Received: 9 June 2020 | Accepted: 23 June 2020

DOI: 10.1002/mgg3.1401

CLINICAL REPORT

Molecular Genetics & Genomic Medicine Open Access WILEY

MYT1 role in the microtia-craniofacial microsomia spectrum

Daniela V. Luquetti[1,2] | **Carrie L. Heike**[1,2] | **Ignacio Zarante**[3,4] | **Andrew E. Timms**[2] | **Jonas Gustafson**[2] | **Harry Pachajoa**[5] | **Gloria L. Porras-Hurtado**[6] | **Paola Ayala-Ramirez**[3] | **Milagros M. Duenas-Roque**[7] | **Natalia Jimenez**[8] | **Lina M. Ibanez**[8] | **Paula Hurtado-Villa**[8]

[1]University of Washington School of Medicine, Seattle, WA, USA

[2]Seattle Children's Research Institute, Seattle, WA, USA

[3]Human Genomics Institute, Pontificia Universidad Javeriana, Bogotá, Colombia

[4]Hospital Universitario San Ignacio, Bogotá, Colombia

[5]Universidad Icesi, Cali, Colombia

[6]Clinica Comfamiliar Risaralda, Pereira, Colombia

[7]Hospital Edgardo Rebagliati Martins, Lima, Peru

[8]Pontificia Universidad Javeriana, Cali, Colombia

Correspondence
Daniela V. Luquetti, 1900 9th avenue C9S-5, Seattle, WA, USA.
Email: Daniela.luquetti@seattlechildrens.org

Funding information
This work was supported by NIH: NIDCD (R00DC011282), NIDCR (RC1 DE 020270, R01 DE022438), NICHD (X01 HL140518-01).

Abstract

Background: Craniofacial microsomia (CFM), also known as the oculo-auriculo-vertebral spectrum, comprises a variable phenotype with the most common features including microtia and mandibular hypoplasia on one or both sides, in addition to lateral oral clefts, epibulbar dermoids, cardiac, vertebral, and renal abnormalities. The etiology of CFM is largely unknown. The *MYT1* gene has been reported as a candidate based in mutations found in three unrelated individuals. Additional patients with mutations in this gene are required to establish its causality. We present two individuals with CFM that have rare variants in *MYT1* contributing to better understand the genotype and phenotype associated with mutations in this gene.

Methods/Results: We conducted genetic analysis using whole-exome and -genome sequencing in 128 trios with CFM. Two novel *MYT1* mutations were identified in two participants. Sanger sequencing was used to confirm these mutations.

Conclusion: We identified two additional individuals with CFM who carry rare variants in *MYT1*, further supporting the presumptive role of this gene in the CFM spectrum.

KEYWORDS
craniofacial microsomia, genetics, hemifacial microsomia, microtia, oculo-auriculo-vertebral spectrum

1 | INTRODUCTION

Craniofacial microsomia (CFM, OMIM: 164210), also known as the oculo-auriculo-vertebral spectrum, hemifacial microsomia, or Goldenhar syndrome, is typically characterized by uni- or bilateral microtia and mandibular hypoplasia in addition to ocular, vertebral, and renal abnormalities (Gorlin, Cohen, & Hennekam, 2001; Heike & Hing, 2009). CFM, like other complex diseases, usually occurs sporadically. In multiplex families, the transmission is usually autosomal dominant, often with incomplete penetrance, although autosomal recessive inheritance has also been postulated for some families (Rollnick & Kaye, 1983; Vendramini-Pittoli & Kokitsu-Nakata, 2009). It is associated with high

inter- and intrafamilial phenotypic variability. Although there is no consensus on the minimum criteria for diagnosis, microtia is generally accepted as the minimum criterion for diagnosis (Beleza-Meireles, Clayton-Smith, Saraiva, & Tassabehji, 2014; Keogh, Troulis, Monroy, Eavey, & Kaban, 2007). The etiology remains largely unknown, however, reports of familial cases as well as the presence of CFM features in individuals with chromosomal aberrations and genomic imbalances suggest that CFM has a genetic basis.

