A Recurrent Variant in *POLR1B*, c.3007C>T; p.Arg1003Cys, Associated with Atresia of the External Canal and Microtia in Treacher Collins Syndrome Type 4

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Established Facts

- Treacher Collins syndrome (TCS) is a heterogenous malformation syndrome characterized by a distinct facial appearance including downslanting palpebral fissures, malar hypoplasia, conductive hearing loss, and mandibular hypoplasia.
- A new causative gene, *POLR1B*, encoding DNA-directed RNA polymerase I subunit RPA2, was identified as a fourth type of TCS (TCS4).

Novel Insights

- We identified a recurrent variant, p.(Arg1003Cys), of *POLR1B* in a male patient with TCS4. Our patient supports the idea that the disease-causing variants are position-specific in TCS4.
- Even though the phenotypic spectrum of TCS4 is common to that of other types of TCS, p.(Arg1003Cys) may contribute to atresia of the external auditory canal.

Keywords

Treacher Collins syndrome · *POLR1B* · Ribosomopathies · Atresia · External auditory canal

Abstract

Treacher Collins syndrome (TCS) is a heterogenous malformation syndrome characterized by a distinct facial appearance including downslanting palpebral fissures, malar hypoplasia, conductive hearing loss, and mandibular hypoplasia. Recently, a new causative gene, *POLR1B*, encoding DNA-di-

rected RNA polymerase I subunit RPA2, was identified as a fourth type of TCS (TCS4). We describe another patient with TCS4 caused by a recurrent *POLR1B* variant, c.3007C>T; p.Arg1003Cys. Including our patient, all 4 patients with p.(Arg1003Cys) had atresia of the external auditory canal and microtia. All of the reported pathogenic variants in *POLR1B* were clustered at only 2 residues. Our patient highlights the genotype-phenotype correlation in TCS4 associated with *POLR1B*.

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Introduction

Treacher Collins syndrome (TCS1, MIM #154500; TCS2, MIM #613717; TCS3, MIM #248390) is a heterogeneous malformation syndrome characterized by a distinct facial appearance including downslanting palpebral

fissures, malar hypoplasia, conductive hearing loss, and mandibular hypoplasia. Atresia of the external canal and microtia are observed frequently in approximately 70% of the patients. In general, genotype-phenotype correlations have not been observed in large studies [Teber et al., 2004; Vincent et al., 2016]. The causative genes *TCOF1* and *POLR1D* show a dominant inheritance pattern, while *POLR1C* and *POLR1D* show a recessive inheritance pattern, accounting for 86, 6, and 1.2% of the cases, respectively. Recently, a new causative gene, *POLR1B*, encoding DNA-directed RNA polymerase I subunit RPA2, was identified as a fourth type of TCS (TCS4), indicating that ribosomopathies further contribute to the underlying mechanism of craniofacial development [Sanchez et al., 2020]. However, information on genotype-phenotype correlations is extremely limited in the previously reported patients with TCS4.

Fig. 1. Photographs of the patient and radiological investigations of the auditory organs. The patient at the age of 2 months (**a**, **b**) and 7 years of age (**c**). The right auricle shows grade 3 microtia (**b**). Mandibular retrognathia, malocclusion, and dental hypoplasia were noted. The left auricle is normal (**c**). **d** An axial computed

tomography image of the right temporal bone shows bony atresia of the external canal with deformity of the malleus and incus. The malformed malleus is fused to the tympanic wall. An axial computed tomography image of the left temporal bone shows malleoincudal fusion. Bilateral cochlea and vestibule are normal.

Here, we present another patient with TCS4 caused by a recurrent *POLR1B* variant, c.3007C>T; p.Arg1003Cys. Combined with our patient, all four patients with p.(Arg1003Cys) had atresia of the external auditory canal and microtia. Our patient highlights a genotype-phenotype correlation in TCS4 associated with *POLR1B*.

