INNSZ

INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN

MÉXICO, D.F., A 18 DE FEBRERO DE 2011.

DRA. JAZMÍN ARTEAGA VÁZQUEZ
INVESTIGADOR PRINCIPAL
DEPARTAMENTO DE GENÉTICA
PRESENTE

Por este medio, me permito informarle que el Comité Institucional de Investigación Biomédica en Humanos, del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, **ha revisado y aprobado** el Protocolo de Investigación clínica, titulado:

"IDENTIFICACIÓN DEL NÚMERO VARIABLE DE COPIAS (CNVS) EN PACIENTES CON CARDIOPATÍAS CONGÉNITAS CONOTRUNCALES AISLADAS Y ASOCIADAS A OTRAS MALFORMACIONES"
REF. 290

Así mismo se solicita que al terminar el estudio se deberá de enviar los resultados con resumen de todos los datos sobresalientes y conclusiones, un informe anual (si la duración del estudio es mayor de un año), donde comunique los avances y resultados parciales de su Investigación.

Sin más por el momento quedo de usted.

ATENTAMENTE,

DR. PATRICIO SANTILLÁN DOHERTY
COORDINADOR
COMISIÓN DE ÉTICA EN INVESTIGACIÓN

INCMNSZ
18 FEB 2011
COMITÉ INSTITUCIONAL DE INVESTIGACIÓN BIO MÉDICA EN HUMANOS

INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN "SALVADOR ZUBIRÁN"
22 FEB 2011
DEPARTAMENTO DE GENÉTICA

Rubén original
22-Febrero-2011
Jazmín Arteaga

c.c.p. Dr. Rubén Lisker Y. Dirección de Investigación.
C.P. Martha Arredondo Urzúa. Jefe del Depto. C.F.E.I.

Investigación
Tradición Servicio
Asistencia PSD/Docencia

• Vasco de Quiroga 15,
• Delegación Tlalpan
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20007700

INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN

Recibí Versión Vigente 24/09/15

ACUSE

MÉXICO, D.F., A 17 DE SEPTIEMBRE DE 2015

DRA. JAZMÍN ARTEAGA VÁZQUEZ
INVESTIGADORA PRINCIPAL
DEPARTAMENTO DE GENÉTICA
INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN, "SALVADOR ZUBIRÁN"
AV. VASCO DE QUIROGA NO. 15
COL. BELISARIO DOMÍNGUEZ SECCIÓN XVI
DEL. TLALPAN, C.P. 14080, MÉXICO, D.F.
PRESENTE

Le informamos que con relación al Protocolo de Investigación Clínica, titulado:

"IDENTIFICACIÓN DEL NÚMERO VARIABLE DE COPIAS (CNVS) EN PACIENTES CON CARDIOPATÍAS CONGÉNITAS CONOTRUNCALES AISLADAS Y ASOCIADAS A OTRAS MALFORMACIONES"
REF. 290

Estos Comités han recibido y revisado la documentación que señala el cierre de estudio, llevado a cabo el 27 de mayo de 2015.

Sin otro particular, reciba un cordial saludo.

ATENTAMENTE,

DR. CARLOS A. AGUILAR SALINAS
PRESIDENTE
COMITÉ DE INVESTIGACIÓN

DR. ARTURO GALINDO FRAGA
PRESIDENTE
COMITÉ DE ÉTICA EN INVESTIGACIÓN

CAAS/AGF/apc

INS. NACIONAL DE CIENCIAS MEDICAS Y NUTRICIÓN "SALVADOR ZUBIRÁN"
17 SEP 2015
COMITÉ DE ÉTICA EN INVESTIGACIÓN

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INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN
SALVADOR ZUBIRAN

Dirección de Investigación

FORMA ÚNICA PARA REGISTRO DE PROYECTOS

No invada las zonas sombreadas

CLAVE: GEN - 290 - 11 / 13 - 1

FECHA DE RECEPCIÓN: 2010-10-27

TÍTULO: "IDENTIFICACIÓN DEL NÚMERO VARIABLE DE COPIAS (CNVs) EN PACIENTES CON CARDIOPATÍAS CONGÉNITAS CONOTRUNCALES AISLADAS Y ASOCIADAS A OTRAS MALFORMACIONES"

INVESTIGADOR RESPONSABLE: JAZMIN ARTEAGA VAZQUEZ

DEPARTAMENTO O SERVICIO: GENETICA

TIPO DE INVESTIGACIÓN:

X	1. Investigación Clínica	(incluye seres humanos o sus productos biológicos)
	2. Investigación Experimental	(incluye animales de investigación o sus productos biológicos)
	3. Investigación Documental	(revisión de expedientes, revisión bibliográfica, informe de casos, etc.)
	4. Desarrollo Tecnológico	(instrumental, equipo, métodos diagnósticos, drogas nuevas, etc)
	5. Investigación Epidemiológica	(estudios en poblaciones, en comunidad o en hospital)
	6. Otros	(organización de eventos, asistencia a reuniones, donativos, etc)

PATROCINADORES:	*Cantidad:*	
CONACYT FONSEC SSA/IMSS/ISSSTE S0008-2010-1	700000	700000
	0	*TOTAL*
	0	0
		Fondo de Apoyo

PERÍODO DE UTILIZACIÓN DE LOS RECURSOS: de mes: 01 año: 2011 a mes: 12 año: 2013

FORMA EN LA QUE SE RECIBIRÁN LOS FONDOS:

	primer trimestre	segundo trimestre	tercer trimestre	cuarto trimestre
Primer año:	400000	0	0	0
Segundo año:	150000	0	0	0
Tercer año:	150000	0	0	0
Cuarto año:	0	0	0	0
Quinto año:	0	0	0	0