Recently, heterozygous variants in *MYT1* (myelin transcription factor 1, OMIM * 600379) were reported in three unrelated individuals; this is the first gene implicated in CFM (Berenguer et al., 2017; Lopez et al., 2016). Two individuals had de novo variants while one had an inherited mutation from a unaffected father. We present two additional individuals with rare disrupting variants in *MYT1* identified through exome and genome sequencing of 128 trios with CFM. We discuss how our cases contribute to the phenotype associated with this gene and how our findings further support the hypothesis that isolated microtia can be part of the CFM spectrum.

2 | METHODS

2.1 | Clinical assessment

We recruited 360 families with a diagnosis of CFM from a network of 10 sites in the US, Colombia, and Peru during multiple NIH-funded studies (RC1 DE 020270, R00 DC011282, R01 DE022438). Our eligibility criteria were based on the presence of at least one of the following phenotypic findings: microtia; mandibular hypoplasia and preauricular tag(s); mandibular hypoplasia and facial tag(s); mandibular hypoplasia and epibulbar dermoid; mandibular hypoplasia and lateral oral cleft (i.e. macrostomia); preauricular tag and epibulbar dermoid; preauricular tag and lateral oral cleft; facial tag and epibulbar dermoid; lateral oral cleft and epibulbar dermoid. Exclusion criteria included diagnosis of a syndrome, abnormal chromosome studies, and/or mandibular hypoplasia due to deformational plagiocephaly or torticollis.

Detailed phenotypic data were ascertained from all cohorts by a standardized series of two-dimensional photographs, and parental interview about the participant's medical history. Physical examination was performed by a medical geneticist or physician in the cohorts from Colombia and Peru. Prenatal and family history was collected to account for exposure to known teratogens and to assess recurrence of the phenotype in the family. Phenotypic and clinical data were reviewed at the coordination center by a medical geneticist and a craniofacial pediatrician. Blood or saliva samples were collected from the proband and available parents. DNA was extracted from blood and saliva using standard procedures.

All human studies were reviewed and approved by the institutional review board (IRB) of the Seattle Children's Hospital and at the local institutions IRB's. An informed consent form was signed by all participants.

2.2 | Genetic analysis

Sequencing was performed in DNA from 128 trios (exome sequencing in 44 trios and whole genome sequencing in 84 trios) using standard procedures.

Paired end reads were mapping to the human genome (hg19 for exomes and hg38 for genomes) using BWA-MEM with default parameters. The Genome Analysis Toolkit (GATK) realigned reads around known indels and recalibrated quality scores to reduce artifacts caused by the sequencing chemistry, and Picard was used to mark duplicate reads. For the exome's variants, the haplotype callers from GATK and Freebayes were used and the variants on the intersection of the two variant callers were reported. For the genomes GATK haplotype caller was used to call variants.

Variants are annotated with SnpEff and loaded into a database using the GEMINI framework, with additional annotations added by annovar. Annotations included predicted functional effect (e.g., splice-site, nonsense, missense), protein position, known clinical associations (OMIM, CLINVAR, COSMIC), conservation score (PhastCons, GERP), and effects protein function (PolyPhen), CADD scores, and population allele frequencies (ExAC, gnomAD).

Tools within GEMINI were used to identify variants confirming to a number of disease models. We identify variants that are rare in the population (Maximum allele frequency (MAF) <0.01 or <0.05), are predicted to have a high impact on the gene and are de novo or transmitted in an autosomal recessive, compound heterozygote or X-linked manner. Bidirectional Sanger sequencing was performed to confirm *MYT1* variants.

3 | RESULTS

3.1 | Clinical report

The first proband (PM1) was a boy, born at term without complications to a nonconsanguineous couple with two healthy children. His birth weight was 2.93 kg (18th centile) with a length of 49 cm (32nd centile). During pregnancy, the mother was treated with cephalexin for a urinary tract infection in the first trimester and folic acid in first and second trimesters. The child was 12 years old at the time of clinical assessment for the study and presented with bilateral microtia (grade II/III), bilateral aural atresia and bilateral clinodactyly of fifth fingers; his neurodevelopment was age appropriate.