Case Report

The proband was a 7-year-old boy. He was born to nonconsanguineous parents after an uneventful pregnancy with a birth weight of 2,966 g (−0.1 SD), length of 50 cm (+0.5 SD), and occipitofrontal circumference of 32.5 cm (−0.6 SD) at 39 weeks of gestation. His father was aged 31 years and his mother was 25 years old. On the basis of his distinct facial appearance with malformed ears, micrognathia, and hypospadias, he was referred to our hospital for further evaluations. On examination at 1 month of age, he was noted to have atresia of the right external auditory meatus and grade 3 microtia, micrognathia, downslanting palpebral fissures, hypertelorism, and a prominent philtrum (Fig. 1a, b). Hypospadias was glandular type. His developmental milestones were normal for his age with hearing aids (Fig. 1c). Evoked response audi-

ometry at 500 Hz revealed a threshold of 70 dB in the right ear and 80 dB in the left ear. Temporal computed tomography showed an unusual shape of the tympanic cavity and a defect of the cartilaginous part of the external auditory meatus; normal structure of the inner ear was observed on the right side, whereas abnormal structures were noted in the ossicles on the left side (Fig. 1d).

Molecular Studies

Genomic DNA was extracted from peripheral blood of the patient and both parents using a QIAcube Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Whole-exome sequencing was performed on the affected patient. Genomic DNA captured by Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) was sequenced on a NovaSeq 6000 platform (Illumina, Inc., San Diego, CA, USA) with 151-base paired-end reads. Candidate variants were confirmed by Sanger sequencing.

We identified a heterozygous variant, NM_019014.5:c.3007C>T; p.Arg1003Cys (hg38), in *POLR1B*. Sanger sequencing confirmed that the c.3007C>T variant was de novo (Fig. 1e). The variant has been reported previously and is recurrent. It was absent from gnomAD (<https://gnomad.broadinstitute.org/>), Jmorp (<https://jmorp.megabank.tohoku.ac.jp/>), and Human Genetic Variation Database (<http://www.hgvd.genome.med.kyoto-u.ac.jp/>) [Murakami et al., 2020].

Table 1. Clinical features of TCS4 in our patient and those previously reported

	Present patient	Sanchez et al., 2020		
		Patients 1, 4, and 6	Patients 2 and 3	Patient 5
cDNA alteration (NM_019014.5)	c.3007C>T	c.3007C>T	c.2046T>A	c.3007C>A
Protein alteration	p.(Arg1003Cys)	p.(Arg1003Cys)	p.(Ser682Arg)	p.(Arg1003Ser)
Inheritance	De novo	De novo	Dominant	Dominant (mosaic in father)
Sex	Male	Female	Female	Female
Clinical features				
Downward-slanting palpebral fissures with malar hypoplasia	+	+	+	+
Conductive deafness	+	+	+	+
Atresia of the external ear canal	+	+	+	−
Microtia	+	+	+	−
Coloboma of the lower lid	+	+	−	+
Intellectual disability	−	−	−	−
Anomaly of the limbs	−	−	−	−
Urogenital anomalies	Hypospadias	NA	NA	NA
NA, not available.

Fig. 2. Schematic representation of POLR1B protein structure (NP_061887.2) by Pfam (<https://pfam.xfam.org/>) and variants in *POLR1B*. **(a)** Two recurrent variants, p.(Ser682Arg) and p.(Arg1003Cys), are shown in the domain structure. Both pathogenic variants were located within domain 6 of RNA polymerase Rpb2. This domain binds the nascent RNA strand/template DNA strand in the Pol II transcription elongation complex. **(b)** The profiles of genetic tolerance in *POLR1B* (ENST00000263331.5, 1135aa) by

MetaDome (<https://stuart.radboudumc.nl/metadome/>). Recurrent variants, Ser682 and Arg1003, were located in highly intolerant areas. RNA_pol_Rpb2_1: RNA polymerase beta subunit; RNA_pol_Rpb2_2: RNA polymerase Rpb2, domain 2; RNA_pol_Rpb2_3: RNA polymerase Rpb2, domain 3; RNA_pol_Rpb2_4: RNA polymerase I, Rpa2-specific domain; RNA_pol_Rpb2_6: RNA polymerase Rpb2, domain 6; RNA_pol_Rpb2_7: RNA polymerase Rpb2, domain 7.

Discussion

We identified a recurrent variant, c.3007C>T; p.Arg1003Cys, in *POLR1B* in a male patient with a typical phenotype of TCS with malar hypoplasia, atresia of the right external canal and microtia, anomalies of bilateral temporal bones, and conductive hearing impairment, but without intellectual disability. This is the second report of TCS4 caused by mutation of *POLR1B* (Table 1).

