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Nativa al abrigo de la sierra
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DELECIONES EN EL GEN *NPHP1* Y SU ASOCIACIÓN CON NEFRONOPTISIS: REPORTE DE CASO

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ABSTRACT

Nephronophthisis is a hereditary kidney disease with considerable phenotypic variability, characterized by chronic tubulointerstitial nephritis and progression to end-stage renal disease. Approximately 20 genes are associated with either isolated (80-90%) or syndromic (10-20%) forms, most of which follow an autosomal recessive inheritance pattern. This study presents the case of an 11-year-old patient, born to healthy non-consanguineous parents, with chronic renal failure of unknown etiology, associated with polycystic kidney disease. A genomic analysis was performed with the aim of detecting single nucleotide variants and copy number variants in a gene panel associated with the clinical suspicion. Two variants in the *NPHP1* gene were identified in a compound heterozygous state: the c.1897_1906del p.(Thr633LeufsTer37) variant and a deletion involving the entire gene. These findings suggested juvenile nephronophthisis associated with *NPHP1* as the most likely etiological diagnosis for this patient. For the final classification of the genetic variants, family segregation information and phenotypic data were integrated, implementing the ClinGen and ACMG/AMP guidelines, allowing an accurate interpretation of their pathogenicity.

Key words: bioinformatic algorithms, classification guidelines, genomic analyses, nephronophthisis

RESUMEN

La nefronoptisis es una enfermedad renal hereditaria con gran variabilidad fenotípica, que se caracteriza por nefritis tubulointersticial crónica y progresión hacia enfermedad renal terminal. Alrededor de 20 genes se asocian a formas aisladas (80-90%) o sindrómicas (10-20%), en su mayoría de herencia autosómica recesiva. En este estudio se presenta el caso de un paciente de 11 años, hijo de padres sanos no consanguíneos, con insuficiencia renal crónica de etiología desconocida, asociada a poliquistosis renal. Se realizó un análisis genómico con el objetivo de detectar variantes de nucleótido único y variantes en el número de copias en un panel de genes asociado a la sospecha clínica. Se identificaron dos variantes en heterocigosis compuesta en el gen *NPHP1*: la variante c.1897_1906del p.(Thr633LeufsTer37), y una deleción que involucraba al gen completo. Estos hallazgos sugirieron nefronoptisis juvenil asociada a *NPHP1* como diagnóstico etiológico más probable para este paciente. Para la clasificación final de las variantes genéticas, se integró información de segregación familiar y datos fenotípicos, implementando las guías de ClinGen y ACMG/AMP, lo cual permitió establecer una interpretación precisa de su patogenicidad.

Palabras clave: algoritmos bioinformáticos, análisis genómicos, guías de clasificación, nefronoptisis

INTRODUCCIÓN

La nefronoptisis (NFP, OMIM 26100) es una enfermedad renal quística autosómica recesiva, y una de las causas genéticas más frecuentes de insuficiencia renal en niños/as y adultos jóvenes (Wolf, 2015). Los síntomas clínicos típicos incluyen poliuria, polidipsia, alteración de la reabsorción de sodio resultante en hipovolemia e hiponatremia, nefritis tubulointersticial crónica y progresión a enfermedad renal terminal (ERT) típicamente antes de los 30 años; aunque también se han informado apariciones más tardías. La NFP se sospecha en ausencia de anomalías congénitas de los riñones y/o del tracto urinario, y cuando se presentan signos o síntomas de enfermedad renal glomerular. La ecografía renal en los estadios iniciales es normal, o revela cambios inespecíficos con un aumento de la ecogenicidad renal, y en la enfermedad renal avanzada se muestra una pobre diferenciación corticomedular; los quistes corticomedulares aparecen en el 70% de los pacientes. En el momento de la presentación inicial, aproximadamente el 80%-90% de las personas con nefronoptisis parecen tener NFP aislada (es decir, todos los hallazgos clínicos evidentes son secundarios a la disfunción renal), y aproximadamente entre 10%-20% de las personas tienen manifestaciones extrarrenales, que pueden ser indicativas de un cuadro sindrómico (Stokman et al., 2023). Estos síndromes presentan características como retinitis pigmentosa (síndrome Senior-Loken, MIM: 266900) o hipoplasia del vermis cerebeloso y otras anomalías multiorgánicas (síndrome de Joubert, MIM: 609583). Otras características extrarrenales incluyen fibrosis hepática, parálisis de la mirada, *situs inversus* y defectos esqueléticos (Simms et al., 2011).

Se reconocen dos fenotipos clínicos generales basados en la edad de inicio para la NFP: un fenotipo de inicio infantil y, más comúnmente, un espectro fenotípico de inicio juvenil, adolescente o adulto. Sin embargo, existe gran variabilidad inter e intrafamiliar, principalmente en la tasa de progresión a ERT (König et al., 2017; Gupta et al., 2021; Stokman et al., 2023).

La prevalencia de esta enfermedad poco frecuente es desconocida. Sin embargo, la incidencia estimada de NFP en Norteamérica es de entre 1/50.000 a 1/100.000 (Luo y Tao, 2018). Además, es la responsable de entre el 2,4-15% de casos de ERT en la infancia. No existe tratamiento específico, por lo que el abordaje se basa en el manejo de la insuficiencia renal crónica y el trasplante renal oportuno (Pitón et al., 2022).

El diagnóstico de NFP se basa en las manifestaciones clínicas y se confirma con una prueba genética positiva. El papel de la biopsia renal en el diagnóstico sigue siendo motivo de debate (Luo et al., 2018). A la fecha, se han identificado variantes genéticas causantes de esta patología en 21 genes en el 50-60% de los individuos afectados con nefronoptisis. La mayoría de estos genes codifican proteínas ciliares agrupadas en diferentes localizaciones subcelulares. Dentro de estos, se encuentran genes mayormente asociados con NFP, como *NPHP1, NPHP4, CEP290, IQCB1, TMEM67, INVS, NPHP3* y genes poco frecuentes asociados a NFP, como *ADAMTS9, ANKS6, CEP83, CEP164, DCDC2, GLIS2, IFT172, MAPKBP1, NEK8, RPGRIP1L, SDCCAG8, TTC21B, WDR19* y *ZNF423* (Gupta et al., 2021).

Dentro de los casos de NFP juvenil, entre el 20 y 25% poseen mutaciones que causan pérdida de función en el gen *NPHP1*. Este gen codifica para la proteína nefrocistina-1, la cual se expresa principalmente en las células epiteliales ureterales y en los precursores tubulares del riñón (Lindström et al., 2018), y se localiza en la zona de transición de la base ciliar. Esta proteína desempeña un rol fundamental en la morfología adecuada de los cilios (Adamiok-Ostrowska y Piekiełko-Witkowska, 2020), y se ha demostrado que las mutaciones en este gen alteran su estructura y función (Leggatt et al., 2023). La expresión a nivel proteico es anormal en células traqueales de pacientes con nefronoptisis, y las células derivadas presentan algunas anomalías sutiles de la motilidad ciliar (Fliegauf et al., 2006).

Existen numerosos informes tanto de deleciones homocigotas como de variantes heterocigotas compuestas (Otto et al., 2008; Halbritter et al., 2013), ocasionadas por una variante de pérdida de función junto a una segunda variante, incluyendo aquellas del tipo *missense* (Caridi et al., 2006; Otto et al., 2008; Halbritter et al., 2013).

A continuación, se describe el caso clínico de un niño con diagnóstico presuntivo de nefronoptisis de presentación infantil.

Presentación del caso

Se presenta al Hospital Pediátrico Dr. Humberto Notti de Mendoza, un niño de 11 años y seis meses, con insuficiencia renal crónica de origen desconocido asociada a poliquistosis renal. Se trata del quinto hijo de una hermandad de seis, de una pareja referida sana no consanguínea. No se rescatan antecedentes familiares renales u otro antecedente de relevancia (Figura 1). El paciente comienza seguimiento multidisciplinario en el mencionado nosocomio desde 2021, por detención del crecimiento, enuresis secundaria, palidez, astenia y dolor óseo, con diagnóstico de anemia y fallo renal. Se constata al examen físico sin dismorfias, baja talla (121,8 cm; percentil <3 y DE: -2,91) y bajo peso (22 kg; percentil <3 y DE: -2,92) con neurodesarrollo acorde a edad.

En su ingreso hospitalario se realizaron múltiples estudios complementarios. Los estudios ecográficos revelaron ecogenicidad aumentada y múltiples imágenes quísticas en ambos riñones (Figura 2). Los valores de laboratorio (Hto: 22%, Hb: 7,3 g/dL, creatinina: 6,53 mg/

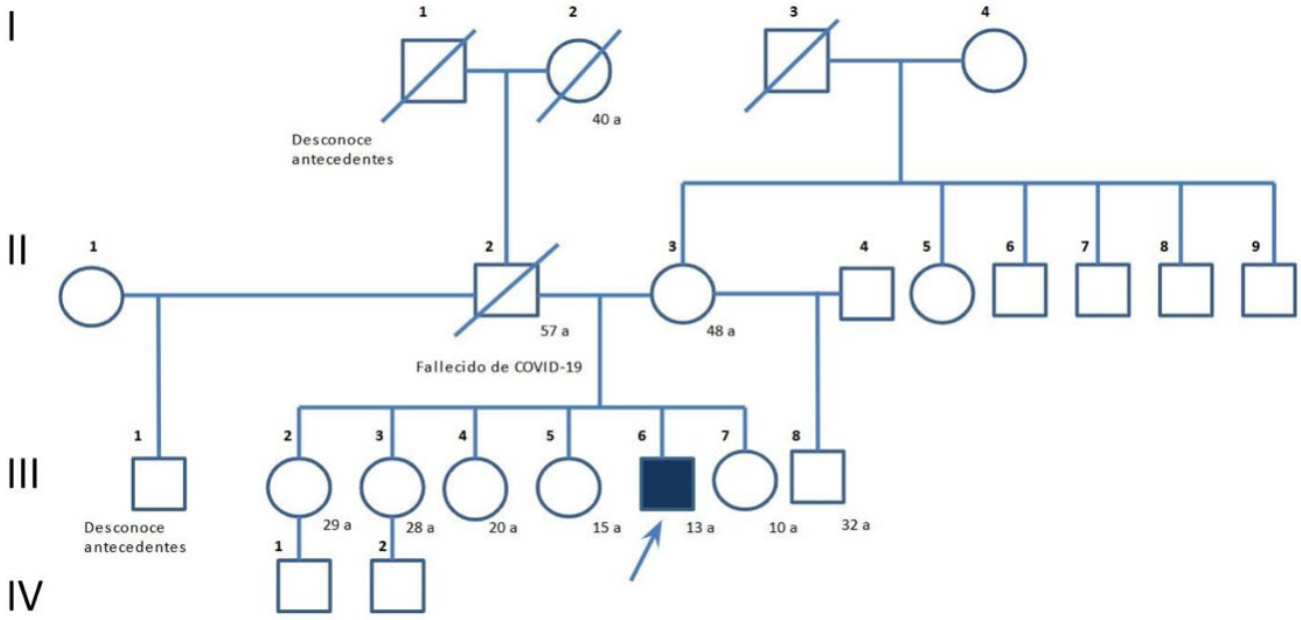

Figura 1. Genealogía. Pedigree de la familia del probando. Con la flecha se señala el paciente en estudio.

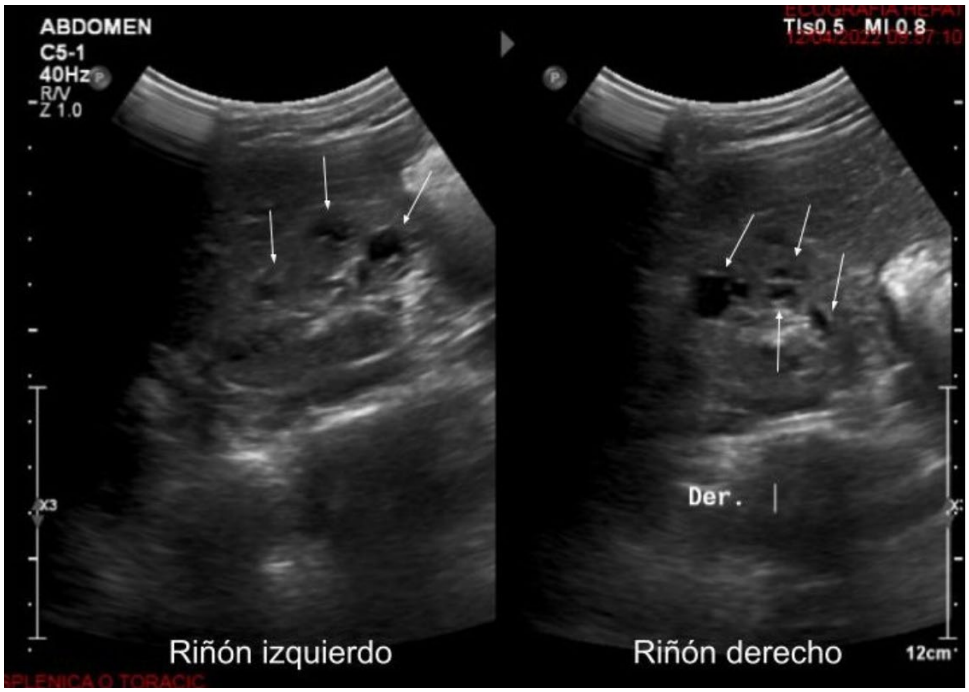

Figura 2. Ecografía renal. Se evidencian quistes pequeños entre corteza y médula en ambos riñones, señalados con flechas.

dL, clearance de creatinina: 10 ml/min/1,73m², uremia: 2,32g/l, K: 4,2 mEq/L, Na: 143 mEq/L, Cl: 91 mEq/L, Ca: 6,9 mg/dL, P: 4,91 mg/dL, Mg: 1,98 mg/dL, estado ácido base -EAB- venoso: pH: 7,19 - PCO_2: 29,7 mmHs - HCO_3: 11,6 mmol/l, hormona paratiroidea -PTH: 964 ng/dL, en orina de 24 h: proteinuria: 16 mg/kg/día, densidad urinaria: 1006) fueron compatibles con insuficiencia renal crónica con requerimiento de terapia de reemplazo renal mediante Diálisis Peritoneal Continua Ambulatoria (DPCA) hasta su trasplante renal con buena respuesta. El fenotipo clínico y los estudios de imágenes orientaron a la sospecha clínica de nefronoptisis.

El objetivo de este estudio fue analizar genes específicos asociados a nefronoptisis en un paciente con sospecha clínica, empleando diversos métodos diagnósticos. La caracterización molecular de la enfermedad contribuirá a un mejor abordaje terapéutico y al asesoramiento genético familiar.