The audiometry demonstrated bilateral severe conductive hearing loss. Computed tomography revealed normal middle and inner ear structures; no other images were performed. He used bilateral bone anchored hearing aids.

The second proband (PM2) was a girl, born at term without complications to a nonconsanguineous couple, with two healthy children. Her birth weight was 2.5 kg (fourth centile), unknown length; she was discharged from the hospital on her second day of life. During pregnancy, the mother used iron supplements during all trimesters and developed a fever during the second trimester. The child was 1-year-old at time of clinical assessment for the study and presented with right microtia grade II and ipsilateral aural atresia, microphthalmia, and left chorioretinal coloboma; her neurodevelopment was age-appropriate. An auditory brainstem response test demonstrated severe mixed (conductive and neurosensorial) hearing loss on her right ear. Abdominal ultrasound and karyotype were normal.

The probands are unrelated and the families (parents and grandparents) are from different regions of Colombia, PM1 family is from Cundinamarca department and PM2 family is from Valle del Cauca and Nariño. Family history of birth defects, and hearing loss was negative in both families.

3.2 | Genetic analysis

The genetic analysis on DNA from blood from the parents and probands (trios) demonstrated that both individuals had maternally inherited heterozygous missense variants in *MYT1* (NM_004535.2:c.232C>A and NM_004535.2:c.2780G>A). The mother of PM2 had a small cleft in the lobe of the right ear (Figure 1), compatible with an autosomal dominant pattern of inheritance with incomplete penetrance. These variants were confirmed by Sanger sequencing in the probands and carrier mothers and were absent in the fathers. No other de novo, homozygous or compound heterozygous variants of interest were observed.

For the variant NM_004535.2:c.232C>A, two Latino individuals were reported as heterozygous in gnomAD (http://gnomad.broadinstitute.org/), with an allele frequency of 0.000008. The other variant, NM_004535.2:c.2780G>A (p.Gly927Glu), has not been reported, but in the same codon two other missense heterozygous variants have been reported; each one in one individual resulting in: p.Gly927Arg (European) and pGly927Val (South Asian), both were considered as "probably damaging" by Polyphen.

We compared the phenotype and genomic characteristics of the probands described here and the three described in previous studies (Tables 1 and 2).

4 | DISCUSSION

The published evidence on the association between *MYT1* and CFM is insufficient, and clinical reports on additional individuals' patients with mutations in this gene are required to establish causality. We identified two additional individuals with CFM who carry rare variants in *MYT1*, further supporting the presumptive role of this gene in CFM. One child presented with microtia only, potentially expanding the phenotype associated with variants in this gene to microtia without other associated birth defects; and the other child presented with eye anomalies that were different than those previously described in individuals with CFM with *MYT1* variants. In a recent study in which *MYT1* was sequenced in 73 individuals with CFM, no pathogenic variants were found (Zamariolli et al., 2019). Of the 128 individuals with CFM sequenced in this study, only two individuals were identified with variants in this gene, and there were no overlapping variants with the three previously reported mutations, supporting the genetic heterogeneity suspected for this condition.

The protein encoded by this gene is known to play a role in the developing nervous system and to bind to the proteolipid proteins of the central nervous system. However, *MYT1* is widely expressed in the mouse and human embryo and there is evidence for gene expression in the 1st and 2nd pharyngeal arches, which give rise to most of CFM involved tissues, during mouse development at embryonic days 10.5, 11.0, and 12.5 (Donaldson et al., 2012). Furthermore, functional studies of *MYT1* have shown that it regulates genes

