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A functional SNP in the promoter region of *TCOF*1 is associated with reduced gene expression and YY1 DNA–protein interaction

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Abstract

Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial malformation caused by null mutations in the *TCOF*1 gene. High inter and intra familial clinical variability, ranging from mild malar hypoplasia to perinatal death due to airway collapse is observed, but, to date, no genotype–phenotype correlation has been reported. Considering haploinsufficiency as the molecular mechanism underlying the disease, we have hypothesized that mutations in the promoter region of the gene, which has never been previously characterized, in *trans* with a pathogenic mutation, could modulate the phenotype. Therefore, the aims of the present study were to determine the *TCOF*1 gene's core promoter and to identify mutations in this region that could contribute to the phenotypic variation observed in this syndrome. We have delimitated the minimal promoter to a region of less than 150 bp, with 63% of identity among 5 different species. We screened 1.2 kbp of the *TCOF*1 5′flanking sequence in the DNA obtained from 21 patients and 51 controls and identified four new single nucleotide polymorphisms (SNPs), one of which (−346C>T), was proved to be functional, as it decreased the promoter activity by 38%. Electrophoretic mobility shift assay (EMSA) analysis demonstrated that the −346T allele impairs DNA-binding to the YY1 transcription factor. This promoter variant represents a candidate allele to explain the clinical variability in patients bearing TCS.

Keywords: *TCOF*1 promoter; Clinical variability in Treacher Collins syndrome; Functional SNP; Modifier allele

1. Introduction

Treacher Collins syndrome (TCS; OMIM 154500) is a craniofacial disorder that affects the development of structures derived from the embryonic first and second branchial arches (Poswillo, 1975). TCS is inherited in an autosomal dominant pattern with 60% of the cases resulting from de novo mutations (Splendore et al., 2003). TCS has variable expressivity and high penetrance, since only one unequivocal case of non-penetrance has been reported to date (Dixon et al., 1994). The syndrome

Abbreviations: *TCOF*1, Treacher Collins–Franceschetti syndrome gene; YY1, Ying Yang 1 transcription factor; EMSA, Eletrophoretic Mobility Shift Assay; SNP, Single Nucleotide Polymorphisms; SSCP, Single Strand Conformation Polymorphism; dHPLC, Denaturing High-Performance Liquid Chromatography; (prime), denotes a truncated gene at the indicated side; UBF, Upstream Binding Factor; bp, basepairs; kb, 1000 basepairs; cpm, counts per minute.

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has an incidence of 1:50.000 live births and the common clinical features include down slanting palpebral fissures with lower eyelid coloboma, hypoplasia of the mandible and the zygomatic complex, malformed ears and conductive hearing loss due to atresia of the external ear canal (Gorlin et al., 2001).

The gene associated with the syndrome, *TCOF*1, contains 28 exons and several alternative splices (The Treacher Collins Collaborative Group, 1996; Edwards et al., 1997; So et al., 2004). The *TCOF*1 product, a nucleolar protein named treacle, plays a role in ribosome biogenesis and interacts with hNop56p in pre-ribosomal ribonucleoprotein complexes and also with the RNA polymerase I transcription factor UBF (Hayano et al., 2003; Valdez et al., 2004). More than 118 different pathogenic mutations were reported in the coding region of *TCOF*1, most of which are family-specific deletions or duplications causing a premature stop codon (Splendore et al., 2005). Absence of the truncated protein in fibroblasts from TCS patients suggests that null mutations in *TCOF*1 probably lead to nonsense mediated mRNA decay (Isaac et al., 2000). Therefore, haploinsufficiency has been proposed as the molecular mechanism underlying the disorder. Identification of families with no pathogenic mutation in the coding region of *TCOF*1 has suggested the possibility of genetic heterogeneity or the existence of different mechanisms leading to the syndrome (Isaac et al., 2000; Splendore et al., 2000, 2002, 2005).

TCS has a high intra and interfamilial phenotypic variation, ranging from perinatal death, due to compromised airways, to individuals that cannot be diagnosed on clinical grounds alone. Several attempts to evaluate the clinical variability in TCS have demonstrated no genotype–phenotype correlation in the syndrome (Edwards et al., 1997; Gladwin et al., 1996; Dixon, 1996). It has also been shown that clinical variability does not depend on the type or location of the mutation, sex or on whether the case is a sporadic or a familial one (Splendore et al., 2000; Teber et al., 2004). A recent study has demonstrated that the genetic background has a major effect on the phenotype of *Tcof*1$^{+/-}$ mice, suggesting that variations in other genes in the same or related pathways affect the function of the protein in different individuals (Dixon and Dixon, 2004).

We hypothesized that clinical variability can be determined by the degree of *TCOF*1 expression regulated by the wild-type allele. Several possible mechanisms could account for variations in treacle's expression, including mutations in the promoter region of the *TCOF*1. In this regard, we have hypothesized that mutations in *cis*-acting elements of the wild-type allele could modulate the phenotype in TCS patients. In the present work, we aimed at delimitating the *TCOF*1 gene's minimal promoter region, identifying functional SNPs in 1.2 kbp of the 5′ upstream region and evaluate any genotype–phenotype correlation in TCS patients.

2. Materials and methods

2.1. Patients and controls

Twenty-one Brazilian unrelated TCS patients with previously identified pathogenic mutation (Splendore et al., 2000, 2002, 2003, 2005) were screened for sequence variation in the promoter region of *TCOF*1. For each identified variation, at least 51 controls were screened. For the −346C>T SNP, 13 additional patients and 162 controls were included in the screening, since this SNP initially exhibited a higher frequency (>20%) in patients as compared to controls. We also screened three other patients (2 familial cases and 1 sporadic) for whom no pathogenic mutation in the coding region of the gene was detected through sequencing or Southern blot analysis (Splendore et al., 2005).

DNA was extracted according to standard techniques (Miller et al., 1988; Richards et al., 1993). The study protocols were previously approved by the ethical committee of our institution and informed consent was obtained from both patients and control subjects or from their legal tutors.

2.2. Mutation screening of the TCOF1 promoter region

The 5′ flanking sequence from the human *TCOF*1 gene was obtained from the Homo sapiens chromosome 5 genomic contig (GenBank accession no. NT_029289) after a BLASTn (http://www.ncbi.nlm.nih.gov/blast/) search, using the exon 1 sequence (NM_000356). Four overlapping segments (P1, P2, P3 and P4), corresponding to 1241 bp of the upstream sequence relative to the first methionine (NT_029289), were amplified with primers available upon request.

PCR products were analyzed through SSCP and dHPLC (Transgenomic). Samples showing mobility shifts or altered peaks were sequenced in both directions in an automated sequencer (MegaBACE 1000, GE Healthcare Bio-Sciences).

To verify whether the −1025G>C and −948G>A SNPs were in *cis*, PCR products were cloned with the TOPO TA™ Cloning Kit (Invitrogen Corporation). Once we observed that they were in linkage disequilibrium, we have screened only the −1025G>C transition in the control population, by PCR, followed by digestion with the *Hinc*II restriction enzyme (New England Biolabs). The −346C>T SNP was screened through Single Nucleotide Primer Extension (SNuPe™ Amersham Pharmacia Biotech) with the 5′-agatgagtaaaacgcagacc-3′ primer.

All the promoter mutations detected in this study were named relative to the start codon of the *TCOF*1 gene (http://www.genomic.unimelb.edu.au/mdi/mutnomen; Den Dunnen and Antonarakis, 2000). The +1 position is located 76 nucleotides downstream of the 5′ end of *TCOF*1 gene (position 109000247 in the reference sequence NT_029289,