COSTOS TOTALES DE LA INVESTIGACIÓN
(ver instrucciones al reverso)

1. Personal: (sueldos y sobresueldos al personal)	0
2. Equipos: (de laboratorio, cómputo, transporte, etc.)	0
3. Materiales: (reactivos, consumibles, desechables, etc.)	550000
4. Animales: (adquisición, cuidado, procedimientos, etc.)	0
5. Estudios: (de laboratorio, gabinete, especiales, etc.)	0
6. Viáticos: (reuniones científicas y trabajo de campo)	68000
7. Publicaciones: (costos directos de publicación, sobrelíneos)	30000
8. Suscripción: (libros, revistas, software, periódico, etc.)	22000
9. Varios: (teléfono, fax, fotocopias, mensajería, etc.)	30000
10. Fondo de Apoyo: (15% de la cantidad total del proyecto)	0
TOTAL:	700000

INSTITUCIONES PARTICIPANTES

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

Instituto Nacional de Cardiología Ignacio Chávez

FIRMAS

Investigador Responsable

Jefe del Departamento

Comité de Investigación en Humanos

Comité de Investigación en Animales

Director de Investigación

Director General

12-04-2011
Fecha de Resolución

ALTERACIÓN EN EL NÚMERO VARIABLE DE COPIAS (CNVs) EN PACIENTES CON TETRALOGÍA DE FALLOT NO SINDRÓMICA MEDIANTE MLPA

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ANTECEDENTES: Las variaciones en el número de copias (CNVs) representan cambios en la estructura del genoma (>1kb) que pueden alterar la expresión génica mediante la disrupción o fusión de genes, alteración en eventos de recombinación, errores de replicación del DNA y haploinsuficiencia [1, 2]. La tetralogía de Fallot (TF) es una malformación congénita generalmente esporádica y de etiología multifactorial, tiene una prevalencia de 1/3000 nacidos vivos[3]. Esta malformación incluye comunicación intraventricular, cabalgamiento de la aorta, estenosis pulmonar/ obstrucción al tracto de salida e hipertrofia del ventrículo derecho. Comprende 3.5-9% de todas las cardiopatías congénitas. Greenway y cols. estudiaron la asociación entre CNVs y TF mediante el análisis de tríos identificaron 7 nuevos CNVs que incrementan el riesgo para TF no sindrómica y han sugerido que hasta 10% de las TF esporádicas son resultado de CNVs *de novo*[3].

OBJETIVO: Estudiar mediante la técnica de MLPA las CNVs de regiones cromosómicas relacionadas a cardiopatías congénitas en trios: casos con TF no sindrómica y sus progenitores.

MATERIAL Y MÉTODOS: La muestra se integró de 28 tríos (caso, madre y padre) provenientes de la consulta externa y hospitalización del Instituto Nacional de Cardiología "Ignacio Chávez". Se extrajo DNA de muestras de sangre periférica o mucosa oral mediante la técnica de precipitación con sales o purificación por columnas de silica. El análisis molecular se realizó mediante la técnica de MLPA (*Multiplex Ligation Probe Amplification*) para los kits P250-(DiGeorge) y P311-(CHD) MRC-Holland con consecuente separación de los productos amplificados mediante electroforesis capilar. Para confirmar los hallazgos de MLPA se realizó la determinación de dosis genica relativa (DGR) por PCR tiempo real. El análisis de fragmentos se realizó con el programa *ChimerMarker* y el análisis de CNVs con el programa *Coffalyser* v9.4.

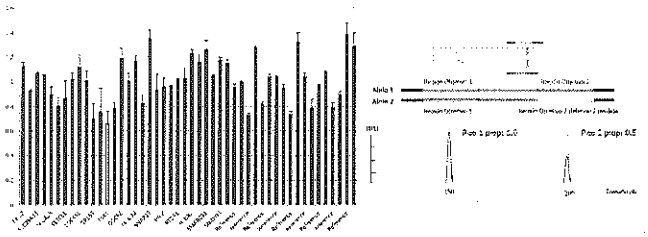

Figura 1. Análisis de CNVs del caso con TF analizado con resultado positivo para la deleción de la sonda correspondiente al gen *TBX1* (marcada en color rojo).

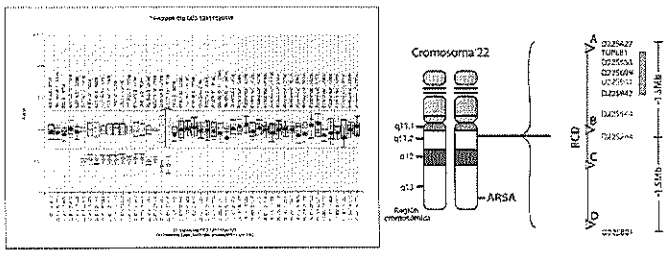

Figura 2. Análisis de CNVs mediante MLPA en un paciente que presenta una deleción típica del cromosoma 22q11.2 propia del síndrome Velocardiofacial.

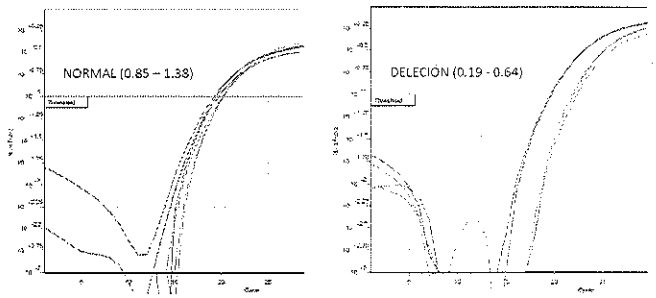

Figura 3. Resultados de la determinación de DGR por qPCR para el exón 2 del gen *TBX1*. a) control normal, DGR ≈1; b) paciente con deleción de *TBX1*; DGR ≈0.5

Figura 4. Estudio de FISH negativo para microdeleción del caso que presentó la deleción del exón 2 de *TBX1*. Se observa la hibridación de ambas señales para la región 22q11.2 y la sonda control.