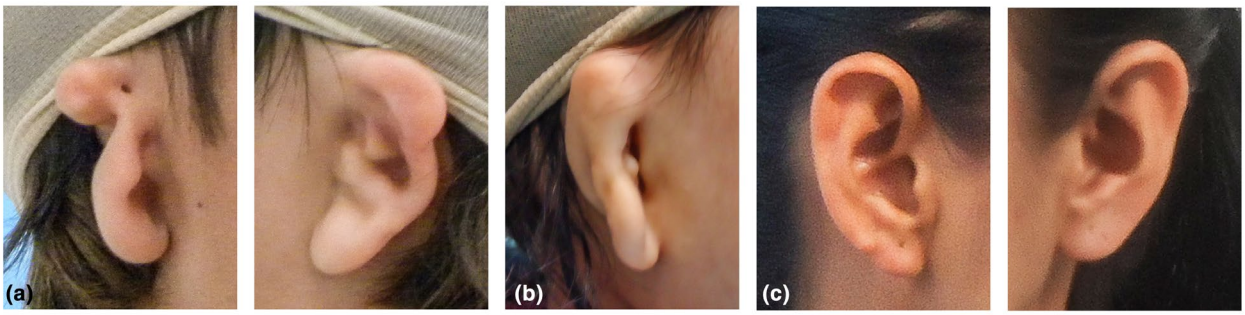

FIGURE 1 Photos of individuals identified with *MYT1* variants. (a) PM1 presented with bilateral microtia. (b) PM2 presented with right microtia. (c) Mother of PM2 presented with an ear lobe cleft on the right ear

TABLE 1 Phenotype of individuals reported here and in the literature with *MYT1* variants

Individual	Microtia	Preauricular tags	Mandibular hypoplasia	Lateral oral cleft	Eye anomalies	Skeletal anomalies	Other anomalies
PM1	Bilateral	—	—	—	—	—	
PM2	Unilateral R	—	—	—	R microphthalmia L chorioretinal coloboma	—	
F1 (Lopez, 2016)	Unilateral R	Unilateral R	Unilateral R	—	R Epibulbar dermoid	Hemivertebrae	Middle ear VSD
F2 (Lopez, 2016)	—	Unilateral L	Unilateral L	Unilateral L	—	Lumbar dysraphism	
P1 (Berenguer et al., 2017)	Bilateral	Unilateral R	Unilateral R	—	—	Cervical fusion	ASD, VSD

Abbreviations: ASD, atrial septum defect; L, left; R, right; VSD, ventricular septal defect.

TABLE 2 *MYT1* variants genomic characteristics and in-silico predictions

Individual	Mutation	Polyphen/SIFT	GERP	CADD phred	Allele Freq (gnomAD v2.1)	Inheritance
PM1	c.232C>A (p.Pro78Thr)	benign/deleterious	5.6	22	2[a]/251,216	Inherited (mat)
PM2	c.2780G>A (p.Gly927Glu)	deleterious	5.0	31	0	Inherited (mat)
F1 (Lopez 2016)	c.25C>T (p.Arg9*)	—	5.3	36	0	de novo
F2 (Lopez 2016)	c.314C>T (p.Ser105Leu)	benign/tolerated	5.6	22	4[b]/251,066	Inherited (pat)
P1 (Berenguer et al., 2017)	c.323C>T (p.Ser108Leu)	benign/deleterious	5.6	25	8[c]/251,082	de novo

Abbreviations: CADD: Combined Annotation Dependent Depletion (http://genetics.bwh.harvard.edu.offcampus.lib.washington.edu/pph2/); GERP: Genomic Evolutionary Rate Profiling (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html); gnomAD: Genome Aggregation Database (https://gnomad.broadinstitute.org/); SIFT: Sorting Intolerant From Tolerant (http://sift-dna.org).

[a]Two Latino individuals.

[b]Two African, one European, and one "other" individual.

[c]Three Latino, four European, and one South Asian individual.

involved in the retinoic acid pathway (*RARA,RARB, RARG, CYP26A1*) and that variants affecting its function could disrupt retinoic acid signaling and thus cause CFM (Berenguer et al., 2017; Lopez et al., 2016). There is also evidence supporting a direct regulation by *Myt1* binding active regulatory regions of *Rara* repressing its expression in neural progenitor cells *(*Vasconcelos et al., 2016).