MATERIALES Y MÉTODOS

Bajo previo consentimiento informado para la realización del estudio genómico, se procedió a la toma de muestra sanguínea y extracción de ADN. Se realizó secuenciación masiva o *Next Generation Sequencing* (SM o NGS) de exoma completo utilizando el kit de captura *Agilent Sure Select V7*. Posteriormente a la secuenciación, se realizó el procesamiento bioinformático de las lecturas, incluyendo el mapeo y el llamado de variantes mediante la herramienta "HaplotypeCaller" del *Genome Analysis Toolkit* (GATK), lo que posibilitó la generación de un archivo en formato VCF. Las variantes fueron anotadas utilizando los paquetes SnpEff/SnpSift, lo que posibilitó vincular cada variante con información biológica proveniente de bases de datos externas. Finalmente, se procedió a la priorización de variantes en genes candidatos asociados con la nefronoptisis y sus diagnósticos diferenciales: *AHI1, ANKS6, ARMC4, ARL13B, ARL6, B9D1, B9D2, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, CC2D2A, CCDC103, CCDC39, CCDC40, CCDC65, CCNO, CEP104, CEP120, CEP164, CEP290, CEP41, CSPP1, C21orf59, C5orf42,CCDC114, CCDC151, DYX1C1, DCDC2, DNAAF1, DNAAF2, DNAAF3, DNAH1, DNAH11, DNAH5, DNAH8, DNAI1, DNAI2, DNAL1, DRC1, DYNC2H1, EVC, EVC2, GAS8, GLIS2, IFT122, IFT140, IFT172, IFT80, INPP5E, INVS, IQCB1, KIAA0586, KIF7, LRRC6, MCIDAS, MKKS, MKS1, NEK1, NEK8, NME8, NPHP1, NPHP3, NPHP4, OFD1, PKD2, PKHD1, RPGR, RPGRIP1L, RSPH1, RSPH3, RSPH4A, RSPH9, SDCCAG8, SPAG1, TCTN1, TCTN2, TCTN3, TMEM138, TMEM216, TMEM231, TMEM237, TMEM67, TRIM32, TTC21B, TTC8, WDPCP, WDR19, WDR34, WDR35, WDR60 ZMYND10, ZNF423.*

Para el *screening* de Variantes en el Número de Copias (o CNVs, del inglés *copy number variants*) se utilizaron los algoritmos bioinformáticos DECoN y ONCOCNV.

Las variantes candidatas fueron clasificadas a partir de las guías de ACMG/AMP (Richards et al., 2015) y las actualizaciones y recomendaciones de ClinGen (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/) en el caso de variantes puntuales (SNVs, del inglés *single nucleotide variants*) y pequeñas inserciones y deleciones (*indels*, del inglés *insertions and deletions*) y las recomendaciones y guías del ACMG-ClinGen (Riggs et al., 2020) en el caso de las CNVs.

Los hallazgos detectados a través de la secuenciación exómica fueron validados. La variante de secuencia, que consistía en una deleción de 10 pb, se confirmó por secuenciación por Sanger siguiendo el protocolo de Soliman et al. (2012). Los cebadores se diseñaron utilizando Primer-BLAST (Ye et al., 2012). La secuencia de los cebadores utilizados se detalla en la Tabla 1. Las amplificaciones de ADN se realizaron en reacciones de 50 µl, utilizando la ADN polimerasa PFU (Inbio Highway, Argentina). Los productos de PCR se purificaron en un gel de agarosa utilizando el kit de recuperación de ADN PuriPrep-GP Kit (Inbio Highway) y fueron secuenciados. La deleción, que involucraba aparentemente a todo el gen, fue confirmada mediante un experimento diseñado específicamente por PCR en tiempo real cuantitativa (qPCR) para determinar la dosis alélica del gen *NPHP1*. Esto se realizó bajo la inspiración del protocolo empleado por Chen et al. (2020) con un cambio en el diseño de cebadores. Los oligonucleótidos se encuentran descriptos en la Tabla 2. Las amplificaciones se realizaron utilizando el kit GoTaq qPCR Master Mix (Promega, WI, EEUU). Las condiciones de ciclado térmico estuvieron compuestas por: 2 min a 50 ºC, seguido de un paso inicial de desnaturalización durante 10 min a 95 ºC, 40 ciclos de 15 s a 95 ºC y 1 min a 60 ºC. Los experimentos se llevaron a cabo por duplicado. La cuantificación

Tabla 1. Secuencia de los cebadores para confirmar la pequeña deleción de 10 pb del gen *NPHP1*.

Cebador	Secuencia 5'-3'
Forward	AATGGCACCCTCCATCCTAC
Reverse	TGAAAAGGCAAAAGCGACTCTGT

Tabla 2. Secuencia de los cebadores para confirmar la deleción del gen *NPHP1* completo.

Cebador	Secuencia 5'-3'
Forward (deleción)	CCAGAAGGTGACGCCATCG
Reverse (para ambos)	AGTGACAGTATGGAACAGAACCA

relativa en la expresión génica se determinó utilizando el método $2^{\Delta\Delta Ct}$ (Livak y Schmittgen, 2001). Utilizando este método, se obtuvieron los cambios en la expresión génica normalizados a un gen de control interno, *GAPDH*.

RESULTADOS

Diagnóstico Genético

En primer lugar, el análisis de los datos de secuenciación del exoma completo del caso permitió identificar a la variante NM_001128178.3:c.1897_1906del - p.(Thr633LeufsTer37) en el gen *NPHP1* en aparente homocigosis. Se trata de una deleción de 10 pb que se predice que causa un corrimiento del marco de lectura con la posterior aparición de un codón *stop* prematuro. La variante se clasificó en primera instancia (agosto de 2022) como de significado incierto (VUS) aplicando los siguientes criterios según las guías mencionadas previamente (Richards et al., 2015):

- Variante que resulta en la pérdida de menos del 10% de la proteína, se predice que escapa al mecanismo NMD (*Nonsense Mediated Decay*) y la pérdida de función proteica constituye un mecanismo conocido asociado al desarrollo de patogenicidad en este gen (criterio PVS1_Moderate).
- Variante que posee baja frecuencia poblacional en la base de datos de gnomAD 4.1 (sin reporte de homocigotas, criterio PM2_Supporting).

Además, al no encontrarse descripta en la literatura, fue considerada una variante novel. Sin embargo, el 15 de julio del 2023 un laboratorio reportó dicha variante como probablemente patogénica en la base de datos clínica ClinVar, aunque sin ningún tipo de descripción adicional.

Dado que no se presentaba consanguinidad en la familia y se trataba de una enfermedad poco frecuente, la probabilidad de que el paciente hubiera heredado la misma mutación de ambos padres era baja. Además, la técnica de secuenciación exómica utilizada en este caso no permite detectar con precisión deleciones de gran tamaño. Por ello, se decidió ampliar la investigación mediante una metodología más adecuada para confirmar la presencia de la variante en homocigosis. Asimismo, en la literatura existen reportes de CNVs en este gen, lo que reforzó la importancia de profundizar en la evaluación del caso. De esta manera, el análisis bioinformático de CNVs a partir de los datos del exoma completo, permitió detectar una deleción en el otro alelo, que involucraba al gen completo de 81.217 pb aproximadamente (Figura 3). Para esta deleción, de acuerdo a las normas de HGVS e ISCN, se utiliza la siguiente nomenclatura:

seq[GRCh38] del(2)(q13q13) NC_000002.12:g.(?_110.123.799)_(110.205.016_?)del.

Dicha deleción se identificó en heterocigosis en el brazo largo del cromosoma 2. La región genómica involucrada en este rearreglo se inicia en la posición 110.123.799 y tiene un extremo final en la posición 110.205.016 aproximadamente, resultando en una monosomía parcial del cromosoma 2 de alrededor de 0,08 Mb (los puntos de ruptura son aproximados debido a las limitaciones de la metodología de análisis).

La variante se clasificó como patogénica, aplicando los siguientes criterios, basados en las guías mencionadas previamente (Riggs et al., 2020):

- La deleción hallada incluye genes codificantes de proteínas u otros elementos funcionalmente importantes conocidos (criterios 1A; 3A).
- Se trata de una variante que solapa completamente con una región genómica recurrente establecida como haploinsuficiente (criterio 2A).

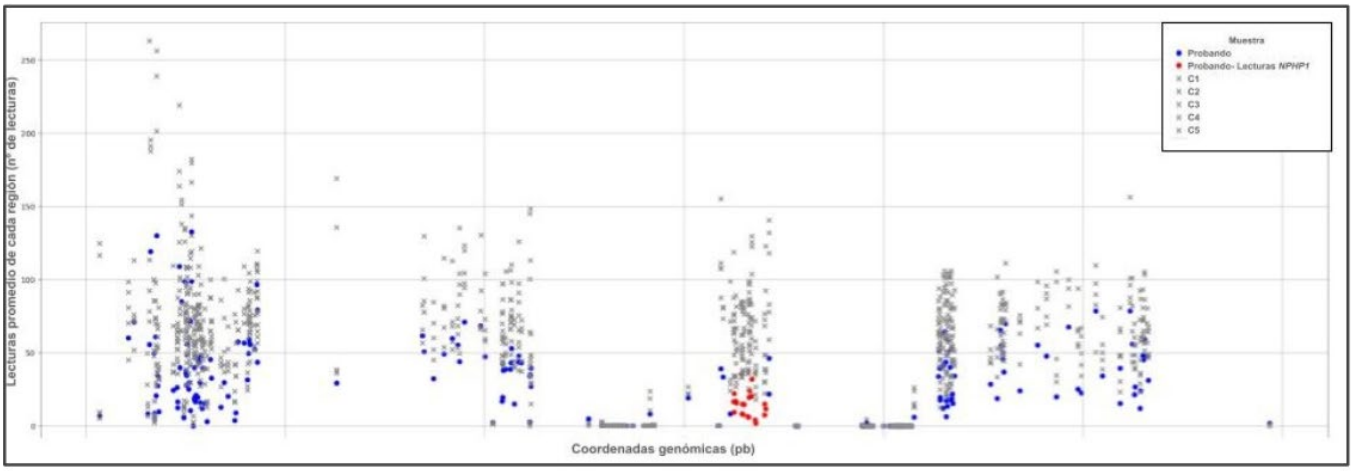

Figura 3. Gráfico de puntos que representa la profundidad promedio de lectura del gen *NPHP1* y sus genes vecinos. Las lecturas del probando se muestran como puntos azules, mientras que las de los controles (individuos sanos) se representan con cruces grises. Se observa una disminución notable en la profundidad de lecturas en la región correspondiente al gen *NPHP1* en la muestra del probando (indicada con puntos rojos), en comparación con los controles (C1 a C5). Cada punto refleja el número de lecturas por región dentro del kit de captura.

- No se dispone de información sobre la herencia o ésta es poco informativa. El fenotipo del paciente no es específico, pero es consistente con lo que se ha descrito en casos similares (criterio 5G).

Cabe destacar que se estudiaron otras variantes tanto de secuencia como en el número de copias en genes asociados al diagnóstico presuntivo, las cuales resultaron no poseer significancia clínica para el caso, descartando otras posibles causas genéticas.

Finalmente, los hallazgos del análisis del exoma completo sugirieron que el diagnóstico etiológico para este cuadro clínico era nefronoptisis juvenil asociada a *NPHP1*, de herencia autosómica recesiva.

Confirmación de las variantes por métodos alternativos

Confirmación de la SNV

En virtud de los hallazgos obtenidos a partir del experimento de secuenciación masiva, se procedió a realizar la confirmación de ambas variantes por métodos alternativos. Como se muestra en la Figura 4, la secuenciación por Sanger confirmó la presencia de una deleción de 10 pb en el gen *NPHP1*. Sin embargo, a pesar de tratarse de una deleción que debería alterar el marco de lectura, no se observó un corrimiento en el mismo, sugiriendo un alelo en homocigosis.

Confirmación de la CNV

El experimento de qPCR, diseñado para detectar la deleción que involucraba al gen completo, permitió confirmar que efectivamente el gen *NPHP1* en el paciente presentaba menos dosis alélica comparada con la muestra control (Figura 5). Cabe destacar que esta técnica no permite definir exactamente los puntos de ruptura de esta variante, pero sugirió que probablemente se extienda más allá de los extremos del gen.

Finalmente, mediante ambos experimentos (secuenciación por Sanger para la pequeña deleción y qPCR para la deleción del alelo completo) se evidenció que ambas variantes estaban presentes en el paciente estudiado, en la configuración alélica exhibida en la Figura 6.

Segregación de variantes

Para evaluar la segregación de las variantes en la familia, se estudiaron a la madre y a las dos mujeres menores de la hermandad. Los resultados del experimento de qPCR mostraron que la deleción del gen completo estaba presente únicamente en el probando y no se detectó en ninguna de sus familiares sanas evaluadas, ya que presentaron dosis alélica similar al control (Figura 7A). Es importante mencionar, que no fue posible estudiar al padre del paciente (por haber fallecido en 2020, sin referencia de patología relacionada a la del probando) ni al resto de sus hermanos.

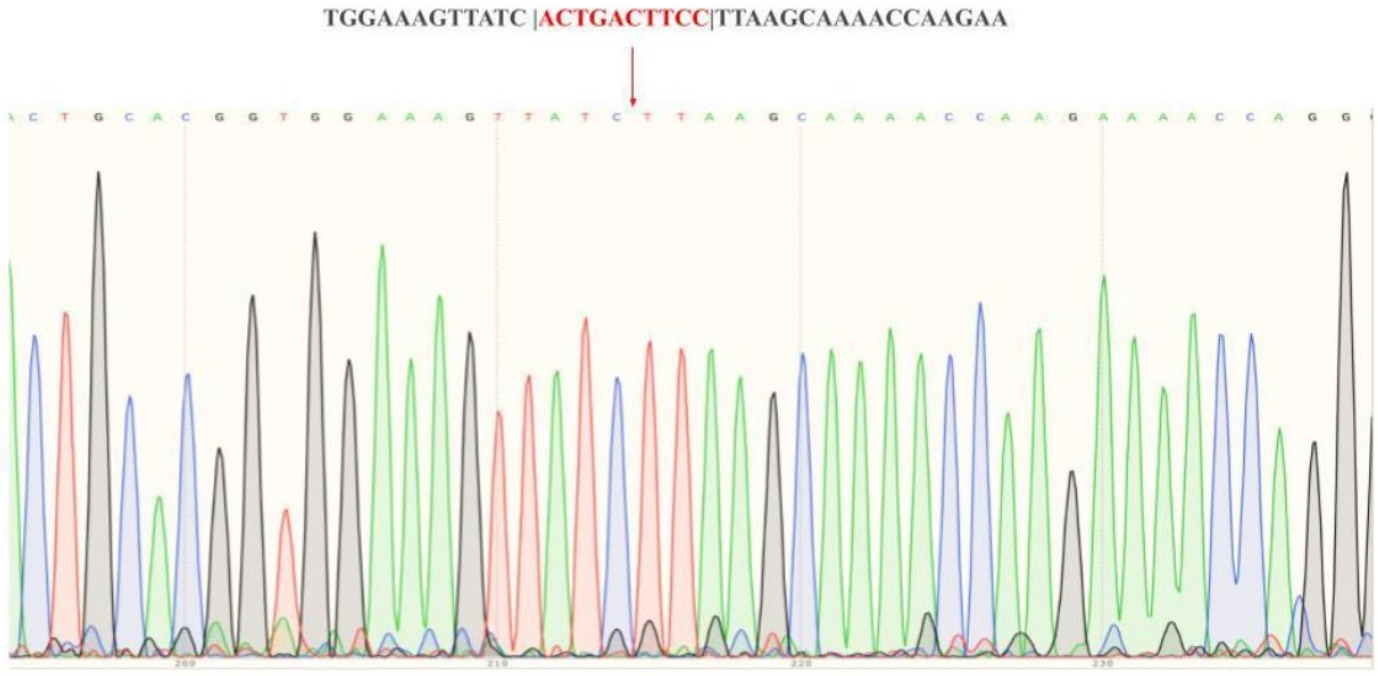

Figura 4. Electroferograma de la secuenciación por Sanger del probando correspondiente a la región del exón 20 donde se detectó la pequeña deleción por NGS. Se observa una deleción homocigota de 10 pares de bases, sin evidencia de corrimiento en el marco de lectura. La flecha señala la ubicación precisa de la deleción: NC_000002.12:g.110123920_110123929del (NM_001128178.3:c.1897_1906del).