RESULTADOS: Se observó una deleción de una región del exon 2 del gen *TBX1* en uno de los 28 casos con TF (Figura1), la cual fue confirmada por PCR en tiempo real (Figura 3). Ninguno de los progenitores de este caso presentó la deleción de *TBX1* y no presentaba otras alteraciones fenotípicas además de la cardiopatía. Para descartar la microdeleción 22q11 clásica en el paciente, se realizó FISH el cual fue negativo (figura 4). En el resto de familias analizadas no se observaron otros cambios en el número de copias.

CONCLUSIONES: En este estudio se identificó una CNV *de novo* del tipo deleción del exón 2 del gen *TBX1* en un caso con TF, la cual no fue detectada por técnicas convencionales. Este hallazgo es similar en frecuencia 3.5%(1/28) al reportado en otras poblaciones (3-6%)[4] y por la importancia funcional durante el desarrollo embriológico temprano de las estructuras cardiacas la deleción observada del gen *TBX1* podría estar asociada con la presencia de la Tetralogía de Fallot como ha sido estudiado.

BIBLIOGRAFÍA:

1. Feuk, L., A.R. Carson, and S.W. Scherer, *Structural variation in the human genome.* Nature Reviews Genetics, 2006. 7(2): p. 85-97.

2. Pollex, R.L. and R.A. Hegele, *Copy number variation in the human genome and its implications for cardiovascular disease.* Circulation, 2007. 115(24): p. 3130-8.

3. Greenway, S.C., A.C. Pereira, et al *De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot.* Nat Genet, 2009. 41(8): p. 931-5.

4. Tomita-Mitchell, A., D.K. Mahnke, C.A. Struble, M.E. Tuffnell, K.D. Stamm, M. Hidestrand, S.E. Harris, M.A. Goetsch, P.M. Simpson, D.P. Bick, U. Broeckel, A.N. Pelech, J.S. Tweddell, and M.E. Mitchell, *Human gene copy number spectra analysis in congenital heart malformations.* Physiol Genomics, 2012. 44(9): p. 518-41.

IDENTIFICATION OF COPY NUMBER VARIATION (CNVs) IN PATIENTS WITH ISOLATED CONOTRUNCAL HEART DEFECTS: A FAMILY TRIO STUDY.

J. Arteaga-Vazquez[1], A. Aguayo[1], Y. Svyryd[1], G. Vargas[2], JE. Calderon[2], C. Zamora[2], O. Mutchinick[1]. 1) Departamento de Genética, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; 2) Instituto Nacional de Cardiología Ignacio Chávez. México, D.F.

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BACKGROUND

Copy number variations (CNVs) initially were recognized as genomic non-functional and rare DNA arrangements but currently around 108,000 CNVs are identified along the human genome and contribute to genome variation between individuals in 5% approximately. CNVs can affect phenotypes by alteration of gene dosages and their products.

Congenital heart diseases are the more frequently birth defects. Besides, Tetralogy of Fallot (ToF) is present in 1/3000 live births and accounts for 60% of conotruncal malformations. Mutations have been detected in early developmental genes associated with ToF: *NKX2-5, NOTCH1, TBX1, JAG1, NOTCH2.* Moreover, Greenway et al. studying nuclear families identified seven CNVs associated with an increased risk for ToF, three of which correspond to genes previously associated with heart disease: *TBX1, NOTCH1* and *JAG1.*

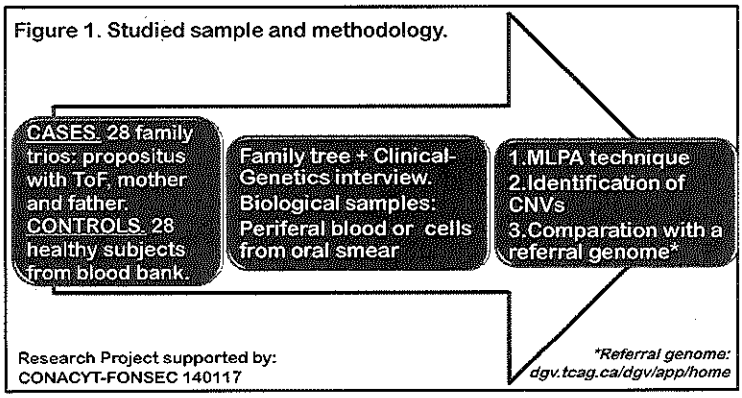
AIM

We decided to study CNVs related to congenital heart disease corresponding to genes *GATA4, NKX2-5, TBX5, BMP4, CRELD1* and other CNVs distributed throughout the chromosomal regions: 10p14, 4q35, 17p13, 22q11 and 22q13 by the MLPA (Multiplex Ligation-dependent Probe Amplification) technique, in a sample of Mexican patients with ToF, their parents and a control group.

MATERIAL AND METHODS

The research project was approved by the Ethics and Human Research Committees from INCMNSZ[1] and INCICH[2]. Twenty eight family trios in which the propositus had the diagnoses of TF and 28 healthy controls *(figure-1)* were analyzed with the MLPA technique using the P311(CHD) and P250 (DiGeorge) kits from MLPA-Holland® *(figure-2).* Real Time-PCR for gene dosage quantification and FISH probe 22q11.2 were used in order to validate the results.

Figure 1. Studied sample and methodology.