Discriminating pathogenic from rare, non-disease-causing variants is a challenge. There is still justification to consider the association between CFM and *MYT1* provisional until further cases are described. Evidence that points against a causal relationship include: (a) according to gnomAD data, *MYT1* is an intolerant gene for loss of function mutations, with the ratio of observed/expected (o/e) variant of 0.18 (90% CI 0.11–0.3), however, it seems to be more tolerant for missense variation o/e: 0.72 (90% CI 0.67–0.78); (b) although two of the variants described are de novo, the other three variants were inherited from nonaffected parents with a negative family history for CFM; (c) considering the prevalence of CFM as 1 in 3,000–5,000, incomplete penetrance, and high genetic heterogeneity, the expected maximum allelic count is one to three, however for two of the variants previously published, the allelic counts are higher than known at the time of publication and potentially "too common" to be causative; (d) the functional validation of the variants were primarily in the up and down regulation effect of the *MYT1* variants in the cellular expression of *CYP26A1*, *RARA*, *RARB,* and *RARG* (Berenguer et al., 2017; Lopez et al., 2016); these tests showing an effect of a candidate mutation at the protein level do not necessarily dictate that this effect is responsible for the disease; and (e) the *Myt1* knock-out homozygous mouse model presents with pancreatic and diaphragmatic abnormalities and neonatal mortality, but no CFM-related birth defects (Hudson, Romm, Berndt, & Nielsen, 2011; Wang et al., 2007). However, it is not uncommon for human and mouse craniofacial phenotypes to differ. Reasons for this include the effect that the genetic background can have on the observed phenotype, such as seen in *Tcof*$^{-/-}$ mice (Dixon & Dixon, 2004); in addition, the functional significance of mutations (gain- or loss-of-function) can also heavily affect modeling of the disease phenotype, such as in the earlier knockout of *Plcb4* in mice (Smits et al., 2005).

Overall, and following the ClinGen clinical validity of gene-disease associations framework, we believe that there

is "moderate evidence" to support a causal role for *MYT1* in CFM based on qualitative description: at least three unrelated probands harboring variants with sufficient supporting evidence for disease causality and moderate experimental data supporting the gene-disease association (semi-quantitative assessment of evidence score: 10.6). Additional sequencing data on CFM cohorts, such as the one presented here, will help us gather more evidence to better understand the involvement of this gene in this condition, for which the etiology remains largely unknown.

ACKNOWLEDGMENTS

We thank the participants and their families and the Project Microtia and FACIAL networks. We also thank Paola Barrágan (in memorian), a Colombian geneticist who greatly contributed to this project with her wonderful attitude towards life and her great dedication to patients.

CONFLICT OF INTEREST

We, the authors, do not have any conflict of interest to declare.

AUTHORS CONTRIBUTIONS

DVL, CLH, IZ, PHV, MMDR, HPL, GLPH, PAR contributed to the concept and study design. DVL, CLH, AT, IZ, and PHV wrote the manuscript. DVL, JG, and AT interpreted the data. IZ, PHV, MMDR, HPL, GLPH, NJ, PAR recruited patients and/or performed the clinical evaluation of patients. JG and PAR prepared the biological samples and laboratory assays. All authors revised and approved the final version. DVL is responsible for the overall content.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Daniela V. Luquetti https://orcid.org/0000-0001-6059-7708

REFERENCES

Beleza-Meireles, A., Clayton-Smith, J., Saraiva, J. M., & Tassabehji, M. (2014). Oculo-auriculo-vertebral spectrum: A review of the literature and genetic update. *Journal of Medical Genetics*, *51*(10), 635–645. https://doi.org/10.1136/jmedgenet-2014-102476

Berenguer, M., Tingaud-Sequeira, A., Colovati, M., Melaragno, M. I., Bragagnolo, S., Perez, A. B. A., … Rooryck, C. (2017). A novel de novo mutation in MYT1, the unique OAVS gene identified so far. *European Journal of Human Genetics*, *25*(9), 1083–1086. https://doi.org/10.1038/ejhg.2017.101

Dixon, J., & Dixon, M. J. (2004). 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. *Developmental Dynamics*, *229*(4), 907–914. https://doi.org/10.1002/dvdy.20004

Donaldson, I. J., Amin, S., Hensman, J. J., Kutejova, E., Rattray, M., Lawrence, N., … Bobola, N. (2012). Genome-wide occupancy links Hoxa2 to Wnt-beta-catenin signaling in mouse embryonic development. *Nucleic Acids Research*, *40*(9), 3990–4001.