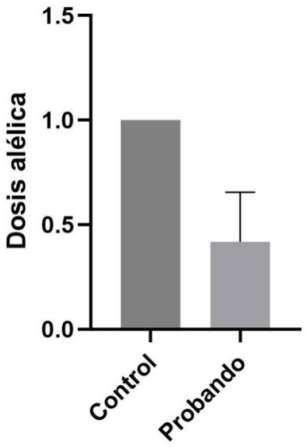

Figura 5. Cuantificación alélica mediante qPCR. Se observa una disminución en la dosis alélica del probando en comparación con el control (individuo sano). El gen *GAPDH* se utilizó como control endógeno (*housekeeping*). El ensayo se realizó por duplicado.

En cuanto a la deleción de 10 pb, se confirmó por secuenciación de Sanger que tanto la madre como una de las hermanas del probando eran portadoras sanas (Figura 7B), lo que es consistente con un patrón de herencia autosómica recesiva.

Reclasificación de las variantes

Luego del estudio de los familiares disponibles, se procedió a reclasificar las variantes detectadas en el paciente. En relación a la deleción g.(?_110.123.799)_(110.205.016_?)del, la herencia resultó no informativa. Si bien la clasificación inicial (agosto de 2022) de la variante fue patogénica, no se adicionaron criterios extra.

En cuanto a la variante c.1897_1906del, inicialmente clasificada como de significado incierto en agosto de 2022, dos criterios fueron reevaluados. Por un lado, se analizó la segregación familiar para determinar si se podría aplicar el criterio PP1 (proveniente de la guía de ACMG/AMP para la clasificación de variantes: *cosegregación con enfermedad en múltiples miembros de la familia afectados en un gen que se sabe definitivamente causa la enfermedad*). A partir de las especificaciones por expertos para sordera, como modelo de aplicación de los criterios ACMG/AMP para enfermedades autosómicas recesivas, la variante alcanzó un puntaje de 0,25 (Oza et al., 2018). Con ello, la variante no consiguió el valor del nivel inferior para la aplicación del criterio PP1, por lo tanto, no se aplicó. Por otro lado, la variante se encontró en un paciente acompañado de una variante patogénica (CNV descripta en este estudio), situación aplicable al criterio PM3 (criterio proveniente de la guía ACMG/AMP: *para trastornos recesivos, detectados en trans con una variante patogénica*). La variante fue identificada en *trans*, confirmada mediante los experimentos de qPCR y secuenciación de Sanger, análisis que corroboraron la presencia de dos alelos distintos. Por lo tanto, de acuerdo con las especificaciones del SVI Clingen para PM3, la variante alcanzó un valor de 1 punto y se aplicó el criterio PM3 con la fuerza moderada por defecto. Con ese puntaje, la variante mantuvo su clasificación de VUS. Asimismo, se analizó la alta probabilidad *a posteriori* (PostP) de

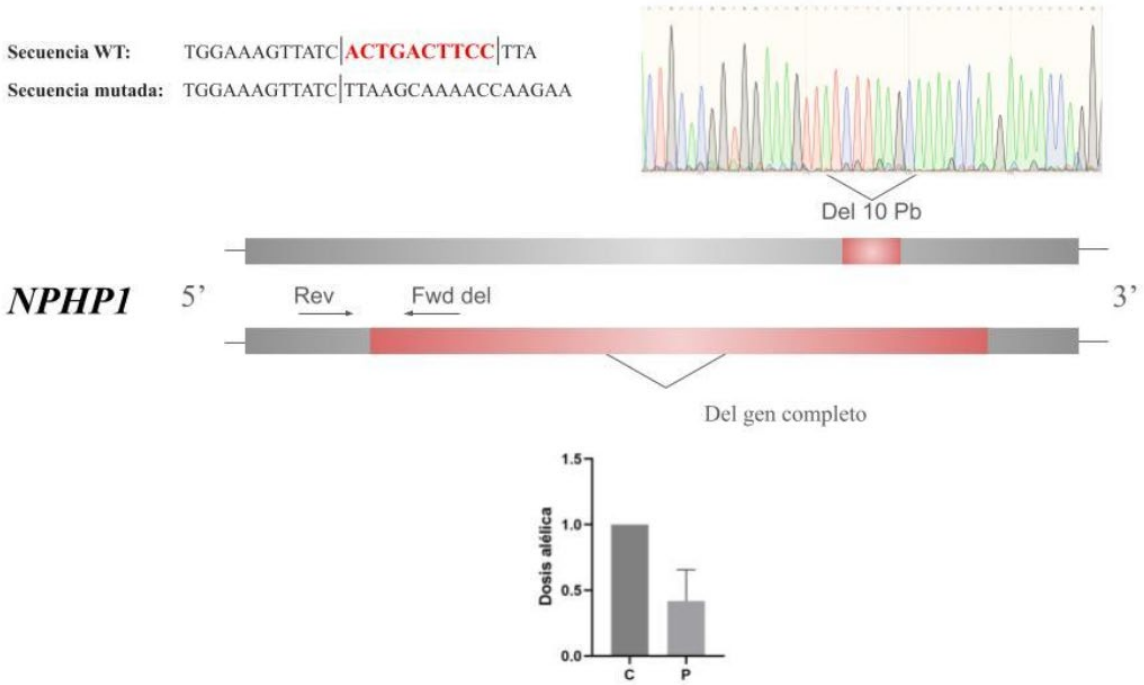

Figura 6. Visualización del diseño del experimento. Se observa el esquema de la configuración alélica de las dos deleciones detectadas en base a ambos experimentos, Sanger y qPCR.

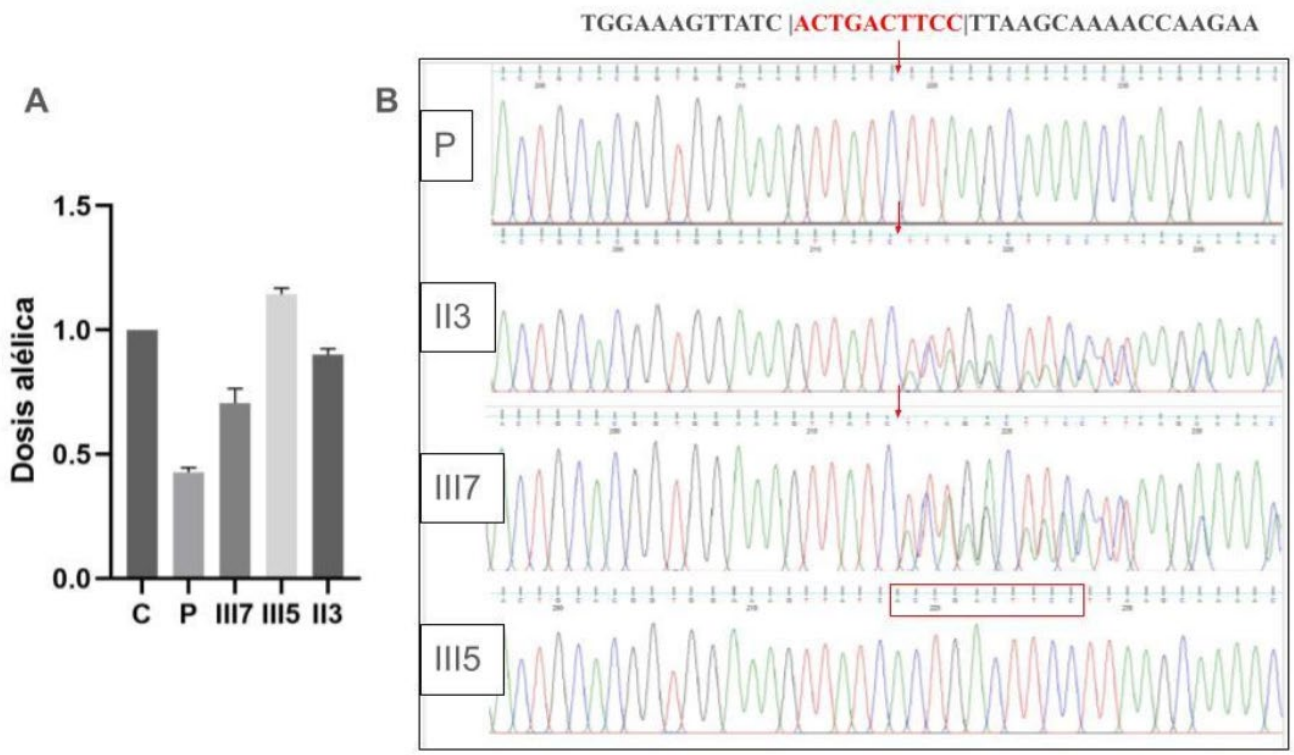

Figura 7. A- Cuantificación alélica obtenida a partir de qPCR. El ensayo fue realizado por duplicado. C: Control, P: Probando, III5: Hermana 1, III7: Hermana 2, II3: Madre. B- Electroferogramas de la secuenciación por Sanger de la deleción pequeña del gen *NPHP1*. P: Probando, III5: Hermana 1, III7: Hermana 2, II3: Madre.

que la variante fuera la causante de la enfermedad, ya que se evaluaron otras causas genéticas de la patología (estudio de Panel de Genes asociados a nefronoptisis) y éstas fueron descartadas. Con lo cual, en una discusión multidisciplinaria con los médicos tratantes, se convino que, tanto por la evolución del paciente como por el fenotipo específico manifestado, la nefronoptisis por *NPHP1* resultó ser la causa más plausible. Asimismo, la evidencia indirecta (no presentar variantes patogénicas o probablemente patogénicas en otro *loci*) apoyó la asignación de patogenicidad de las variantes en un locus específico. Por lo tanto, de forma conservadora se aplicó el criterio PP4 (criterio de la guía ACMG/AMP: *el fenotipo del paciente o los antecedentes familiares son altamente específicos de una enfermedad con una única etiología genética*) en su fuerza por defecto de "*Supporting*" (Biesecker, 2024).

Finalmente, la deleción de 10 pb en el gen *NPHP1* se reclasificó sumando los siguientes criterios: PVS1_Moderate, PM2_Supporting, PM3, PP4; alcanzando un valor de acuerdo al modelo de Tavtigian et al. (2020) de 6 puntos, clasificación de probablemente patogénica (noviembre de 2024).

Tratamiento, evolución y seguimiento

Desde el punto de vista clínico, el paciente recibió un trasplante renal en marzo de 2023 y ha evolucionado favorablemente con buena adherencia a controles y tratamiento, manteniendo en la actualidad su función renal conservada. Se encuentra recibiendo la inmunosupresión indicada por el trasplante (tacrolimus, micofenolato sódico y meprednisona, esta última a dosis baja). No ha presentado episodios de rechazo.

En los años post trasplante no ha sufrido episodios de infecciones urinarias, uno de los principales indicadores de patología urológica. Tampoco se ha evidenciado proteinuria en los análisis de orina, manifestación que puede asociarse a recurrencia de glomerulopatía. Estos datos refuerzan el diagnóstico de nefronoptisis como causa de la enfermedad renal que lo llevó a cronicidad y terminalidad.

Luego del trasplante y a raíz de los resultados obtenidos del estudio genético, el paciente fue valorado por oftalmología, con resultados de fondo de ojos normal y retina sin particularidades, sin presencia de signos sugestivos de retinosis pigmentaria hasta la fecha. Queda aún pendiente el electrorretinograma.

DISCUSIÓN

Dentro de las patologías renales de origen genético, la nefronoptisis es una de las causas más frecuentes de insuficiencia renal (Arango, 2023). Sin lugar a dudas, obtener un diagnóstico preciso permite un seguimiento

y asesoramiento oportuno del paciente. La importancia de estudiar genéticamente a estos pacientes es crucial para practicar una verdadera medicina personalizada.

Dentro de los genes asociados a esta enfermedad, *NPHP1* resulta un gen desafiante a la hora de ser estudiado, no sólo por su longitud sino porque en sus extremos contiene regiones repetitivas que impiden un correcto mapeo/alineamiento con el genoma de referencia.

En la bibliografía, se ha señalado que los diagnósticos clínicos de pacientes con deleciones en *NPHP1* presentan un riesgo significativo de subdiagnóstico de la enfermedad asociada en la práctica clínica (Snoek et al., 2018).

Por otro lado, es importante destacar el valioso significado de seguir profundizando en variantes que despiertan interés. En este caso particular, se analizó a un paciente con una variante en aparente homocigosis detectada por secuenciación masiva. La evidencia indica que cuando se detectan variantes en homocigosis sin antecedentes de consanguinidad, es altamente probable estar ante la presencia de una deleción solapante del otro alelo. Esto derivó en el estudio de deleciones en el gen completo, descritas en la literatura para *NPHP1*, con lo cual se descartó la primera hipótesis, que consistía en que la variante estuviera en homocigosis y llevó a proponer un modelo de heterocigosis compuesta de una deleción de 10 pb y una de aproximadamente 0,08 Mb en el otro alelo.

Es importante estudiar la presencia de CNVs al identificar variantes raras en homocigosis cuando no se refiere consanguinidad o pertenencia a una comunidad cerrada. Los algoritmos bioinformáticos, como los mencionados en materiales y métodos, para la detección de CNVs pudieron certificar que la nueva hipótesis planteada era correcta. Sin embargo, estos no dejan de ser un cribado que debe ser confirmado por una técnica *Gold Standard*. Lo recomendado para estos casos es realizar un estudio de MLPA (*Multiplex Ligation-dependent Probe Amplification*) o Array CGH *(Comparative Genomic Hybridization)*. Debido a la falta de cobertura por la obra social del paciente y al alto costo de este tipo de estudios, se optó por un método alternativo para poder confirmar la aparente deleción del gen completo que los algoritmos informáticos detectaron en una primera instancia. Es así que, bajo la inspiración del gran trabajo de Chen *et al.* (2020), se logró verificar a través de una qPCR que efectivamente uno de los dos alelos estaba completamente delecionado. Es importante señalar que, para una caracterización completa del paciente, sería ideal complementar este análisis con técnicas validadas para establecer genotipos complejos como el presente caso.