Research Project supported by: CONACYT-FONSEC 140117

RESULTS

One propositus showed a de novo CNV in the *TBX1* gene, involving 2-7 exons *(figure-3),* both parents were normal. We did not observe any change in the analyzed CNVs in 28 healthy subjects with negative familial history of ToF. qPCR confirmed the *TBX1* deletion *(figure-4).*FISH study was not able to detect the CNV in *TBX1.*

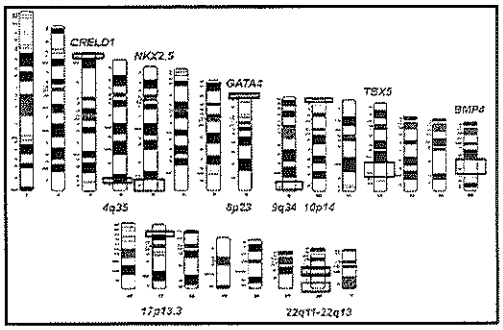


Figure-2. Chromosomal regions and genes explored with the MLPA-Holland® probes P311 (CHD) and P250 (DiGeorge).

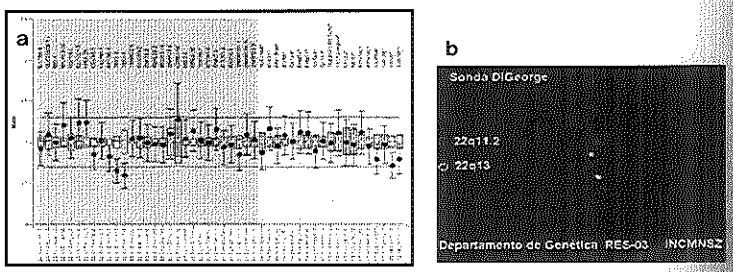


Figure-3. a. Analysis of amplified fragments showing a diminished fluorescence relation for *TBX1* gene (red dots). ChimerMarker and Coffalyser.Net softwares were used. b. Figure-4. FISH analysis in interphase nuclei with TUPLE LSI TUPLE1 SO/LSI ARSA SGn probe by ABBOT®, was normal.

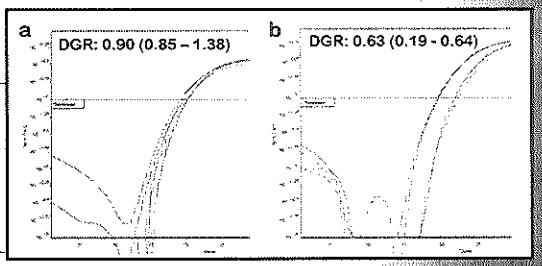


Figure-4. Relative quantification of gene dosage (DGR) by qPCR for exon 2 of *TBX1.* a) Normal control b) Patient with ToF.

CONCLUSIONS

1.We identified a *TBX1* gene deletion in 1/28 cases with ToF.

2.The proportion of cases with de novo CNVs in our sample is according to the observed by other research group, 3-6%(1).

3.The *TBX1* variant in our patient could explain the presence of ToF due to the important participation of this gene in early cardiac development.

4.The MLPA technique allows the detection of chromosomal alterations such as CNVs insertion/deletion type not identified by conventional karyotype or FISH analysis, at a low cost and with a fast performance.

REFERENCES

1. Tomita-Mitchell A, et al. Human gene copy number spectra analysis in congenital heart malformations. *Physiol Genomics*, 2012. 44(9): p. 518-41

2. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. *Nature Reviews Genetics*, 2006. 7(2): p. 85-97.

3. Pollex RL, Hegele RA. Copy number variation in the human genome and its implications for cardiovascular disease. *Circulation*, 2007. 115(24): p. 3130-8.

4. Greenway, S.C., et al. De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. Nat Genet, 2009. 41(8): p. 931-5.

Pediatr Cardiol (2015) 36:1642–1646
DOI 10.1007/s00246-015-1210-9

ORIGINAL ARTICLE

Identification of Copy Number Variations in Isolated Tetralogy of Fallot

Adolfo Aguayo-Gómez[1] · Jazmín Arteaga-Vázquez[1] · Yevgeniya Svyryd[1] · Juan Calderón-Colmenero[2] · Carlos Zamora-González[3] · Gilberto Vargas-Alarcón[4] · Osvaldo M. Mutchinick[1]

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Abstract Tetralogy of Fallot (ToF) is one of the most common and severe congenital heart defects (CHD). Recently, unbalanced structural genomic variants or copy number variations (CNVs) were proposed to be involved in the etiology of many complex diseases, including CHDs. The aim of this study was to investigate the frequency of CNVs in a region with a high density of CNVs, 22q11.2, and other regions with CHD-related genes in a sample of 52 Mexican mestizo patients with isolated ToF and negative fluorescence in situ hybridization staining for 22q11. CNVs were studied using two multiplex ligation-dependent probe amplification (MLPA) kits, SALSA P250-B1® (DiGeorge gene region) and SALSA MLPA P311-A1® CHD-related gene regions (*GATA4*, *NKX2-5*, *TBX5*, *BMP4*, and *CRELD1*). The MLPA assay detected a de novo CNV deletion of the probes located in exons 2 and 7 of the *TBX1* gene in one of the 52 patients studied; this result was confirmed by real-time quantitative polymerase chain reaction. This deletion was not present in the patient's parents and 104 chromosomes from healthy control subjects. Our results clearly suggest a possible etiologic association between the *TBX1* deletion and the ToF in our patient.

Keywords Tetralogy of Fallot · DNA copy number variations · *TBX1* gene

Introduction

Tetralogy of Fallot (ToF) is one of the most common and severe congenital heart defects (CHDs); it has a prevalence of approximately 1/3600 live births, and most frequently occurs sporadically in families without other congenital anomalies [2]. ToF is a multifactorial congenital defect that has been associated with rubella embryopathy, maternal diabetes, anticonvulsant ingestion during pregnancy, and other maternal exposures such as metronidazole [11]. ToF occurs during the first 8 weeks of human embryogenesis. According to reported experimental ablations of the secondary heart field (SHF) and neural crest, conotruncal defects such as ToF are result of a short outflow tract, anterocephalad deviation of the outlet ventricular septum, and a lack of conotruncal rotation [3, 16].