Gorlin, R. J., Cohen, M. M., & Hennekam, R. C. M. (2001). *Syndromes of the head and neck*. Oxford [England]; New York: Oxford University Press.

Heike, C. L., & Hing, A. V. (2009). In Pagon, R. A. B. T., Dolan, C. R., & Stephens, K. (Eds.), *GeneReviews*. 2010/03/20 ed. Seattle, WC: University of Washington.

Hudson, L. D., Romm, E., Berndt, J. A., & Nielsen, J. A. (2011). A tool for examining the role of the zinc finger myelin transcription factor 1 (Myt1) in neural development: Myt1 knock-in mice. *Transgenic Research*, *20*(4), 951–961. https://doi.org/10.1007/s11248-010-9470-x

Keogh, I. J., Troulis, M. J., Monroy, A. A., Eavey, R. D., & Kaban, L. B. (2007). Isolated microtia as a marker for unsuspected hemifacial microsomia. *Archives of Otolaryngology - Head and Neck Surgery*, *133*(10), 997–1001. https://doi.org/10.1001/archotol.133.10.997

Lopez, E., Berenguer, M., Tingaud-Sequeira, A., Marlin, S., Toutain, A., Denoyelle, F., … Rooryck, C. (2016). Mutations in MYT1, encoding the myelin transcription factor 1, are a rare cause of OAVS. *Journal of Medical Genetics*, *5*, 752–760. https://doi.org/10.1136/jmedgenet-2016-103774

Rollnick, B. R., Kaye, C. I., & Opitz, J. M. (1983). Hemifacial microsomia and variants: Pedigree data. *American Journal of Medical Genetics*, *15*(2), 233–253. https://doi.org/10.1002/ajmg.1320150207

Smits, S. M., Van Der Nobelen, S., Hornman, K. J., Von Oerthel, L., Burbach, J. P., & Smidt, M. P. (2005). Signalling through phospholipase C beta 4 is not essential for midbrain dopaminergic neuron survival. *Neuroscience*, *136*(1), 171–179. https://doi.org/10.1016/j.neuroscience.2005.07.053

Vasconcelos, F. F., Sessa, A., Laranjeira, C., Raposo, A., Teixeira, V., Hagey, D. W., … Castro, D. S. (2016). MyT1 counteracts the neural progenitor program to promote vertebrate neurogenesis. *Cell Reports*, *17*(2), 469–483. https://doi.org/10.1016/j.celrep.2016.09.024

Vendramini-Pittoli, S., & Kokitsu-Nakata, N. M. (2009). Oculoauriculovertebral spectrum: Report of nine familial cases with evidence of autosomal dominant inheritance and review of the literature. *Clinical Dysmorphology*, *18*(2), 67–77. https://doi.org/10.1097/MCD.0b013e328323a7dd

Wang, S., Zhang, J., Zhao, A., Hipkens, S., Magnuson, M. A., & Gu, G. (2007). Loss of Myt1 function partially compromises endocrine islet cell differentiation and pancreatic physiological function in the mouse. *Mechanisms of Development*, *124*(11–12), 898–910. https://doi.org/10.1016/j.mod.2007.08.004

Zamariolli, M., Colovati, M., Moyses-Oliveira, M., Nunes, N., Caires Dos Santos, L., Alvarez Perez, A. B., …Melaragno, M. I. (2019). Rare single-nucleotide variants in oculo-auriculo-vertebral spectrum (OAVS). *Molecular Genetics & Genomic Medicine*, *7*, e959.

How to cite this article: Luquetti DV, Heike CL, Zarante I, et al. *MYT1* role in the microtia-craniofacial microsomia spectrum. *Mol Genet Genomic Med*. 2020;00:e1401. https://doi.org/10.1002/mgg3.1401