Se estudiaron también a los familiares del probando para poder evaluar la segregación de ambas variantes en la familia. Esto resultó de vital importancia, ya que permitió reclasificar a las variantes y sumar más evidencia que apoyara un diagnóstico genético. De todas maneras, el estudio funcional de la deleción de 10 pb, sería de utilidad para poder sumar aún más evidencia. Asimismo, en este trabajo se ha demostrado la manera de utilizar las herramientas bioinformáticas en combinación con técnicas moleculares para arribar a un diagnóstico confiable.

Por otro lado, se ha realizado una implementación rigurosa de las guías de ACMG/AMP, las revisiones por ClinGen y paneles de expertos para la clasificación de las variantes detectadas. Estas herramientas de interpretación del diagnóstico son indispensables para el abordaje posterior del tratamiento y seguimiento del paciente.

Finalmente, y a modo de conclusión, en pacientes con fenotipos asociados a tan amplia heterogeneidad genética y fenotípica, la identificación de la etiología genética del cuadro permite establecer un pronóstico, adecuar el seguimiento y el eventual tratamiento específico. La comunicación fluida y el intercambio entre los médicos tratantes y el equipo de diagnóstico es fundamental para alcanzar mejores resultados para los pacientes y sus familias.

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CONTRIBUCIONES DE AUTORES

Lugones A.C., y Martí M.A., contribuyeron a la concepción y diseño del estudio. Lugones A.C. y Aguirre N.M. redactaron el manuscrito. Iglesias García L., Erra L., Martinez Mayer J., Waldman M., Pérez Millán M.I. y Recabarren A. realizaron experimentos. Guillamondegui M.J, Fernandez Robetto A., Tardivo A. y Julián P. realizaron el trabajo clínico, recopilaron datos y participaron en el cuidado del paciente. Todos los autores han revisado el manuscrito en busca de contenido intelectual importante y han aprobado la versión final.

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A NOVEL *DLGAP2* VARIANT IDENTIFIED AFTER EXOME SEQUENCING OF AN INFANT WITH AUTISM SPECTRUM DISORDER (ASD)

UNA NUEVA VARIANTE DEL GEN *DLGAP2* IDENTIFICADA TRAS LA SECUENCIACIÓN DEL EXOMA DE UN NIÑO CON TRASTORNO DEL ESPECTRO AUTISTA (TEA)

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ABSTRACT

Autism spectrum disorder (ASD) encompasses various conditions related to neurological development alterations, affecting millions of people worldwide according to the World Health Organization. ASD displays a multifactorial etiology arising from complex interactions between genetics, epigenetics, and environmental factors. ASD diagnosis is primarily based on behavioral and neuropsychological evaluation; recent years have seen an increase in the use of various genomic analysis technologies to attempt to generate a map of potentially implicated genes. This study focused on an 8-year-old boy with a complex clinical history from birth. The patient was diagnosed at the age of four with ASD, level 3, due to difficulties in all areas of development (language, social interaction, play, cognition, and behavior). Given the severity of his phenotype, various genetic studies of increasing sensitivity were conducted including a targeted exome sequencing enriched using the Twist Human Customized Core Exome Kit. The objective of this study was to identify genetic variants potentially associated with the reported clinical diagnosis of ASD. Data were normal for karyotype and for *FMR1* gene exon 1 sequencing. Comparative genomic hybridization (CGH) array and targeted exome sequencing were also performed. These additional studies identified several genetic changes compared to reference genomes, 49.41 kb deletion (coordinates 94,621,121-94,670,533) encompassing part of the *GPC* gene in band 13q31.2, as well as heterozygous changes in the coding sequence of the *DHDDS* and *DLGAP2* genes with uncertain clinical significance according to reports from the laboratory responsible. However, the detection of the variant in the *DLGAP2* gene seems not to be a random occurrence since a relevant number of publications are pointing out a connection with ASD diagnosis. The extensive genetic characterization performed allowed the detection of several variants in his genome. Our evaluation of the clinical data and the identified genomic changes confirms the relevance of the new *DLGAP2* gene variant and are compared with the literature to suggest phenotype-genotype correlations.

Key words: autism spectrum disorder, whole exome sequencing, rare variants, *DLGAP2* gene

RESUMEN

El trastorno del espectro autista (TEA) engloba diversas patologías relacionadas con alteraciones del desarrollo neurológico que afectan a millones de personas según la Organización Mundial de la Salud. Su etiología multifactorial surge de interacciones complejas entre genética, epigenética y factores ambientales. El diagnóstico se basa principalmente en estudios conductuales y evaluación neuropsicológica pero últimamente ha habido un aumento en el uso de diversas tecnologías de análisis genómico para intentar generar un mapa de posibles genes implicados. Este estudio se centró en un niño de 8 años con una historia clínica compleja desde el nacimiento. El paciente fue diagnosticado a los 4 años con TEA, grado 3, por dificultades en todas las áreas del desarrollo (lenguaje, interacción social, juego, cognición y conducta). Por la gravedad de su fenotipo, se realizaron estudios genéticos de creciente sensibilidad, incluyendo una secuenciación dirigida de exoma, enriquecida mediante el kit *Twist Human Customized Core Exome*. El objetivo de este estudio fue identificar variantes genéticas que puedan explicar el diagnóstico TEA descrito. El cariotipo y la secuenciación de exón 1 del gen *FMR1* fueron normales. El análisis mediante hibridación genómica comparativa (CGH-array) y la secuenciación dirigida de exoma identificaron cambios genéticos, incluyendo una deleción de 49,41kb (coordenadas 94.621.121-94.670.533) que abarca parte del gen *GPC*, en banda 13q31.2, así como cambios heterocigotos en la secuencia codificante de los genes *DHDDS* y *DLGAP2*, de significado clínico incierto según los informes del laboratorio. Sin embargo, la detección de una variante en *DLGAP2* no parece algo aleatorio ya que un número relevante de publicaciones sugieren una conexión con un diagnóstico TEA. La amplia caracterización genética realizada permitió la detección de diversas variantes en su genoma. Nuestra evaluación sobre los datos clínicos y sobre los cambios genómicos identificados confirma la relevancia de la nueva variante del gen *DLGAP2* y son comparados con la literatura para sugerir correlaciones fenotipo-genotipo.

Palabras clave: trastorno del espectro autista, secuenciación del exoma completo, variantes raras, gen *DLGAP2*

INTRODUCTION

Autism spectrum disorder (ASD), commonly known as autism, comprises a group of different neurodevelopmental disorders, generally with early onset and an average age of diagnosis of five years (van 't Hof et al., 2021). Its clinical diagnosis is characterized by significant heterogeneity and consists of identifying typical deficiencies in social interaction, verbal and non-verbal communication, and preference for repetitive interests or behaviors (Singui and Smith-Hicks, 2023; Tajik-Parvinchi et al., 2023). Significantly, most individuals diagnosed with ASD also present other neurodevelopmental disorders (e.g., intellectual disability), neurological disorders (e.g., frequent manifestation of epileptic processes), and/or mild neurological motor signs, making differential diagnosis complex as many mental health and neurodevelopmental conditions present overlapping symptoms with autism (Liu et al., 2017; Peall et al., 2024).

The overall prevalence of ASD is 27.6 per 1,000 (one in 36 children) at eight years old, and it is 3.8 times more frequent in boys than in girls (4% vs. 1%) (Leow et al., 2024). However, this prevalence varies substantially across studies, depending on racial and ethnic groups (Maenner et al. 2023). Different explanations for this marked difference have been suggested, including greater public awareness and increased availability and sensitivity of diagnosis (Zeidan et al., 2022). There is evident clinical heterogeneity and developmental trajectories of underlying pathologies in ASD, even among monozygotic twins, with behavioral manifestations strongly influenced by genetic factors (Szatmari et al., 2016; Ho et al., 2022). This establishes a complex etiology for ASD, positing the theory of the existence of de novo germline mutations and rare inherited variants possibly converging on similar pathways affecting neuronal and synaptic homeostasis (Cantado et al., 2024), without excluding the presence of environmental risk factors (Chaste and Leboyer, 2012). At present, the genetic contribution to ASD is strongly supported by an increasing number of studies, with high heritability estimates, such as concordance rates of 70-90% among monozygotic twins (Ho et al., 2022; Schaefer and Mendelsohn, 2013). However, the potential influence and/or functional connection of multiple changes found in the genome of individuals with ASD with the manifestation of this disorder is still unclear. As a result, it is challenging to define specific variants directly causing ASD (Wain et al, 2018; Myers et al., 2020). Disorders with a well-defined genetic basis, such as Down syndrome (trisomy of chromosome 21), Rett syndrome (alterations in the *MECP2* gene), and several pathologies defined by expansions of tandem repeat sequences, such as Fragile X syndrome (FXS), myotonic dystrophy type 1 (DM1), or Huntington's disease (HD), have been associated with some syndromic forms of ASD, providing the first direct evidence of a genetic etiology or defect in the clinical manifestations of autism. However, a clear monogenic cause has been identified in less than 20% of patients, with most of our current knowledge about ASD genetics derived from the identification and analysis of rare variants (Bicks and Geschwind, 2024; Escudero and Sepúlveda, 2024; Krishnan et al., 2015; Angeard et al., 2018; Piras et al., 2020). The identification of rare but relevant genetic factors in individuals diagnosed with ASD has been conducted over the last decade thanks to the development of new genetic tools capable of "reading" changes in the genome with high precision. These include arrays and next-generation sequencing (NGS) (Vicari et al., 2019; Choi and An, 2021). Large-scale genomic studies using these tools have shown that approximately 20-40% of ASD cases have a defined genetic variant associated. Specifically, the presence of large genomic changes (deletions, duplications, or translocations) has been observed in 3% of ASD cases, but using arrays, the presence of copy number variations (CNVs) has been detected in 10-30% of ASD patients. These CNVs include microdeletions or micro duplications of more than 1 kb in specific regions of the genome, involving the loss or gain of genes present in these regions. Many genetic and critical genomic CNVs have been revealed in ASD and are commonly found in patients with neurodevelopmental disorders (Takumi and Tamada, 2018). The correlation between CNVs and ASD has been established for multiple loci, with the identification of potentially clinically relevant genes based on their known biological function, such as *SHANK2*, *SHANK3*, *NRXN1*, *NLGN4*, *PCDH10*, *DIA1*, *NHE926*, and *PARK2*. On the other hand, the incorporation of new genome sequencing techniques (NGS) in the study of ASD is allowing the discovery of new rare gene variants associated with this diagnosis, detecting single nucleotide polymorphisms (SNPs) in the sequence of a specific gene. Even with minimal changes, theoretically, SNPs can alter the production of specific proteins with a possible connection to ASD pathogenesis. It has been estimated that the proportion of ASD associated with SNPs is between 17% and 60% (Gaugler et al., 2014), suggesting a significant contribution through the identification of numerous variants associated with ASD that provide new insights into the genomic architecture of this pathology (Trost et al., 2022).

This article presents a clinically diagnosed case of ASD with continuous functional follow-up, for which various genetic studies have also been performed. The results have revealed specific changes in the genome of the studied individual with uncertain and/or unknown clinical significance. In-depth analysis of these changes aims to foster a better understanding of genotype-phenotype correlation in individual ASD cases and to optimize their clinical management.

MATERIALS AND METHODS

Case presentation

We present the case of an 8-year-old boy, the result of a natural pregnancy, without threats of miscarriage or preterm birth, delivered by cesarean section at 40 weeks due to lack of progression. The newborn (NB) had normal parameters (weight=3410 g, length=48.5 cm, head circumference (HC)=36.5 cm, and Apgar 9/10). The father was 41 years old and the mother 35 at the time of birth, with a single and first pregnancy, no previous miscarriages, and one live birth (GAV 1/0/1), i.e., no history of miscarriages and/or pregnancy complications (normal ultrasound series and blood pressure), normal neonatal screening for congenital diseases and otoacoustic emissions (OAE). Since birth, the child presented a distinctive clinical picture, with feeding difficulties and gastroesophageal reflux diagnosis. At 21 months of age, he underwent surgery for left cryptorchidism. He was under nephrology follow-up for intermittent hematuria until discharge at age 6, and in the Child Mental Health Unit (CMHU) from age 2, with ongoing clinical follow-up and currently on guanfacine 1 mg/day medication. At age 3, ASD was suspected due to characteristic patterns, such as a tendency for isolation, significant difficulties in speech and language development, and poor school performance. He showed no social interest, and his play was limited and stereotyped. By age 4, communication, language, and social skills were significantly underdeveloped relative to his chronological age (CA). The E. Schopler Psychoeducational Profile (PEP) showed scores ranging from one to one and a half year below his CA of 4 years (overall development= 2 years and 8 months). Finally, scores on the Reynell III Language Development Scale Module I for preverbal or single-word children were very low (comprehension scale= 1 year and 10 months and expression scale= 1 year and 9 months), surpassing the cut-off points associated with an ASD diagnosis. Based on the results of ADOS-2, ADI-R algorithm, and other assessments, a grade 3 ASD diagnosis was concluded at age 4. Over the years, the child continued to experience language difficulties, requiring supervision in daily tasks, with play remaining individual and repetitive. At age 7, spontaneous manual, digital, and postural movements appeared in various activities and routines, predominantly in the distal upper left limb. Brain MRI, EMG, and sensory and motor nerve conduction studies (ENG) showed no significant lesions, with results within normal limits. A diagnosis of Stereotypic Movement Disorder was concluded, characterized by repetitive movements without purpose or intent. At age 9, neurophysiology tests recorded a normal EEG, while quantitative EEG (qEEG) mapping indicated signs of cerebral immaturity for his age due to increased absolute (AP) and relative (RP) power values in the delta band and increased frontal-occipital coherence in the same frequency range. He also showed moderate impairments in visual and auditory attention, evidenced by increased amplitudes in Visual and Auditory Cognitive Evoked Potentials (403.14 ms/1.63µV and 377.99 ms/4.99µV, respectively, with normal values: 261.8-386.6ms/25.78µV and 248.7-373.96ms/21.3µV). At age 8, social and speech/language difficulties intensified, with challenging and aggressive behaviors (hair-pulling, pushing, slapping). His speech was repetitive and echolalic (constant and intense repetition). In a recent follow-up at age 10, it was confirmed that the child does not have reduced mobility but rather profound motor disinhibition, requiring constant adult supervision to prevent him from running off and getting hit. He needs continuous parental supervision. He still shows behavioral regulation deficits, intense frustration, crying, and complaints without apparent reason during simple activities, even when accompanied by his parents. His spontaneous speech is limited and characterized by echolalia of short phrases, with slow responses to verbal demands, and occasionally answers briefly after much insistence. Notably, early work with various speech and occupational therapy professionals, along with intense maternal collaboration in anticipating activities and school excursions as well as clinical visits, helps reduce his intense anxiety after three to four sessions, allowing him to self-regulate, control, and start collaborating and learning receptively. Under these conditions, his language shows social characteristics and he maintains better eye contact. He is currently under clinical follow-up by Neuropediatrics and CMHU, with pharmacological treatment of 20 mg/day MPH EFG (Rubifen®) and 0.5 mg/day risperidone (Risperdal®).