Some genes have been associated with ToF including *JAG1* (MIM 601920), *NKX2-5* (MIM 600584), *GATA4* (MIM 600576), *ZFPM2* (MIM 603693), *GDF1* (MIM 602880), *TBX1* (MIM 602054), and *GATA6* (MIM 601656); additionally, CNVs in the genes *MID1*, *ADAMTS2*, *CNN2*, *GJA5*, among others were associated with ToF [5, 19]. Recently, unbalanced structural genomic variants or copy number variations (CNVs) were proposed to be involved in the etiology of many diseases including CHD [6, 13]. The participation and effect of CNVs in the etiology of ToF are

Electronic supplementary material The online version of this article (doi:10.1007/s00246-015-1210-9) contains supplementary material, which is available to authorized users.

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poorly understood. Previous studies, applying low-resolution techniques, suggested that CNVs might be involved in the mechanisms of ToF [13]. Greenway et al. [6] studied CNVs in 114 patients with ToF and their parents using a high-resolution microarray and observed that 10 % of sporadic nonsyndromic ToF cases resulted from de novo CNVs. Moreover, seven new CNV loci were identified that were associated with an increased risk of sporadic ToF; this included three loci in genes that were previously associated with CHD: *TBX1*, *NOTCH1*, and *JAG1* [6].

The multiplex ligation-dependent probe amplification (MLPA) assay is a high-throughput and low-cost technique that allows the analysis of over 40 target genome sequences in a single reaction and detects multiple CNVs [22]. This technique has been recommended to screen 22q11.2 deletions in patients with conotruncal heart defects [1, 20].

Herein, we report the results of our search for CNVs in a region with a high density of CNVs, 22q11.2, and other regions with CHD-related genes in a sample of Mexican mestizo patients with isolated ToF and their parents.

Materials and Methods

Study Participants

All participants were recruited from patients attending the Adult and Pediatric Congenital Heart Defects Clinics and from patients referred for surgical CHD repair to the National Institute of Cardiology "Ignacio Chávez." In all cases, ToF was confirmed by an expert cardiologist, and other congenital malformations or clinical phenotypes of the 22q11 spectrum syndrome were assessed by a qualified medical geneticist. Case enrollment criteria included the confirmation of the isolated ToF diagnosis based on clinical, imaging, and hemodynamic studies; the agreement of both parents to participate in the study; and that the four grandparents were born in Mexico. Patients with DiGeorge syndrome or velocardiofacial syndrome confirmed by fluorescence in situ hybridization (FISH) were excluded. Signed informed consent agreement was obtained from all participants and from the respective parents of those patients who were less than 18 years of age. The institutional research and ethics board committees approved the protocol. For all cases, perinatal information about illnesses and risk factors for CHD was collected, and a family pedigree of all participating families was built.

MLPA Analysis

Blood or saliva samples were collected from all participants for genomic DNA extraction from blood lymphocytes or saliva by standard procedures. CNV studies were performed using two kits: SALSA MLPA P311-A1® Congenital Heart Defects and SALSA P250-B1® DiGeorge (MRC-Holland, Amsterdam, The Netherlands). The P311-A1 CHD probe mix contains 45 DNA probes, which includes 35 probes to the *GATA4*, *NKX2-5*, *TBX5*, *BMP4*, and *CRELD1* genes and 10 control probes. The P250-B1 DiGeorge probe mix contains 48 MLPA probes; of these, 29 probes correspond to the 22q11 region (LCR22-A to LCR22-D), and the other probes target the 22q13, 10p14, 8p23.1, 4q34-qter, 9q34.3, and 17p13.3 chromosomal regions. The MLPA assay was performed according to the standard protocol supplied by MRC-Holland® [18]. The copy number ratio determination was performed using the Coffalyser.Net v.131211 free software. Both MLPA analyses were performed on 52 (104 chromosomes) individuals with no history of any type of CHD to analyze CNVs in a group of healthy blood donors. Real-time quantitative polymerase chain reaction (qPCR) was used to confirm MLPA findings (Supplemental material).

Results

A total of 52 patients consisting of 27 males (51.9 %) and 25 females (48.1 %) with ToF were included in the study. The mean maternal and paternal age at birth of the patients was 24.5 ± 7.4 and 28.2 ± 7.6 years, respectively. Delivery by cesarean section was observed in 11 pregnancies, prematurity occurred in two cases, and first trimester-threatened abortion was reported in five cases. No other CHD maternal risk factors were observed. During pregnancy, 30 mothers of cases consumed dietary supplements of folic acid, but only 16 mothers consumed folic acid supplements during the periconceptional time. Two mothers of cases used nonsteroidal anti-inflammatory drugs, and one mother smoked cigarettes during the first trimester of pregnancy. Other environmental exposures during the first trimester of pregnancy were not reported.

The MLPA assay detected a heterozygous deletion of the probes 05408-L07614 and 10810-L14347, which are located in exons 2 and 7 of the *TBX1* gene in one patient (Fig. 1). This result was confirmed by qPCR for both exons (Fig. 2). The size of the imbalance was estimated between 6177 and 6241 bp on chromosome 22 between coordinates 18127111 and 18133352 (GRC36 [hg18]) region according to the MLPA P250-B1 kit. The case was a 19-year-old female with normal intellectual and social behavior and no other clinical manifestation related to the 22q11 spectrum phenotype. The MLPA study of her parents was normal. The observed CNV deletion was absent in the analysis of 104 chromosomes from 52 healthy control subjects.