To date, 6 patients with TCS4 have been reported [Sanchez et al., 2020] with variants at 2 amino acid residue positions in *POLR1B* at 1003Arg and 682Ser in 4 and 2 individuals, respectively. Of note, p.(Arg1003Cys)

is the most frequent variant (3/6) in TCS4. Our patient is the fourth individual found to harbor a missense mutation p.(Arg1003Cys) in *POLR1B*. Our patient was of a different ethnicity from the previously described individuals, further supporting the idea that this variant is the most recurrent in *POLR1B* associated with the TCS4 phenotype.

Perturbation of ribosome biogenesis, resulting in p53-dependent neural crest cell apoptosis and abnormal migration, has been shown to be the underlying mechanism of craniofacial anomalies in TCS [Noack Watt et al., 2016]. *PolI*, *II*, and *III* are major factors of ribosome biogenesis in eukaryotes. In this report, we described

another patient with a de novo pathogenic variant of *POLR1B*, which is a core subunit of *PoII*. The core subunits of *PoII* consist of *POLR1A*, *POLR1B*, *POLR1C*, and *POLR1D*. Loss-of-function mutation in *POLR1A* causes acrofacial dysostosis, Cincinnati type [Weaver et al., 2015]. Loss-of-function mutations in *POLR1C* and *POLR1D* cause TCS2 and TCS3, respectively [Noack Watt et al., 2016]. According to these studies, all of the *PoII* core subunits have crucial roles in craniofacial development and contribute to distinct types of ribosomopathies in humans.

According to large studies [Dauwerse et al., 2011; Vincent et al., 2016], the incidence of auditory canal atresia is generally high in TCS1, TCS2, and TCS3 with a rate of 70%. However, atresia of the auditory canal is a distinct phenotype that is common among all of the patients with the p.(Arg1003Cys) variant in *POLR1B* (Table 1). These results indicated that p.(Arg1003Cys) may have a crucial pathogenic role in the development of auditory canal atresia in TCS4. However, most of the phenotypic spectrum of TCS4 is common in other types of TCS [Sanchez et al., 2020], and the number of patients with TCS4 is limited. Therefore, it is difficult to discuss the genotype-phenotype correlations of *POLR1B* in TCS4. Non-craniofacial anomaly of our patient was hypospadias of glandular type. Genital anomaly is a rare involvement of the patients with TCS [Writzl et al., 2008]. The underlying mechanism of hypospadias in our patient remained unclear.

Sanchez et al. [2020] showed that loss of function of *POLR1B* was the underlying mechanism of TCS4 by reproducing an abnormal craniofacial phenotype in zebrafish following knockdown of *polr1b*. The identification of patients with only 3 recurrent variants converging on 2 amino acid residues makes it highly likely that the affected nucleotides, especially c.3007C>T; p.Arg1003Cys, are mutational hot-spots. Ser682 and Arg1003 are located in domain 6 of RNA polymerase Rpb2, which represents the hybrid-binding domain and the wall domain, and performs several important functions such as catalyzing the DNA-dependent polymerization of RNA (Fig. 2a). The hybrid-binding domain binds the nascent RNA strand/template DNA strand. To determine why the specific variants are clustered in *POLR1B*, we investigated the profiles of genetic tolerance in *POLR1B* by MetaDome (<https://stuart.radboudumc.nl/metadome/>) [Wiel et al., 2019]. Recurrent variants, Ser682 and Arg1003, were in highly intolerant areas (Fig. 2b). These results suggested that the Ser682

and Arg1003 have crucial functions in domain 6 of RNA polymerase Rpb2.

In conclusion, we identified a recurrent variant, p.(Arg1003Cys), in *POLR1B* in a male patient with TCS4. Our patient provides support for the hypothesis that the disease-causing variants are position-specific in TCS4. Even though the phenotypic spectrum of TCS4 is common in other types of TCS, p.(Arg1003Cys) may contribute to atresia of the external auditory canal. Further detailed studies based on the genotype-phenotype correlations in TCS4 patients with *POLR1B* variants will provide a molecular basis for craniofacial development and ribosomopathies.

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Statements of Ethics

Clinical information was collected after obtaining written informed consent from the patient's family. This study was approved by the institutional review board of Kanagawa Children's Medical Center (approval No. 2018-1).

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

E.Y., Y.T., and K.K. provided molecular genetic data and wrote the manuscript. M.T., S.K., M.I., K.F., T.K., H.M., and K.K. provided clinical data. All authors analyzed and interpreted the data and approved the manuscript in its final form.

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