Karyotype

Venous blood samples (5-10 mL) were collected in sterile tubes containing heparin as an anticoagulant. The samples were transported to the laboratory at room temperature and processed within 24 hours of collection to ensure optimal cell viability, following standard protocol that included cells fixation, slide preparation (cells spreading and Giemsa staining), and microscopic analysis of metaphases, as described in Arsham et al., 2017. The karyotype was analyzed for numerical and structural chromosomal abnormalities. The analysis followed the International System for Human Cytogenetic Nomenclature (ISCN) guidelines to ensure standardized reporting.

Genomic DNA Extraction for genetic analysis

Peripheral venous blood samples (5 mL) were collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Genomic DNA was extracted from all of the blood

samples using the QIAamp DNA Blood Mini Kit (Qiagen) with the Qiacube or EZ2 equipment. DNA quantification was performed with the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and QIAexpert System (Qiagen, Hilden, Germany).

FMR1 CGG repeat amplification

To detect mutations in the *FMR1* gene, genomic DNA (gDNA) was amplified by PCR targeting the CGG repeats in the 5' untranslated region of the *FMR1* gene. The size of the amplifies fragment was then genotyped using the ABIPRISM 3130 automated sequencer and analyzed with the GeneMapper software. The measurement range for the repeats is 5 to 200 CGG, with normal alleles considered to be approximately between 5 and 44 CGG repeats.

CGH array

The Genome-Wide Human CytoScan 750K Array (Affymetrix, CA, United States) was used to analyze genomic alterations according to the manufacturer's protocol and as summarized in Ghazali et al., 2023. Fragments of DNA (200 µg) were hybridized with pre-equilibrated Affymetrix Chip CytoScan 750K at 50 °C for 18 h. The arrays were washed and stained using GeneChip Fluidics Station 450 and Affymetrix GeneChip Command Console Software, followed by data analysis using Affymetrix Chromosome Analysis Suite (ChAS) software. The molecular karyotype (or array CGH) aims to detect chromosomal imbalances too small (e.g., deletions or duplications) to be visualized by conventional karyotyping (detection limit >5 Mb). Array CGH can detect genomic imbalances with a diagnostic resolution of 50-100 kb in an individual's genome.

Next-generation sequencing (targeted exome)

To achieve a higher sensitivity level, targeted exome sequencing was performed using next-generation sequencing (NGS), aiming to detect point variants and possible copy number variations (CNV) in the entire genome, prioritizing changes in genes associated with autism clinical phenotypes (HP:000017, HP:0000753, HP:0000729) and intellectual disability (HP:0001249, HP:0001526, HP: 0002187, HP:0002342, HP: 0006887, HP: 0006889, HP:0010864). Specifically, the coding regions (exons) of the gene and adjacent intronic regions (16 nucleotides upstream and downstream of each exon) were analyzed using the Twist Human Exome Enrichment system (Twistbioscience) and ultrasequencing (2x100bp, Novaseq 6000 platform, Illumina) and the results were analyzed with the Varsome Clinical platform. The variants detection limit was 80% with >100X and 20% with >50X coverage.

RESULTS

Based on the identified clinical picture, genetic studies were proposed for the patient by obtaining his genomic DNA and conducting various tests in chronological order, classified by increasing sensitivity, to identify the potential origin of the different pathologies observed, even before his ASD diagnosis.

At age 3, two genetic studies were conducted. First, a normal 46XY karyotype was confirmed without any evident chromosomal changes. Secondly, the 5' non-coding region of the *FMR1* gene was also analyzed, amplifying the CGG repeat size in this region via PCR. This test is regularly done because it is confirmed a connection between ASD and FXS. Between 2-6% of children diagnosed first with autism have FXS, and approximately 3% of children diagnosed first with FXS have autism. When the CGG repeat expands beyond 200 repetitions, this sequence changes to a methylated status on the cytosines, ultimately repressing *FMR1* protein expression. Sizes between 6-55 CGG repeats are considered normal within a population. In the patient under study, amplification revealed a pattern corresponding to an allele with 30 (+/-1) CGG repeats, which is within normal limits. It's important to note that this analysis does not detect other types of mutations causing FMR1 loss of function, reported in rare cases from point mutations or intragenic deletions, or by a pathogenic expansion in the adjacent *FRAXE* gene, resulting in a clinical outcome similar to FXS. An expression study of *FMR1* and/or *FRAXE* genes should be included to complement the PCR approach, fully ruling out these genes' involvement in the ASD diagnosis. The negative results from these two approaches led to broader and more sensitive exploratory genetic studies (Willemsen et al., 2011).

In this case, the clinical laboratory report provided (hg19 version for genome comparison) describes detecting a heterozygous deletion of uncertain significance in the 13q.31.2 region, with genomic coordinates (94,621,125-94,670,533) and a size of 49.41kb (at the detection limit for this technique). This region, also according to the report, contains part of the glypican 6 gene (*GPC6*, OMIM 604404) sequence. No other gene is involved. We reviewed the ClinVar repository for sequence changes described for the *GPC6* gene and its potential connection to autism or intellectual disability phenotypes. Although there are pathogenic variants of this gene, they are mainly related to autosomal recessive omodysplasia, unrelated to neurodevelopmental disorders (Bayat et al., 2020). The rest of the array results were normal, concluding that the identified deletion is highly unlikely to be clinically relevant to the described ASD clinical picture.

NGS analysis (Varsome Clinical Platform) and interpretation of the results (Gestlab-Cointec software)

did not identify any point variant described as pathogenic or likely pathogenic. However, two heterozygous gene variants were detected. Specifically, the c.995C>G(p.Ser332Ter) change in the *DHDDS* gene, and the c.2210>T(p.Thr737Met) change in the *DLGAP2* gene (Table 1). The provided report defines both identified changes as of uncertain clinical significance based on the American College of Medical Genetics (ACMG) pathogenicity criteria after consulting databases and additional literature on these genes and their association with reported clinical variants (Richards et al., 2015). In the following sections, we provide a description and evaluation of this information, along with our own additional and updated data.

For the dehydrodolichyl diphosphate synthase subunit gene (*DHDDS*, Gene ID 79947), which is involved in the synthesis and glycosylation of dolichol in the endoplasmic reticulum, a heterozygous nonsense mutation was identified, resulting in the appearance of a premature termination codon in the resulting protein's synthesis. The mutation, a cytosine (C) to guanine (G) substitution, occurs at nucleotide 995 of its mature mRNA (995C>G) and causes a premature stop codon at amino acid (aa) position serine 332 (Ser332Ter). This gene has several isoforms of the final protein, ranging in size from 240 to 334 aa. The identified mutation is located near the terminal end of its amino acid sequence, eliminating the last two aa from the sequence for those variants >332 aa. Therefore, in conjunction with its heterozygous manifestation, it seems unlikely that the observed change introduces any relevant functional alterations in the final protein or activates cellular degradation processes (nonsense-mediated decay), although *in silico* predictors for this variation suggest higher pathogenic potential compared to non-pathogenic (Varsome). This variant (annotated as rs770732789) is present in the population (gnomAD 0.0009%), recently added to the repository of annotated clinical variants (ClinVar) in February 2023, following a study in a patient with Retinitis Pigmentosa, a pathology not compatible with the symptoms developed by the patient under study. Currently, there are 369 annotated clinical variants for the *DHDDS* gene, associated with both autosomal dominant (AD) pathologies, where the patient's heterozygous state could be sufficient for possible symptom development, and recessive (AR) pathologies, where the heterozygous state would not be sufficient to cause symptoms. Initially, two de novo, nonsense, pathogenic mutations were described in this gene (R37H and R211Q) in five unrelated patients, associated with developmental delay and seizures with or without movement abnormalities (DEDSM, OMIM:617836), a dominant inheritance pathology variant (Richards et al., 2015). However, we have not identified any potential association between these and the variant found in the patient diagnosed with ASD and evaluated here, with no such clinical manifestation to date. In conclusion, it is highly unlikely that this identified genetic variation in the *DHDDS* gene has clinical relevance to the described intellectual disability as ASD clinical picture, although it is classified as a variant of uncertain significance (VUS). To entirely rule out this possibility, it is recommended to conduct a study quantifying mRNA and/or protein levels to confirm no significant change in this gene's products. Since obtaining brain tissue is unfeasible, this gene is expressed in similar amounts throughout the body, including the skin, making a study from epithelial mucosa cells equally informative (Hamdan et al., 2017).

The second variant was found in *DLGAP2* (Gene ID 9228), a gene primarily expressed in the brain and testes. Specifically, a heterozygous missense mutation was identified, involving a cytosine (C) to thymine (T) substitution at position 2210 of the mature mRNA. This change translates to the substitution of threonine (Thr) with methionine (Met) at amino acid position 737 in the amino acid sequence (Thr737Met). This mutation occurs in a central region of the primary protein isoform, which is 1055 amino acids long. This variant is identified for the first time in the *DLGAP2* gene in this work, adding to the previously 237 identified variants (ClinVar). A detailed review of the previously described variants revealed three in this gene that involve the same amino acid change (threonine to methionine) due to a single nucleotide change, but occurring in three different regions of the sequence (Thr664Met, Thr679Met, Thr941Met). These three variants were recently identified, with Thr664Met

Table 1. Genetic variants detected by targeted exome sequencing

Gen	Transmission	Genotype	Variant (c.DNA,Prot)	Classification	Consequence	DbSNP ID
DHDDS	AD/AR*	het	NM-205861.3:c.995>G NP-995583.1:p.Ser332Ter	VUS	Nonsense	rs770732789
DLGAP2	Unknonwn	het	NM-001346810.2:c.2210>T NP-001333739.1:p.Thr737Met	VUS	Missense	rs973854776

VUS: variant of uncertain significance; AD: autosomal dominant; AR: autosomal recessive

described as a benign variant, and Thr679Met and Thr941Met as VUS, so the pathogenicity of the same change identified in the ASD patient evaluated here is not immediately apparent. However, the type of change, introducing a new protein synthesis site in the sequence, and its location in the middle of the protein, suggest a more relevant role in this case, that should be further studied. Moreover, published information about this gene positions it as an interesting candidate with potential cause-effect relevance in ASD. This gene encodes a membrane protein that may play a role in synaptic organization and neuronal cell signaling, and has been described as an imprinted gene, meaning it is expressed in a parent-specific manner, in this case, only expressing variants from the father. Variations in *DLGAP2* have been described in individuals with complex autosomal dominant neurodevelopmental disorders, including ASD, schizophrenia spectrum disorder, intellectual disability, developmental coordination disorder, and attention-deficit/hyperactivity disorder (Poquet et al., 2017). Currently, there is strong evidence that the sequence of this gene frequently displays changes after the detection of genomic alterations within the chromosome 8 in individuals with ASD with severe/intellectual disability. At least 33 heterozygous variants within or including *DLGAP2* have been described in nine publications and several public databases, although not all of them have been functionally implicated. However, the number is high and continues to grow. Many of the reported variants are copy number variants (CNVs) involving *DLGAP2* along with other genes. Since the effects of the additional genes could not be ruled out as potential contributors to the patients' phenotypes, these cases were not qualified (Woodbury-Smith et al., 2020; Zarrei et al., 2019). The exome analysis report provided by the laboratory describes information on some of these studies, mentioning that this gene is relatively intolerant to loss-of-function variation (gnomAD v2.1.1).

Given all this information, the ClinGen Syndromic Disorders Gene Curation Expert (GCEP) opted to qualify cases described in the literature as evidence supporting the gene-disease relationship of variants in this gene, although they ultimately did not annotate any of the reported nonsense variants without proof of their functional impact on the protein (Cukier et al., 2014). There is indirect experimental evidence supporting the gene-disease relationship, including studies of DLGAP2 interactions with other proteins and studies with animal models. Thus, DLGAP2 interacts with DLG3, DLG4, SHANK1, SHANK2, SHANK3, NLGN4, and CASK, all factors encoded by well-known genes with variations associated with susceptibility to ASD and intellectual disability, as predicted by STRINGdb (Horner et al., 2021). Very recently, a *Dlgap2* KO mice exhibited olfactory dysfunction and impaired hippocampus-related cognitive functions, such as spatial memory, both signs of common co-occurring conditions in patients with ASD (Hsieh et al., 2023; Chen et al., 2025).

DISCUSSION AND CONCLUSIONS

All this information suggests that, although the identified genetic variant in the *DLGAP2* gene is classified as clinically uncertain in relation to the described ASD clinical picture, it would be highly interesting to conduct additional tests on the segregation of this variant and to evaluate its functionality in the patient under study, as well as in close relatives, since the implication of this gene in ASD is solid, based on other published studies. However, obtaining samples for the proposed studies would require additional familiar consents and ethical permits. To facilitate this, one option would be to attract and put together additional individuals with ASD, already reported to carry variants in the *DLGAP2* gene, for a larger study. Next experimental steps should include the design of *in silico* studies to predict the structural changes that might be caused in the protein by the introduction of the extra methionine using, for example, Alpha Fold open software (https://alphafold.com/). In case the variant identified causes relevant perturbations in the 3D structure of the protein, we would recommend the quantification of *DLGAP2* mRNA and/or protein levels to confirm whether there is a significant change in these products in the individual(s) under study. Although this second part would be challenging to execute since the gene is almost exclusively expressed in the brain and testes, with very low expression in other parts of the body, making access to relevant tissue difficult.

As a conclusion to the detailed description of this clinical case, we want to emphasize that although current traditional psychiatry research primarily relies on diagnoses based on well-characterized symptomatology and functional aspects, it is highly relevant to introduce genetic studies routinely and in parallel from the initial stages of diagnosis to support the understanding of childhood neurodevelopment processes. This should not be limited to cases with known hereditary parental pathology or immediate prenatal and postnatal evidence due to severe neonatal hypotonia, dysmorphic or malformative syndromes (such as trisomy 21, Cornelia de Lange syndrome, Phelan-McDermid syndrome, Noonan syndrome, neurofibromatosis type 1, among others). Technological advancements in genomic techniques must reach the vulnerable pediatric population.

As we have described for the *DLGAP2* gene, there are genetic factors with a high susceptibility to manifest variations in ASD phenotypes, suggesting a potential cause-effect correlation. Genetic studies will lay the groundwork for advancing to studies in which the next step is to appropriately modify these genes to

ultimately evaluate whether it is possible to modulate the pathology itself. A child correctly assessed and diagnosed with ASD should not see efforts in genetic research dwindle; rather, these efforts should serve to improve the care of the child and their family. The better the information and professional support shared, the lower the probability of parental mental health issues due to guilt, despair, and/or depression (Da Paz et al., 2018), and the fewer the errors and waste of time and money on pseudoscientific and even harmful therapies, such as chelation and hyperbaric oxygen therapy.

