5] This supports the observation that this region may be prone to replication errors. The clinical implication of such a 'hotspot' is that when trying to detect the mutation in a given family, SSCP analysis should be started in this area. However, if an SSCP shift is not found at this site then systematic screening of the other exons is necessary.

In this study a 40 base pair deletion was found in exon 20, this is by far the largest sequence change detected in *TCOF1* to date. Sequence changes reported previously have tended to be small with deletions ranging from between only one and ten base pairs. Like most deletions this 40 base pair deletion results in the introduction of a premature termination codon. The significance of the particularly large size of the deletion is unknown.

No insertion mutations were found in this study. This is unusual since insertions are the second most commonly reported mutation type (11 out of 105). Insertions, like deletions, cause a frame shift, which normally results in a premature termination codon at variable distances downstream from the mutation site.

Nonsense and mis-sense mutations both occur as result of a single base change, but have very different affects on the final protein product. Nonsense mutations, which have now been reported nine times, lead to an immediate termination of translation, causing a shortened protein. The length of the protein will depend on how early or late in the coding sequence the mutation occurs. However, it is likely that the shortened protein will be degraded due to the process of nonsense mediated protein degradation.[13] By contrast, mis-sense mutations, which have now been reported four times, result in a normal length protein, but with one of the amino acids changed. The mis-sense mutation W53C in exon 2 will result in an amino acid change from tryptophan to cysteine, two dissimilar amino acids. Interestingly, a mis-sense mutation that affects the same codon has already been reported. Mutation W53R was found by Edwards *et al*. and results in a tryptophan to arginine substitution.[11] Both these mutations are likely to lead to a structural change in the protein, however, since these mis-sense mutations occur close to the end of exon 2 and there is a small possibility that these mutations could interfere with splicing.

The TCS gene has been found to be highly polymorphic,[7,12] with single nucleotide changes occurring every 210 base pairs.[7] Consideration must therefore be given to the question of whether the mis-sense changes found in this study are actually disorder causing.

Consequences of mutation detection

The importance of detection of mutations in the TCS gene are two-fold. The first area and the one where mutation detection is likely to be of greatest value, is in postnatal diagnosis in family members. In a family with an affected individual, the identification of the site and type of mutation can allow rapid and accurate screening of all family members. This information allows informed genetic counselling, particularly of parents and siblings. For the apparently unaffected parent with an affected child, this can have a significant impact on the decision to have further children. If the mutation is found to have arisen *de novo*, there is a considerably reduced risk of a second TCS child, although the possibility of germinal mosaicism may still mean there is a small (2–3 per cent) risk. However, if the mutation is detected in either of the parents, there is a 50 per cent risk for each future child. Examples of families where postnatal diagnosis has allowed such genetic counselling have been illustrated in this paper.

The second area where mutation testing is of value is in prenatal diagnosis. Previously, prenatal diagnosis of TCS has been reported by means of sonography,[14,15] fetoscopy[16] and combined linkage analysis and ultrasound.[17] Direct mutation testing has advantages over previous prenatal TCS diagnostic techniques in that where the family specific mutation site is already known, simple and accurate diagnosis is possible early in pregnancy. Families with small numbers can be studied as well as the larger families on whom it was possible to perform linkage analysis.[17] Mutation analysis does, however, have several limitations. The first is the risk of obtaining the foetal sample for analysis that occurs with chorionic villus sampling. The second is that mutation testing gives no indication of the degree of severity of the syndrome. The third is because most mutations are

family specific prior screening of at least one family member is necessary to identify the area of the *TCOF1* gene to study. Molecular prenatal diagnosis cannot be offered in families where prior mutational screening has not been carried out at this present time.

Conclusions

This study reports 36 mutations in the TCS gene. The detection of these mutations can now be used for genetic counselling of individuals and their families both to confirm the presence of the syndrome in mildly affected individuals and to predict the chances of passing on the syndrome to future offspring. In addition, now that the family specific site is known in these individuals, if a prenatal diagnosis is requested rapid testing can be carried out to predict whether the foetus is affected or unaffected.

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