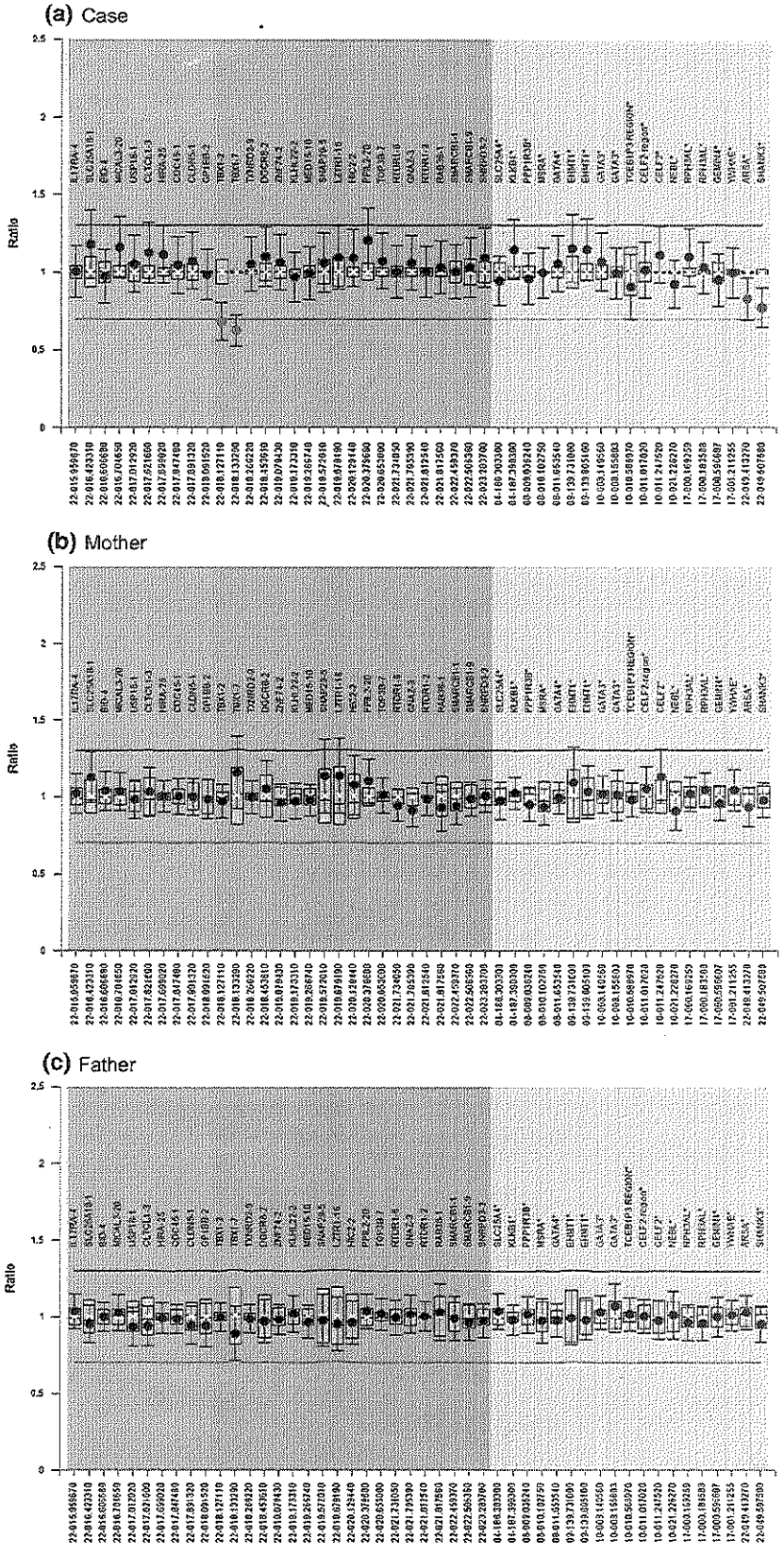

Fig. 1 Multiplex ligation-dependent probe amplification (MLPA) analysis. MLPA analysis in the patient (**a**) positive for deletion of the *TBX1*-2 and *TBX1*-7 probes located in the *TBX1* gene (*red dots*) and her parents (**b**, **c**)

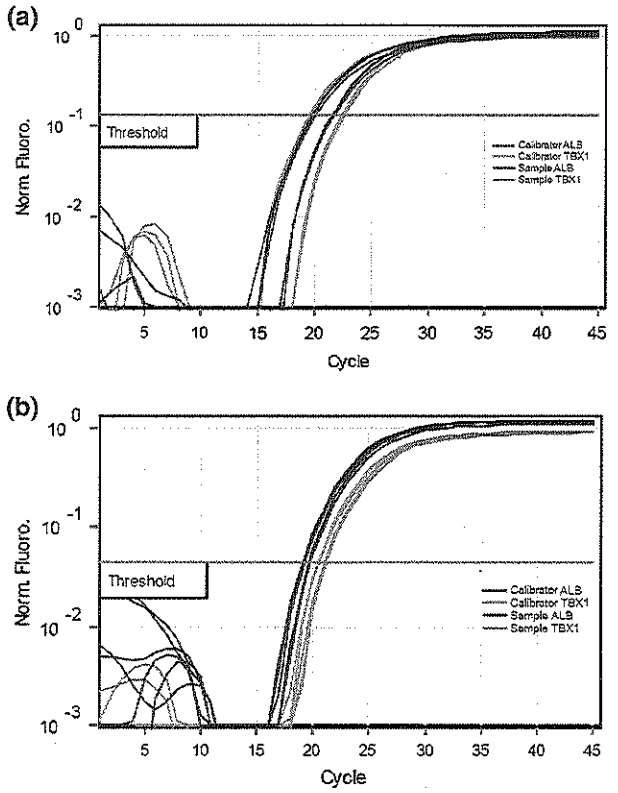

Fig. 2 Confirmation of *TBX1* deletion. **a** Real-time quantitative PCR (qPCR) for *TBX1* exon 2 ($2^{-\Delta\Delta C_t} = 0.63$)). **b** qPCR for *TBX1* exon 7 ($2^{-\Delta\Delta C_t} = 0.61$)). *ALB*, albumin was used as a normal copy reference gene