An important problem associated with ASD diagnosis is that when families start noticing symptoms in one of their children, it triggers a very distressing event that commonly disrupts personal and family well-being and decision-making (Medici, 2021). Finally, the inclusion of biomarkers, which are an objective way to identify and measure biological anomalies, can provide clues for the diagnosis and treatment of ASD and could predict symptom improvements from specific clinical interventions. Additionally, they can indicate if such interventions are altering or targeting an active biomedical process related to the subject's response at that time (Jensen et al., 2022).

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CONFLICT OF INTEREST

The authors declare that they have not conflict of interest.

ETHICAL DISCLOSURES

Protection of human and animal subjects.
The authors declare that no experiments were performed on humans or animals for this study.

CONFIDENTIALITY OF DATA

The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

RIGHT TO PRIVACY AND INFORMED CONSENT

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GENETIC AND CARDIO-METABOLIC RISK FACTORS OF ESSENTIAL HYPERTENSION: A STUDY IN A CENTRAL ARGENTINEAN POPULATION

FACTORES DE RIESGO GENÉTICOS Y CARDIOMETABÓLICOS EN LA HIPERTENSIÓN ESENCIAL: ESTUDIO EN UNA POBLACIÓN DEL CENTRO ARGENTINO

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ABSTRACT

Essential hypertension is a multifactorial disease influenced by both genetic and cardiometabolic factors, with variable prevalence and risk profiles among different populations. Polymorphisms in genes of the renin-angiotensin-aldosterone system (RAAS) and endothelial function have been widely studied for their role in blood pressure regulation and the development of cardiovascular complications. The aim of this study was to investigate the ACE I/D, AT1R A1166C, AGT M235T and eNOS Glu298Asp polymorphisms, together with traditional risk factors, in relation to essential hypertension in a population from San Luis, Argentina. A total of 208 hypertensive patients and 150 normotensive subjects were included. Demographic, anthropometric and biochemical data were collected and analyzed. Genotypic and allelic frequencies of the polymorphisms were determined by PCR-RFLP. Significant differences were found in body mass index (BMI), age, fasting glucose, total cholesterol, HDL-C, triglycerides, and dyslipidemia, with higher levels in hypertensive subjects. Advancing age, overweight, elevated fasting glucose and triglyceride levels were identified as metabolic risk factors. Genotypic and allelic frequencies of the studied polymorphisms did not differ significantly between hypertensive and control groups. No association was found between the studied polymorphisms and hypertension in our population. Age, overweight, elevated fasting glucose, and elevated triglycerides were identified as significant predictors of hypertension in this population.

Key words: endothelial nitric oxide synthase, genetic polymorphisms, hypertension, renin–angiotensin-aldosterone system, risk factors

RESUMEN

La hipertensión arterial esencial es una enfermedad multifactorial influenciada por factores genéticos y cardiometabolicos, con una prevalencia y perfiles de riesgo variables entre diferentes poblaciones. Los polimorfismos en genes del sistema renina-angiotensina-aldosterona (RAAS) y de la función endotelial han sido ampliamente estudiados por su rol en la regulación de la presión arterial y en el desarrollo de complicaciones cardiovasculares. El objetivo de este estudio fue investigar los polimorfismos ACE I/D, AT1R A1166C, AGT M235T y eNOS Glu298Asp, junto con los factores de riesgo tradicionales de hipertensión arterial esencial, en una población de San Luis, Argentina. Se incluyeron 208 pacientes hipertensos y 150 sujetos normotensos. Se recolectaron y analizaron datos demográficos, antropométricos y bioquímicos. Las frecuencias genotípicas y alélicas de los polimorfismos se determinaron mediante la técnica de PCR-RFLP. Se observaron diferencias significativas en índice de masa corporal (IMC), edad, glucosa en ayunas, colesterol total, HDL-C, triglicéridos y dislipidemia, con valores más elevados en los sujetos hipertensos. La edad avanzada, el sobrepeso, la glucemia en ayunas elevada y los niveles aumentados de triglicéridos fueron identificados como factores de riesgo metabólicos. Las frecuencias genotípicas y alélicas de los polimorfismos estudiados no mostraron diferencias significativas entre los grupos hipertensos y control. No se encontró asociación entre los polimorfismos analizados y la hipertensión en esta población. La edad, el sobrepeso, la glucemia en ayunas elevada y los triglicéridos aumentados se identificaron como predictores significativos de hipertensión arterial esencial en esta muestra.

Palabras clave: factores de riesgo, hipertensión, óxido nítrico sintasa endotelial, polimorfismos genéticos, sistema renina–angiotensina-aldosterona

INTRODUCTION

Arterial hypertension (HT) is a common chronic condition that affects approximately one in three adults worldwide, as reported by the World Health Organization (WHO). Nearly half of adults with HT are unaware of their condition (NCD Risk Factor Collaboration, 2021). This non-communicable disease is the primary risk factor for cardiovascular disease and a major cause of premature death worldwide.

While age and genetic factors contribute to HT occurrence, other modifiable factors such as high salt intake, physical inactivity, and harmful habits (smoking and alcohol consumption) also increase HT risk (Mills *et al.*, 2020; Zhou *et al.*, 2021).

Globally, HT prevalence doubled between 1999 and 2019, with a higher incidence in low- and middle-income countries. In the Americas, HT prevalence is around 35%, though it varies widely by region due to genetic and environmental differences (Delucchi *et al.*, 2017; Zhou *et al.*, 2021; Ordunez *et al.*, 2022).

The RENATA 2 study, which surveyed 5,931 adults in 25 cities across Argentina, found a hypertension prevalence of 36.3% (Delucchi *et al.*, 2017). More recent data showed a combined HT prevalence of 46.6%, whereas only 34.7% of individuals self-reported HT. In San Luis province, prevalence reached 40.9%, the highest in its region (Lamelas *et al.*, 2019; Ministerio de Salud de la Nación, 2019; Giunta *et al.*, 2023).

HT is considered a complex trait, with disease predisposition resulting from interactions among multiple genes (Lind and Chiu, 2013; Horani *et al.*, 2015). In 90% of patients, HT etiology is unknown, and genetic factors are significant, with heritability estimated at 30–50% (Agarwal *et al.*, 2005; Padmanabhan and Dominiczak, 2021).

The renin–angiotensin system (RAAS) plays a key role in cardiovascular homeostasis and HT pathogenesis. Extensive evidence links single nucleotide polymorphisms (SNPs) in RAAS genes with HT (Agarwal *et al.*, 2005; Singh *et al.*, 2010). These polymorphisms can alter gene function and predispose individuals to HT. Among the most studied are the angiotensin-converting enzyme (ACE) insertion/deletion (I/D), angiotensinogen (AGT) M235T, and angiotensin II type 1 receptor (AT1R) A1166C polymorphisms. Although RAAS genetic variants have been widely studied, results across different populations are inconsistent and sometimes contradictory (Agachan *et al.*, 2003; Companioni Nápoles *et al.*, 2007; Bautista *et al.*, 2008; Bonfim-Silva *et al.*, 2016; Isordia-Salas *et al.*, 2023; Al-Eitan *et al.*, 2024).

The vascular endothelium is structurally simple but functionally complex, regulating blood flow and vascular homeostasis. In HT, endothelial dysfunction promotes pathological vascular changes. Evidence associates eNOS polymorphisms with HT, coronary disease, stroke, and preeclampsia (Moe *et al.*, 2006; Oliveira-Paula *et al.*, 2017; Gallo *et al.*, 2022). The clinically relevant eNOS variants include the Glu298Asp (G894T) polymorphism, the T-186C polymorphism and the 27 bp VNTR (variable number of tandem repeats) in intron 4 (Oliveira-Paula *et al.*, 2017). Understanding genetic and molecular mechanisms in HT pathophysiology is essential to improve prevention, diagnosis, and treatment strategies (Franks, 2009).

Genetic variability among human populations significantly influences disease pathogenesis, posing challenges for the development of tailored therapies. The aim of the present study is to investigate the association of the ACE I/D, AT1R A1166C, AGT M235T and eNOS Glu298Asp polymorphisms with essential hypertension and risk factors, in a population from the central region of Argentina.

The research is justified by the existing knowledge gap regarding HT genetics in our region. We seek to determine whether these polymorphisms are more prevalent in hypertensive patients, with the intent to draw conclusions applicable to the general population, provide data, and unify diagnostic criteria.

MATERIALS AND METHODS

Study design

A case-control study was conducted at Hospital *Juan Gregorio Vivas* in San Luis, Argentina. The sample comprised 358 individuals: 208 hypertensive patients (HT group) and 150 normotensive subjects (control group).

The study was approved by the Institutional Ethics Committee of the Faculty, and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Demographic and clinical data were collected from both hypertensive patients and controls subjects.

Inclusion criteria: hypertensive patients were aged 18–75 years old with systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90 mmHg. All patients had a diagnosis of essential hypertension and were receiving antihypertensive treatment. The control group comprised healthy individuals aged 18–75 years with no history of hypertension and normal physical and mental health.

Exclusion criteria: individuals outside the specified age range were excluded. Pregnant women and patients with secondary HT were also excluded.

Blood pressure measurement

Blood pressure was measured using a validated arm cuff sphygmomanometer. Three consecutive readings

were averaged. Hypertension was defined as a mean SBP ≥140 mmHg or DBP ≥90 mmHg, measured on three separate occasions, or as a reported diagnosis. The diagnosis of hypertension was based on the Seventh Joint National Committee criteria (Chobanian *et al.*, 2003) and in accordance with the Argentine Consensus of Hypertension 2025. Although threshold values for defining hypertension have been revised in recent years, it is recommended to reserve the definition of hypertension for office-measured SBP values ≥140 mmHg and/or DBP ≥90 mmHg, as from these levels onward the clinical benefit of hypertension treatment is undeniable (Aquieri *et al.*, 2025).

Body mass index (BMI)

Height and weight were measured to calculate BMI (kg/m²) according to the World Health Organization guidelines. Patients were categorized as underweight (<18.5 kg/m²), normal weight (18.5 to 24.9 kg/m²), overweight (25 to 29.9 kg/m²), obese I (30 to 34.9 kg/m²), obese II (35 to 39.9 kg/m²), and obese III (≥40.0 kg/m²).

Biochemical parameters

The biochemical parameters determined were fasting plasma glucose (Glu) and lipid profile components including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). All measurements were performed using standardized enzymatic methods.

Genetic analysis

Genomic DNA was extracted from leukocytes using DNAzol™ (Invitrogen), quantified by spectrophotometry (Epoch Biotek), and stored at -20 °C. Polymorphisms were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) on a Smart Gradient PCR T960C thermocycler (Hangzhou Jingle Sci Int, China). Amplicons or digested fragments were separated on 2–3% agarose gels with GelRed™ and visualized under UV.

Amplification of the Insertion/Deletion (I/D) polymorphism in the ACE gene (rs4646994)

Genotyping for I/D polymorphism was performed using the PCR protocol (Rigat *et al.*, 1992); with the following primers: forward (*fw*): 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and reverse (*rv*): 5′-GATGTGGCCATCACATTCGTCAGAT-3′. The cycling conditions were as follows: initial denaturation at 94°C for 1 min, followed by 30 cycles of annealing at 58°C for 1 min, extension at 72°C for 2 min, denaturation at 94°C for 1 min, and final extension at 72°C for 10 min. PCR amplified products were I allele: 490bp and D allele: 190 bp.

Samples identified as DD homozygotes on the gel required a second PCR due to preferential amplification of the D allele and inefficient amplification of the I allele. Primers used were *fw:* 5′-TGGGACCACAGCGCCCGCCACTAC-3′ and *rv:* 5′-TCGCCAGCCCTCCCATGCCCATAA-3′ (Lindpaintner *et al.*, 1995). The thermal conditions were: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min; followed by a final extension at 72 °C for 10 min. Only the I allele produced a 335 bp fragment; DD homozygote samples showed no amplification.

Amplification of the A1166C polymorphism in the AT1 receptor gene (rs5186)

The A1166C polymorphism was detected by PCR-RFLP as described previously (Frishberg *et al.*, 1998). The following primers were used: *fw* 5'-AATGCTTGTAGCCAAAGTCACCT-3' and *rv* 5'-GGCTTTGCTTTGTCTTGTTG-3'. The cycling conditions were: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min; followed by a final extension at 72 °C for 10 min. The PCR products of 850 bp were digested with the restriction endonuclease *DdeI* (Promega, USA) overnight at 37°C. The enzyme generated two fragments (600 and 250 bp) and recognizes an additional site at nucleotide 1166 in the C allele, that cuts the 250 bp fragment into two smaller bands. Digested products were: A allele-600 and 250 bp; C allele-600, 140 and 110 bp.

Amplification of the M235T polymorphism in the AGT gene (rs699)

The M235T polymorphism was detected by PCR-RFLP (Yuan *et al.*, 2009). The following primers were used: *fw*5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3' and *rv*5'-CAGGGTGCTGTCCACACTGGACCCC-3'. The thermal conditions were an initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products of 162 bp were digested with the restriction endonuclease *Tth111I* (Thermo Scientific™) for 6 h at 37°C. The M allele remained uncut (162bp) and the T allele produced fragments of 141 and 21 bp.

Amplification of the Glu298Asp polymorphism in the eNOS gene (rs1799983)

Determination of Glu298Asp polymorphism was performed by PCR–RFLP as described by Pereira *et al.* (2006). The following primers were using:

fw 5′-AAGGCAGGAGACAGTGGATGGA-3′ and *rv* 5′-CCCAGTCAATCCCTTTGGTGCTCA-3′. The cycling conditions were: initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 63 °C for 1 min and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The resulting 248 bp product was digested overnight at 37 °C with Mbo I (Promega, USA). The G allele remained uncut (248 bp), whereas the T allele produced fragments of 158 bp and 90 bp.

Statistical Analysis

All data were expressed as means ± standard deviation (SD), frequency, or percentage as appropriate. Distribution of genotype and allele frequencies of ACE I/D, AT1R A1166C, AGT M235T and eNOS Glu298Asp polymorphisms among hypertensive and normotensive patients were assessed by the Chi square ($\chi 2$) test. Intergroup comparisons were made using Student's t-test. For continuous variables with normal distribution, ANOVA was used to evaluate differences among groups, followed by Tukey post-test. Odds ratio (OR) was used as a measure of association. All data were analyzed using SPSS software version 27.0 (SPSS Inc., Chicago, IL). A p-value <0.05 was considered statistically significant.

RESULTS

A total of 358 subjects (208 hypertensive patients and 150 controls) were examined in the present case-control study. The demographic, anthropometric, and biochemical characteristics of all the participants are described in Table 1. Among the hypertensive group (HT), 52.4% were male and 47.6% were female. The mean BMI values were significantly higher in HT than in controls (p<0.0001), exceeding the normal range in both groups. Hypertensive and normotensive subjects showed significant differences in age, fasting glucose, total cholesterol, HDL-C, and triglycerides, which were higher in the HT group. In addition, dyslipidemia values were significantly higher in the HT group than in the control group (p<0.01).

The results of the OR analysis for traditional hypertension risk factors are shown in Table 2. The main risk factors for hypertension were overweight/obesity (BMI≥25kg/m²; OR9.44; 95%CI4.81–18.50; p<0.001) and age ≥50 years (p<0.001). Fasting glucose (≥100mg/dl) and elevated TG (≥150mg/dl) were also associated with an increased risk of hypertension.

The distribution of RAAS polymorphisms was investigated in order to find possible associations with the development of HT. Table 3 details the distribution of genotypes and allelic frequencies of RAAS polymorphisms. No significant genotypic and allelic differences were found between hypertensive patients and control subjects.

Genotypic and allelic frequencies of eNOS Glu298Asp polymorphism are also shown in Table 3. No significant genotypic or allelic differences were found between the HT and control groups. The ORs for all polymorphisms were non-significant.

The analyses of BMI, glucose, and lipid profiles according to the genotypes of RAAS polymorphisms in hypertensive and control subjects, stratified by sex, are summarized in Table 4.

Regarding the ACE I/D polymorphism, hypertensive individuals exhibited significantly higher BMI and triglyceride levels across genotypes. The ID genotype was associated with elevated glucose levels in hypertensive subjects, while the DD genotype was linked to lower HDL-C, particularly among women.

Table 1. Baseline characteristics and clinical data in hypertensive (HT) and normotensive (control) patients

	HT (n=208)	Control (150)	*p* value
Sex (% Male/Female)	52.4 / 47.6	31.3 / 68.7	—
Age (years)	54.3 ± 9.6	39.75 ± 13.56	<0.001
BMI (kg/m²)	31.3 ± 5.9	26.8 ± 4.7	<0.001
SBP (mm Hg)	153.2 ± 16.3	117.9 ± 11.7	<0.001
DBP (mm Hg)	90.5 ± 10.2	71.1 ± 9.7	<0.001
Glucose (mg/dl)	91.3 ± 14.3	84.7 ± 11.6	<0.001
Total cholesterol (mg/dl)	199.6 ± 36.3	189.1 ± 40.3	<0.02
HDL-C (mg/dl)	48.3 ± 14.5	51.7 ± 11.8	<0.04
LDL-C (mg/dl)	119.9 ± 34.5	115.7 ± 38.7	NS
Triglycerides (mg/dl)	179.4 ± 96.8	126.1 ± 60.5	<0.001
Dyslipidemia (%)	59.13	44.91	<0.01

Presentation of values: mean ± standard deviation (SD). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. NS: non significant (p > 0.05).

Table 2. Odds ratio (OR) analysis for classic risk factors for hypertension

Risk factor	OR (95% CI)	*p* value
Age ≥50 years	7.45 (4.75 – 11.68)	<0.001
BMI ≥25 kg/m²	9.44 (4.81 – 18.50)	<0.001
Glucose ≥100 mg/dl	3.77 (1.40 – 10.11)	0.005
Total cholesterol ≥200 mg/dl	1.20 (0.73 – 1.97)	NS
LDL-C ≥130 mg/dl	1.15 (0.68 – 1.93)	NS
Triglycerides ≥150 mg/dl	2.76 (1.61 – 4.74)	<0.001

OR: odds ratio, CI: confidence interval. BMI: body mass index, LDL-C: low-density lipoprotein cholesterol. NS: non significant (p > 0.05).

Table 3. Distribution of genotypic and allelic frequencies of the ACE I/D, AT1R A1166C, AGT M235T, and eNOS Glu298Asp polymorphisms in the hypertensive group (HT) and normotensive group (Control)

Polymorphism	Genotype/ allele	HT (n=208)		Control (n=150)		*p* value	OR (95% CI)		*p* value
		n	%	n	%				
AT1R A1166C	AA	110	52.88	81	54		AA vs. AC+CC	0.95 (0.62–1.45)	NS
	AC	78	37.50	55	36.6	NS	AC vs. AA+CC	1.03 (0.67–1.6)	NS
	CC	20	9.62	14	9.3		CC vs. AA+AC	1.03 (0.5–2.11)	NS
	Allele A	298	71.63	217	72.3	NS	C vs. A	0.96 (0.74–1.44)	NS
	Allele C	118	28.36	83	27.6				
ACE I/D	II	57	27.40	38	25.3		II vs. ID+DD	1.11 (0.69–1.79)	NS
	ID	120	57.70	79	52.6	NS	ID vs. II+DD	1.22 (0.8–1.86)	NS
	DD	31	14.9	33	22		DD vs. II+ID	0.62 (0.36–1.06)	NS
	Allele I	234	56.3	155	51.6	NS	D vs. I	0.83 (0.60–1.15)	NS
	Allele D	182	43.8	145	48.4				
AGT M235T	MM	31	14.90	26	17.3		MM vs. MT+TT	0.83 (0.47–1.47)	NS
	MT	100	48.08	79	52.6	NS	MT vs. MM+TT	0.83 (0.54–1.26)	NS
	TT	77	37.02	45	30		TT vs. MM+MT	1.37 (0.87–2.14)	NS
	Allele M	162	38.94	131	43.6	NS	T vs. M	0.82 (0.60–1.11)	NS
	Allele T	254	61.06	169	56.4				
eNOS Glu298Asp	GG	106	50.96	78	52		GG vs. GT+TT	0.95 (0.63–1.46)	NS
	GT	85	40.86	61	40.6	NS	GT vs. GG+TT	1.00 (0.65–1.54)	NS
	TT	17	8.18	11	7.3		TT vs. GG+GT	1.12 (0.51–2.47)	NS
	Allele G	297	71.39	217	72.3	NS	G vs. T	0.95 (0.68–1.32)	NS
	Allele T	119	28.61	83	27.7				

OR: odds ratio, CI: confidence interval. NS: non significant ($p > 0.05$).

In the AT1R A1166C polymorphism, the AA and AC genotypes were associated with increased BMI, glucose, and triglyceride levels in hypertensives; additionally, AA carriers showed higher total cholesterol and HDL-C, especially in women.

For the AGT M235T polymorphism, hypertensive individuals with the MM genotype had higher BMI and total cholesterol levels; those with the MT genotype had higher BMI, glucose, total cholesterol, LDL-C, and triglycerides levels; and TT carriers displayed higher BMI, glucose, and triglyceride levels, compared with the control group.

Considering the NOS Glu298Asp polymorphism (Table 4), the GG genotype was significantly associated with most parameters, especially in women. While the TT genotype was linked to higher glucose level.

Since BMI values exceeded the normal range in both study groups (hypertensive and normotensive) the relationship between BMI and the genotypes was evaluated in the whole population (Table 5).

DISCUSSION

HT is a major public health issue in Argentina and globally. We examined demographic, anthropometric, biochemical, and genetic factors in a central Argentine population.

Hypertension is a complex polygenic disease, and identifying the genes involved in its etiology can contribute to a better understanding of the main pathogenic mechanisms, target organ complications, and interactions with environmental factors.

Recent Genome-Wide Association Studies (GWAS) have identified a large number of loci related to HT by examining common genetic variations. However, it is important to note that a major limitation has been the difficulty in linking SNPs to causal gene and function. GWAS identify genetic regions linked to HT but do not pinpoint the exact causal gene or mechanism (Padmanabhan and Dominiczak, 2021).

Although polygenic risk scores have been used to predict hypertension, their utility in the clinical setting remains uncertain (Kauko *et al.*, 2021).

The renin-angiotensin-aldosterone system is one of the most relevant systems in the regulation of blood pressure. Several studies have reported different components of this system as candidates for the genetic basis of essential hypertension (Bautista *et al.*, 2008; Singh *et al.*, 2010; Lind and Chiu, 2013).

In the present study, we investigated the possible association of RAAS genes polymorphisms with HT.

Table 4. Anthropometric and biochemical parameters according to genotypes of the ACE I/D, AT1R A1166C, AGT M235T, and eNOS Glu298Asp polymorphisms in the whole population (hipertensive and normotensive groups)

Anthropometric/ biochemical parameters	Polymorphism	Genotype	Whole population (n=358)			Male (n=157)	Female (n=201)
			HT (n=208)	Control (n=150)	*p* value	*p* value	*p* value
BMI (kg/m²)	ACE I/D	II	30.85 ± 4.79	26.02 ± 4.91	0.001	0.059	0.001
		ID	31.79 ± 5.08	27.59 ± 5.03	0.001	NS	0.001
		DD	31.04 ± 4.86	26.00 ± 3.79	0.001	0.04	0.001
	AT1R A1166C	AA	31.64 ± 4.56	26.47 ± 4.54	0.001	0.005	0.001
		AC	30.77 ± 5.25	26.47 ± 4.25	0.001	0.02	0.001
		CC	32.83 ± 5.80	30.27 ± 6.65	NS	NS	0.07
	AGT M235T	MM	31.01 ± 4.53	26.03 ± 4.83	0.001	NS	0.02
		MT	30.94 ± 4.54	26.68 ± 4.22	0.001	0.04	0.001
		TT	32.29 ± 5.63	27.50 ± 5.54	0.001	NS	0.001
	eNOS Glu298Asp	GG	32.28 ± 5.36	27.13 ± 4.90	0.001	NS	0.001
		GT	30.54 ± 4.26	26.42 ± 4.30	0.001	0.03	0.001
		TT	31.43 ± 4.97	27.03 ± 6.84	NS	NS	NS
Glucose (mg/dl)	ACE I/D	II	89.45 ± 15.98	84.35 ± 8.67	NS	NS	NS
		ID	92.67 ± 13.26	84.55 ± 12.60	0.001	NS	0.001
		DD	89.77 ± 14.78	85.32 ± 12.42	NS	NS	NS
	AT1R A1166C	AA	91.51 ± 14.62	82.54 ± 10.76	0.001	NS	0.002
		AC	91.08 ± 13.40	85.18 ± 10.49	0.02	NS	0.07
		CC	91.44 ± 16.49	95.56 ± 16.19	NS	NS	NS
	AGT M235T	MM	95.44 ± 21.21	81.50 ± 10.80	0.01	NS	NS
		MT	89.76 ± 12.74	86.60 ± 12.60	NS	NS	0.06
		TT	91.67 ± 12.41	83.40 ± 10.41	0.001	NS	0.003
	eNOS Glu298Asp	GG	92.45 ± 13.66	84.21 ± 12.45	0.001	NS	0.004
		GT	89.80 ± 15.50	86.26 ± 10.72	NS	NS	NS
		TT	90.92 ± 12.67	78.86 ± 10.46	0.04	NS	0.04
Total cholesterol (mg/dl)	ACE I/D	II	202.10 ± 28.98	190.04 ± 43.75	NS	NS	0.01
		ID	198.65 ± 39.27	189.64 ± 38.78	NS	NS	NS
		DD	198.54 ± 37.70	187.08 ± 41.84	NS	NS	NS
	AT1R A1166C	AA	199.57 ± 35.20	187.25 ± 41.95	0.06	NS	0.07
		AC	200.23 ± 37.00	191.92 ± 41.44	NS	NS	0.07
		CC	197.33 ± 41.08	188.33 ± 25.48	NS	NS	NS
	AGT M235T	MM	200.11 ± 38.91	179.82 ± 37.79	0.09	NS	0.09
		MT	198.11 ± 33.46	187.59 ± 37.51	NS	NS	0.02
		TT	201.33 ± 39.23	195.97 ± 45.11	NS	NS	NS
	eNOS Glu298Asp	GG	200.84 ± 34.69	189.12 ± 33.29	0.05	NS	0.08
		GT	196.58 ± 37.93	186.43 ± 43.24	NS	NS	0.04
		TT	205.69 ± 41.04	208.83 ± 68.35	NS	NS	NS
HDL-C (mg/dl)	ACE I/D	II	49.96 ± 21.04	52.87 ± 12.89	NS	NS	NS
		ID	48.15 ± 11.32	50.67 ± 11.86	NS	NS	NS
		DD	46.17 ± 10.11	53.08 ± 10.91	0.02	NS	0.02
	AT1R A1166C	AA	47.66 ± 11.75	53.60 ± 10.18	0.003	NS	0.009
		AC	49.18 ± 18.12	50.95 ± 11.63	NS	NS	NS
		CC	49.00 ± 13.07	44.33 ± 18.52	NS	NS	NS
	AGT M235T	MM	47.89 ± 10.94	49.88 ± 10.37	NS	NS	NS
		MT	46.63 ± 11.68	52.40 ± 12.70	0.01	NS	NS
		TT	50.91 ± 18.58	51.82 ± 11.43	NS	NS	NS
	eNOS Glu298Asp	GG	48.52 ± 14.86	53.00 ± 13.23	0.07	NS	NS
		GT	48.10 ± 13.92	49.77 ± 10.42	NS	NS	NS
		TT	48.62 ± 15.00	57.00 ± 3.80	NS	NS	NS

Table 4 (continues). Anthropometric and biochemical parameters according to genotypes of the ACE I/D, AT1R A1166C, AGT M235T, and eNOS Glu298Asp polymorphisms in the whole population (hipertensive and normotensive groups)

Anthropometric/ biochemical parameters	Polymorphism	Genotype	Whole population (n=358)			Male (n=157)	Female (n=201)
			HT (n=208)	Control (n=150)	*p* value	*p* value	*p* value
LDL-C (mg/dl)	ACE I/D	II	118.72 ± 33.10	118.57 ± 44.55	NS	0.08	0.02
		ID	119.82 ± 35.12	115.58 ± 37.29	NS	NS	NS
		DD	122.58 ± 36.15	113.50 ± 37.30	NS	NS	NS
	AT1R A1166C	AA	119.70 ± 36.24	112.81 ± 38.30	NS	NS	0.08
		AC	119.62 ± 33.03	118.86 ± 42.79	NS	0.09	NS
		CC	121.94 ± 32.81	120.44 ± 21.47	NS	NS	NS
	AGT M235T	MM	119.48 ± 39.40	104.41 ± 33.26	NS	NS	NS
		MT	119.15 ± 32.26	115.94 ± 37.30	NS	NS	0.09
		TT	121.11 ± 35.77	121.21 ± 42.85	NS	NS	NS
	eNOS Glu298Asp	GG	119.73 ± 33.22	113.60 ± 34.08	NS	NS	NS
		GT	117.59 ± 35.53	114.89 ± 40.83	NS	NS	NS
		TT	132.54 ± 38.83	145.20 ± 58.45	NS	NS	NS
Triglycerides (mg/dl)	ACE I/D	II	182.77 ± 99.41	128.04 ± 58.50	0.02	0.06	NS
		ID	177.16 ± 94.66	128.82 ± 59.53	0.001	NS	0.002
		DD	182.08 ± 95.80	118.63 ± 66.17	0.009	NS	0.004
	AT1R A1166C	AA	167.03 ± 81.12	123.33 ± 52.81	0.001	NS	0.001
		AC	200.36 ± 99.23	124.65 ± 71.25	0.001	0.07	0.01
		CC	164.78 ± 99.81	148.56 ± 55.92	NS	NS	NS
	AGT M235T	MM	163.56 ± 68.34	150.35 ± 81.82	NS	NS	NS
		MT	172.60 ± 94.47	117.04 ± 54.30	0.001	NS	0.001
		TT	195.38 ± 98.61	126.62 ± 54.61	0.001	0.01	0.051
	eNOS Glu298Asp	GG	175.77 ± 89.56	129.68 ± 58.37	0.001	NS	0.001
		GT	185.09 ± 98.57	120.60 ± 65.35	0.001	0.056	0.01
		TT	177.62 ± 97.40	138.40 ± 38.76	NS	NS	NS

Values are presented as mean ± standard deviation (SD). BMI: body mass index.
NS: not significant ($p > 0.05$). HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol.