Discussion

The MLPA assay identified a de novo CNV in the *TBX1* gene that was not detected by FISH. The frequency of CNVs in CHD-related genes in our study was lower than that in previous reports (3–10 %) [23]. *TBX1* has been studied as a critical gene in the early development of heart structures and as one of the most important factors in the pathogenesis of 22q11.2 deletion syndrome [9]. The absence of amplification of the MLPA probes corresponding to exons 2 and 7 in *TBX1* suggests the possibility of an almost complete gene deletion that could affect the normal function of the allele. This particular CNV and the derived haploinsufficiency might explain the CHD present in the patient because the first eight exons of the gene are shared by the three human alternative splicing transcripts of *TBX1* [7]. The above-mentioned hypothesis is supported by reports of CNVs in the *TBX1* gene that were associated with conotruncal heart defects in a mouse model [8, 17]. Mouse studies showed that haploinsufficiency of cardiac transcription factor genes such as *NKX2–5*, *TBX1*, *TBX5*, and *GATA4* was related to ToF and other CHDs [8].

TBX1 was identified as a major candidate gene in the etiology of most of the described phenotypes observed in the 22q11.2 deletion syndrome, which is characterized by a spectrum of abnormalities including conotruncal heart defects [10]. In mice, *Tbx1* is expressed mostly in the SHF, pharyngeal endoderm, and head mesenchyme, and it contributes to the outflow tract myocardium, right ventricle, endocardium, and mesenchymal cushions [14]. *Tbx1* is known to play a role in proliferation and differentiation in the SHF. The loss of *Tbx1* affects SHF development, leading to hypoplasia of the outflow tract at midgestation and a common arterial trunk at the fetal stage [24].

A previous study [21] reported a CNV frequency of 1.2 % (5/402) in the 22q11.2 region in isolated CHD cases using the MLPA assay; however, the type of the heart malformation was not specified. Of three duplications and two deletions observed in that study, only one deletion involved the *TBX1* gene. We hypothesize that the heterozygous presence of the deletion involving the *TBX1* gene resulted in the haploinsufficiency of *TBX1* in our patient. The absence of this deletion in both normal parents and 104 chromosomes from healthy controls strongly suggests an etiologic association with ToF in the patient. We are not able to exclude CNVs and point mutations in other ToF-related genes such as *NOTCH1*, *JAG1*, and *HAND2* , because of the limitations of the MLPA assay. Although all the patients studied showed a normal phenotype except for the heart defect present in all of them, other genetic abnormalities cannot be ruled out.

The haploinsufficiency of *TBX1* causing CHD has been suggested to be a result of point mutations [25], deletions [12], or duplications [15]. Our findings agree with those reported by Chen et al. [4], who used comparative genomic hybridization to analyze 45 fetal samples with conotruncal heart defects that were negative for del22q11.2 by FISH. Their results showed an 846-bp de novo deletion including exon 2 of the *TBX1* gene in one patient with ToF. Interestingly, this DNA fragment overlaps with the deletion observed in our patient, which also comprises the exon 2 of the *TBX1* gene.

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Agergaard P, Olesen C, Ostergaard JR, Christiansen M, Sørensen KM (2012) The prevalence of chromosome 22q11.2 deletions in 2,478 children with cardiovascular malformations. A population-based study. Am J Med Genet A 158A(3):498–508. doi:10.1002/ajmg.a.34250
2. Apitz C, Webb GD, Redington AN (2009) Tetralogy of Fallot. Lancet 374(9699):1462–1471. doi:10.1016/S0140-6736(09)60657-7
3. Bailliard F, Anderson RH (2009) Tetralogy of Fallot. Orphanet J Rare Dis 13(4):2. doi:10.1186/1750-1172-4-2
4. Chen M, Yang YS, Shih JC, Lin WH, Lee DJ, Lin YS, Chou CH, Cameron AD, Ginsberg NA, Chen CA, Lee ML, Ma GC (2014) Microdeletions/duplications involving TBX1 gene in fetuses with conotruncal heart defects which are negative for 22q11.2 deletion on fluorescence in situ hybridization. Ultrasound in obstetrics & gynecology: the official journal of the International Society of. Ultrasound Obstet Gynecol 43(4):396–403. doi:10.1002/uog.12550
5. Fahed AC, Gelb BD, Seidman JG, Seidman CE (2013) Genetics of congenital heart disease: the glass half empty. Circ Res 112(4):707–720. doi:10.1161/CIRCRESAHA.112.300853
6. Greenway SC, Pereira AC, Lin JC, Depalma SR, Israel SJ, Mesquita SM, Ergul E, Conta JH, Korn JM, McCarroll SA, Gorham JM, Gabriel S, Altshuler DM, De Lourdes Quintanilla-Dieck M, Artunduaga MA, Eavey RD, Plenge RM, Shadick NA, Weinblatt ME, De Jager PL, Hafler DA, Breitbart RE, Seidman JG, Seidman CE (2009) De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. Nat Genet 41(8):931–935
7. Heike CL, Starr JR, Rieder MJ, Cunningham ML, Edwards KL, Stanaway IB, Crawford DC (2010) Single nucleotide polymorphism discovery in TBX1 in individuals with and without 22q11.2 deletion syndrome. Birth Defects Res A Clin Mol Teratol 88(1):54–63. doi:10.1002/bdra.20604
8. Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, Jurecic V, Ogunrinu G, Sutherland HF, Scambler PJ, Bradley A, Baldini A (2001) Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. Nature 410(6824):97–101
9. Merscher S, Funke B, Epstein JA, Heyer J, Puech A, Lu MM, Xavier RJ, Demay MB, Russell RG, Factor S, Tokooya K, Jore BS, Lopez M, Pandita RK, Lia M, Carrion D, Xu H, Schorle H, Kobler JB, Scambler P, Wynshaw-Boris A, Skoultchi AI, Morrow BE, Kucherlapati R (2001) TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. Cell 104(4):619–629
10. Parisot P, Mesbah K, Théveniau-Ruissy M, Kelly RG (2011) Tbx1, subpulmonary myocardium and conotruncal congenital heart defects. Birth Defects Res A Clin Mol Teratol 91(6):477–484
11. Patel SS, Burns TL (2013) Nongenetic risk factors and congenital heart defects. Pediatr Cardiol 34(7):1535–1555. doi:10.1007/s00246-013-0775-4
12. Paylor R, Glaser B, Mupo A, Ataliotis P, Spencer C, Sobotka A, Sparks C, Choi CH, Oghalai J, Curran S, Murphy KC, Monks S, Williams N, O'Donovan MC, Owen MJ, Scambler PJ, Lindsay E (2006) Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. Proc Natl Acad Sci USA 103(20):7729–7734. doi:10.1073/pnas.0600206103
13. Pollex RL, Hegele RA (2007) Copy number variation in the human genome and its implications for cardiovascular disease. Circulation 115(24):3130–3138. doi:10.1161/CIRCULATIONAHA.106.677591
14. Rana MS, Christoffels VM, Moorman AF (2013) A molecular and genetic outline of cardiac morphogenesis. Acta Physiol 207(4):588–615. doi:10.1111/apha.12061
15. Rauch R, Hofbeck M, Zweier C, Koch A, Zink S, Trautmann U, Hoyer J, Kaulitz R, Singer H, Rauch A (2010) Comprehensive genotype-phenotype analysis in 230 patients with tetralogy of Fallot. J Med Genet 47(5):321–331. doi:10.1136/jmg.2009.070391
16. Restivo A, Piacentini G, Placidi S, Saffirio C, Marino B (2006) Cardiac outflow tract: a review of some embryogenetic aspects of the conotruncal region of the heart. Anat Rec A Discov Mol Cell Evol Biol 288(9):936–943. doi:10.1002/ar.a.20367
17. Scambler PJ (2010) 22q11 Deletion syndrome: a role for TBX1 in pharyngeal and cardiovascular development. Pediatr Cardiol 31(3):378–390
18. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30(12):e57
19. Soemedi R, Wilson IJ, Bentham J, Darlay R, Töpf A, Zelenika D, Cosgrove C, Setchfield K, Thornborough C, Granados-Riveron J, Blue GM, Breckpot J, Hellens S, Zwolinkski S, Glen E, Mamasoula C, Rahman TJ, Hall D, Rauch A, Devriendt K, Gewillig M, O'Sullivan J, Winlaw DS, Bu'lock F, Brook JD, Bhattacharya S, Lathrop M, Santibanez-Koref M, Cordell HJ, Goodship JA, Keavney BD (2012) Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease. Am J Hum Genet 91(3):489–501. doi:10.1016/j.ajhg.2012.08.003
20. Sørensen KM, Agergaard P, Olesen C, Andersen PS, Larsen LA, Ostergaard JR, Schouten JP, Christiansen M (2010) Detecting 22q11.2 deletions by use of multiplex ligation-dependent probe amplification on DNA from neonatal dried blood spot samples. J Mol Diagn 12(2):147–151. doi:10.2353/jmoldx.2010.090099
21. Sørensen KM, El-Segaier M, Fernlund E, Errami A, Bouvagnet P, Nehme N, Steensberg J, Hjortdal V, Soller M, Behjati M, Werge T, Kirchoff M, Schouten J, Tommerup N, Andersen PS, Larsen LA (2012) Screening of congenital heart disease patients using multiplex ligation-dependent probe amplification: early diagnosis of syndromic patients. Am J Med Genet A 158A(4):720–725. doi:10.1002/ajmg.a.35214
22. Stuppia L, Antonucci I, Palka G, Gatta V (2012) Use of the MLPA assay in the molecular diagnosis of gene copy number alterations in human genetic diseases. Int J Mol Sci 13(3):3245–3276. doi:10.3390/ijms13033245
23. Tomita-Mitchell A, Mahnke DK, Struble CA, Tuffnell ME, Stamm KD, Hidestrand M, Harris SE, Goetsch MA, Simpson PM, Bick DP, Broeckel U, Pelech AN, Tweddell JS, Mitchell ME (2012) Human gene copy number spectra analysis in congenital heart malformations. Physiol Genomics 44(9):518–541. doi:10.1152/physiolgenomics.00013.2012
24. Xu H, Morishima M, Wylie JN, Schwartz RJ, Bruneau BG, Lindsay EA, Baldini A (2004) *Tbx1* has a dual role in the morphogenesis of the cardiac outflow tract. Development 131(13):3217–3227. doi:10.1242/dev.01174
25. Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, Minoshima S, Ichida F, Joo K, Kimura M, Imamura S, Kamatani N, Momma K, Takao A, Nakazawa M, Shimizu N, Matsuoka R (2003) Role of TBX1 in human del22q11.2 syndrome. Lancet 362(9393):1366–1373. doi:10.1016/S0140-6736(03)14632-6