There is evidence suggesting an association between these genes and HT (Horani *et al.*, 2015; Lamelas *et al.*, 2019), however, these variants have yielded contradictory results across different populations (Agachan *et al.*, 2003; Moe *et al.*, 2006; Companioni Nápoles *et al.*, 2007; Bonfim-Silva *et al.*, 2016).

The potential role of the AT1R gene in the predisposition to hypertension is controversial, and conflicting results have been reported (Agachan *et al.*, 2003; Bautista *et al.*, 2008; Sharma *et al.*, 2024). The present study found no association between the AT1R A1166C polymorphism and hypertension. This finding is consistent with our previous results of this SNP in a population from San Luis (Lapierre *et al.*, 2006). Similarly, Bautista *et al.* (2008) and Isordia-Salas *et al.* (2023) found no association between AT1R A1166C polymorphism and hypertension.

The ACE I/D polymorphism is one of the most widely studied genetic variants of the RAAS genes. Several authors have reported that ACE DD genotype is associated with HT (Agachan *et al.*, 2003; Bautista *et al.*, 2008; Isordia-Salas *et al.*, 2023). However, we could not find a relationship between this variant and the HT in our population, which could be explained by the interactions of different factors. In fact, according to a recent genetic report for central Argentina, the genetic context cannot be analyzed without taking into account the different historical, migratory, socioeconomic and demographic processes that result in the particular genetic characteristics of each region (García *et al.*, 2015).

Regarding AGT M235 Tpolymorphism, our results revealed no differences in genotype and allele frequencies between both groups. This is consistent with some previous reports (Bautista *et al.*, 2008; Yuan *et al.*, 2009). In contrast, other studies have reported a strong association between the TT genotype and HT (Agachan *et al.*, 2003; Isordia-Salas *et al.*, 2023).

Table 5. Relationship between body mass index (BMI) and the genotypes of the ACE I/D, AT1R A1166C, AGT M235T, and eNOS Glu298Asp polymorphisms in the whole population (hipertensive and normotensive groups)

Parameter	Polymorphism	Genotype	Whole population (n=358)	*p* value
BMI (kg/m²)	ACE I/D	II	28.97 ± 5.36	
		ID	30.16 ± 5.45	0.05
		DD	28.42 ± 4.96	
	AT1R A1166C	AA	29.43 ± 5.21	
		AC	29.06 ± 5.28	0.03
		CC	31.85 ± 6.17	
	AGT M235T	MM	28.76 ± 5.25	
		MT	29.10 ± 4.89	0.05
		TT	30.55 ± 5.99	
	eNOS Glu298Asp	GG	30.16 ± 5.75	
		GT	28.78 ± 4.71	NS
		TT	28.96 ± 5.64	

Values are presented as mean ± standard deviation (SD). NS: non significant ($p > 0.05$).

The Glu298Asp polymorphism is one of the most widely studied variants of the eNOS gene. In our population we found no association between the Glu298Asp genotype and HT. Consistent with our results, Moe *et al.* (2006) reported that this eNOS polymorphism was not associated with hypertension.

Gene–environment interactions in HT are complex. While some studies report associations between RAAS and eNOS SNPs and HT, others do not, likely due to genetic and environmental heterogeneity (Horani *et al.*, 2015; Lamelas *et al.*, 2019).

The lack of association observed in our population may reflect underlying genetic heterogeneity. In addition, genetic, epigenetic, and environmental factors may interact to influence the hypertensive phenotype. Another possible explanation includes epistatic gene–gene interactions in which the effect of the studied gene is masked by the effect of other susceptible genes.

A limitation of this study is the relatively small sample size. Future research involving larger, more diverse cohorts is necessary to validate or refine these preliminary findings.

When we compared the demographic characteristics, the anthropometric and biochemical parameters of the hypertensive and control groups, we observed significant differences in fasting plasma glucose, total cholesterol, HDL-C, triglycerides, and dyslipidemia, with higher levels in the hypertensive group. These findings reinforce the importance of metabolic factors as key determinants of the risk of high blood pressure. It is noteworthy that the BMI was significantly higher in hypertensive patients than in controls; however, in both groups, the BMI was above the normal range, indicating a worrying fact about the existence of overweight throughout our study population. The pervasive overweight status within both groups underscores a broader health challenge that needs to be addressed.

We analyzed traditional cardiovascular risk factors in our population. The Odds Ratio (OR) analysis revealed that the age ($p<0.001$), the overweight ($p<0.001$), the elevated fasting glucose ($p<0.005$), and elevated triglycerides ($p<0.001$) as significant predictors of HT. The risk factors involved in the development of high blood pressure have been reportedin numerous prior studies, and our findings are consistent with most of them (Pereira *et al.*, 2006; Oliveira-Paula *et al.*, 2017; Lamelas *et al.*, 2019; Mills *et al.*, 2020; Zhou *et al.*, 2021; Giunta *et al.*, 2023).

Evidence highlights that hypertension is not an isolated problem, but is intrinsically linked to metabolic disorders (such as glucose and lipid abnormalities), forming a cardiometabolic risk complex that worsens the patient's prognosis, increasing vascular damage and the risk of serious cardiovascular events. Therefore, hypertension must be viewed in the context of other metabolic factors, as their combined management is

crucial for improving prognosis and reducing long-term cardiovascular risk (Mills *et al.*, 2020; Ordunez *et al.*, 2022).

A stratified analysis by sex was performed to study the association between the different genotypes with BMI, fasting glucose, and lipid profile parameters.

Overall, the results in Tables 4 and 5 indicate how different genotypes modulate the anthropometric and biochemical profile of our population, with more pronounced effects in women and specific variations according to each polymorphism. These findings suggest a possible interaction between genetic variants and adverse metabolic profiles associated with hypertension, with a pattern of greater susceptibility in women. This is consistent with Kauko *et al.* (2021), which suggests that the effect of genetics on hypertension risk, compared to that of modifiable factors, appears to be greater in women than in men.

Studies on the role of gene polymorphisms in the renin-angiotensin system and the eNOS gene in the development of hypertension are limited in the Argentine population.

To our knowledge, this is the first study of RAAS and eNOS polymorphisms conducted in a primary health care center attending population in San Luis; our prior work focused solely on the AT1R A1166C polymorphism and was performed in a private clinic setting (Lapierre *et al.*, 2006).

This research contributes to the cumulative growth of knowledge, leading to a more comprehensive understanding of hypertension and the genetic and cardiometabolic factors that characterize populations in our region.

CONCLUSION

These findings suggest that the ACE I/D, AT1R A1166C, AGT M235T, and eNOS Glu298Asp polymorphisms are not associated with the development of hypertension in the studied population.

Age, overweight, elevated fasting glucose, and elevated triglycerides are significant predictors of HT in this population.

Given HT's polygenic nature, larger studies in our region are needed to confirm these results.

Understanding the distribution of genetic polymorphisms and identifying their relationship to cardiometabolic risk factors is crucial for developing targeted public health strategies for hypertension.

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AUTHOR CONTRIBUTION

LBF and MEA designed the study. MMC and LBF collected data. MMC performed genotyping and statistical analyses. MMC, MEA, and LBF drafted and revised the manuscript. All authors approved the final version.

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The authors declare no financial conflicts of interest.

DECLARATION OF GENERATIVE AI USE

During the preparation of this work, the authors used ChatGPT in order to assist in the writing process, improving readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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NOTA AL EDITOR - NOTE TO THE EDITOR

PROFESSIONAL BODIES: HOW BEST TO PROMOTE AND SUPPORT INDIVIDUALS WORKING IN HUMAN GENETICS AND GENOMICS

ASOCIACIONES PROFESIONALES: LA MEJOR MANERA DE PROMOVER Y APOYAR EL TRABAJO DE LOS PROFESIONALES DE LA GENÉTICA Y GENÓMICA HUMANAS

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Professional bodies are dedicated to the advancement of the knowledge and practice of professions through developing, supporting, regulating and promoting professional standards for technical and ethical competence (Speight, 2014). They seek to maximise the public benefit of the work undertaken by their members and support the reputation of their professional members. In the UK, the Science Council defines a Professional Body as "*an organisation with individual members, practicing a profession or occupation in which the organisation maintains an oversight of the knowledge, skills, conduct and practice of that profession or occupation.*" Some professional bodies are regulated by the executive at a national level to ensure that professional titles are only used by individuals who are registered and entitled to use these e.g., medical doctors. However, most professional bodies are independent membership organisations that coordinate the activities of a particular profession or a group of closely related professions and represent the interests of their members. It is into this latter group that human genetics and genomics professional bodies fall.

Since the formation of the American Society for Human Genetics in 1948 (Hirschhorn, 2008), professional bodies for human genetics have played a fundamental role in setting standards for their members, ensuring quality, and advancing the field through education, ethical guidelines, and advocacy. They facilitate professional development for clinical and laboratory geneticists, genetic counsellors and genetic nurses, scientists and researchers, as well as allied health professionals. They provide resources in genetics and genomics for all healthcare professionals (Godard *et al.*, 2003) and guide public policy on complex issues like genetic privacy, data use, reproductive technologies, gene therapies, precision medicine (Sasongko et al., 2025), and cloning. Their activities include developing educational programs (Tobias *et al.*, 2021), disseminating information on genetic

testing, and establishing best practices to support patient care and research (Harper, 2017; Wasant *et al.*, 2019).

Professional bodies, societies or associations in human genetics and genomics have developed in different geographical regions to act at a national and continental level (see table 1). Many societies have seen their primary responsibilities as providing mentorship and training opportunities to the next cadre in the profession; developing new professional roles e.g. bioinformaticians, and promoting advances in knowledge through annual conferences, training programs and public outreach. Efforts to increase access to educational resources through webinars, hybrid conferences and educational apps are all improving the reach and influence of the societies (Tobias *et al.*, 2021). Many societies place particular emphasis on supporting the development of genomics clinical services across their continent (Rotimi, 2004).

Recognising the global importance of genomics, and the opportunity for shared learning and coordination of efforts between multi-nation societies to maximise the impact and benefit of genomics, the International Federation of Human Genetics Societies (IFHGS, https://www.ifhgs.org) was formed in 1996 as an umbrella organisation (Pembrey, 2017). The Middle Eastern and North African Medical Genetics Association (MENA-MGA) was adopted to membership of the IFHGS in 2025.

The bylaws of the Federation state that:

"*The purpose of The International Federation of Human Genetics Societies (the Federation) is to provide a forum for organized groups dedicated to all aspects of human genetics, including research, clinical practice, and professional and lay education. The Federation will enable communication between its member groups and encourage interaction between workers in genetics fields and in related sciences and will make itself available to promote meetings and publications and other forums which support human genetics research and practice.*"

Every five years the IFHGS hosts a congress which brings together members from all continents to discuss genomics, share research, experiences and perspectives of delivering genomic healthcare. The next meeting will be in Guadalajara, Mexico, 2-6 March 2027 (https://ic.relagh.org).

Building bridges and forging alliances between the IFHGS and other international institutions is essential for consolidating strategic pathways to progress. In this regard, the Human Genome Organisation (HUGO) plays a key role by bringing together genomics professionals to advance research and education, a mission it has pursued for more than three decades. The Federation is an active member of the HUGO Forum (https://www.hugo-international.org/hugo-forum/).

The maturity, size and resources differ between societies, sometimes reflecting the level of investment in genomics in their countries or regions and the political and public attitudes to genomics. However, many of the ambitions and challenges remain similar.

Professional organisations face challenges in ensuring that the perspective and expertise of their membership is reflected in national debate and policy making relevant to human genomics. To be responsive to the 24/7 news cycle and social media, knowing when to engage in the debate and how to contribute in a constructive manner is difficult, especially when for many societies, leadership is undertaken by individuals on a voluntary basis in addition to their primary professional roles.

Table 1. Constituent groups of the International Federation of Human Genetics Societies.

Society	Membership (Approximate number of members)	Founded	Website
African Society of Human Genetics (AfSHG)	>1000	2003	https://www.afshg.org
American Society of Human Genetics (ASHG)	8000	1948	https://www.ashg.org
Asia-Pacific Society of Human Genetics	214	2006	https://apshg.info
East Asian Union of Human Genetics Societies (EAUHGS)	9050*	2001	https://www.eauhgs.asia
European Society of Human Genetics (ESHG)	3500	1967	https://www.eshg.org
Human Genetics Society of Australasia	1200	1977	https://www.hgsa.org.au
Latin American Network of Human Genetics Societies (RELAGH)	2500**	2001	https://www.relagh.org
Middle Eastern and North African Medical Genetics Association (MENA-MGA)	1000	2022	https://mena-mga.org

* Membership at EAUHGS: 6750 in Japanese Society of Human Genetics, 2000 in Chinese Society of Medical Genetics, 300 in Korean Society of Medical Genetics

** From 24 societies of the Latin American Human Genetics Networks

Strategic collaboration among human genetics societies is imperative for effective impact. Such concerted action enables the consolidation of clear guidance for their members and, critically, positions the scientific community as an authoritative voice in public and policy discourse. In a context of concerns regarding information integrity, these organizations have a social responsibility to serve as a trusted source of information, providing evidence-based data and expert analysis to the general public and decision-makers. This serves a dual objective: first, to empower the public to form well-founded opinions, and second, to ensure that national, regional and global public policies are built upon a foundation of rigorous scientific knowledge rather than on unsubstantiated claims.

It is vital that societies act as forums for rich, respectful debate about the future of genomics. Creating opportunities for diverse attitudes and opinions to be heard is essential to ensure that the societies represent the professions and communities which they endeavour to serve. In addition, it is vital that young members are encouraged to participate and assume leadership roles (Riccardi *et al.*, 2022; Avram *et al.*, 2023).

We encourage individuals working in human genomics to join their respective national, regional and continental genomics societies, contribute to the leadership, attend conferences to meet colleagues and network working effectively across regions, countries and continents, ensuring that genomics is made available for the benefit of all.

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