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Identificación de nuevos polimorfismos del Síndrome de Marfan, un estudio de caso.

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RESUMEN

Antecedentes: El síndrome de Marfan (SMF) es un trastorno autosómico dominante, causado por mutaciones del gen que codifica la *fibrilina-1*, es un componente de glucoproteína de las fibras elásticas que tiene importantes funciones estructurales y reguladoras en la matriz extracelular, lo que lleva a una desregulación de la señalización del factor de crecimiento - beta transformante (TGF-β), que afecta los sistemas esquelético, ocular y cardiovascular, del tejido conectivo. Estos pacientes afectados llevan una esperanza de vida reducida, en gran medida dependiente de complicaciones cardiovasculares. Su buen pronóstico dependerá de un eficiente diagnóstico, tratamiento y el conocimiento del paciente de su enfermedad.
Objetivos: Identificar los polimorfismo que influyen en la expresión del fenotipo y calidad de vida de un individuo con Síndrome de Marfan.
Materiales y Métodos: Para ello tomamos el resultado del estudio molecular realizado por el paciente, mediante el método de secuenciación Sanger, el cual fue procesado y sus características de rendimiento fueron determinadas por el Laboratorio de Diagnóstico Cardiovascular Jhon Welsh (Baylor College of Medicine (BCM) Houston, Texas). Este laboratorio está certificado bajo las enmiendas de mejora del laboratorio clínico de 1988 (CLIA-88), que permitió conseguir las secuencias del resultado del estudio. A los resultados de la secuenciación se les realizó un análisis bioinformático para relacionar los polimorfismos encontrados en el paciente con el Síndrome de Marfan, por medio de herramientas como: El modelamiento por homología que consiste en alinear la secuencia de aminoácidos de la proteína con secuencias de proteínas de las cuales ya se conoce su estructura, partiendo de observaciones donde proteínas con funciones similares tienen

estructuras similares (proteínas homologas), se usan estas proteínas de las cuales ya se conoce su estructura para plegar la proteína problema, las proteínas usadas como plantillas son obtenidas de la base de datos públicas como PDB. En algunos casos la proteína problema presenta mutaciones que son de nuestro interés y precisan ser estudiadas y analizadas, para ello se realizaron pasos adicionales en el modelamiento de la proteína para añadirle estas mutaciones y así verificar como estas afecta a la proteína en sí. Estas mutaciones previamente identificadas con técnicas como secuenciación y alineamiento fueron luego reproducidas en nuestra estructura tridimensional.

Resultados: Al realizar el análisis de los resultados obtenidos nos muestra un par de mutaciones no reportadas y las cuales no estaban relacionadas con la enfermedad. Tenemos una mutación cambiando una *Serina* por una *Glicina* (S713G) se evidencia que no se da dentro de ningún dominio, se encuentra en un loop, donde tenemos un cambio de un aminoácido hidrofóbico por otro aminoácido hidrofóbico y la aparición de un codón de parada prematuro a causa de la mutación de un ácido glutámico (E2097X). La aparición de este codón de parada, dejaría la proteína incompleta, dejando por fuera 774 aminoácidos, este corte se da en un dominio tipo TB y dejaría por fuera 10 dominios de distintos tipos (TB, EGF_CA, vWFA y cEGF), este hallazgo es lo que más afecta en el fenotipo del paciente, por ser una proteína de varios dominios. Por ser una proteína de 2871 aminoácidos muy larga para su análisis de fragmenta por segmentos de entre 200-400 aminoácidos y estos fueron modelados usando el software Modeller y para zonas donde Modeller presentaba problemas se usó el servidor web Swiss Model, de esta manera se modelaron los distintos segmentos de la proteína. Al tener todos estos segmentos cada uno fue unido usando el sofware Chimera, hasta completar la proteína.

Conclusiones: Se encontró que al alinear las dos estructuras completas, la wildtype con la mutada, el RMSD es 0.034 Å, un cambio imperceptible, mientras que en el loop de la mutación S713G tenemos un RMSD de 0.700 Å, lo cual es un cambio minino, sin embargo quedan pendientes estudios futuros para evaluar con dinámica molecular si esta mutación en este punto afecta en el tiempo la estructura de la proteína, el resultado de la proteína con una estructura bastante compleja, grande, alargada muy difícil de analizar.

De momento la principal mutación que afectaría a la proteína sería la del codón prematuro de parada, lo que puede explicar los rasgos fenotípicos del paciente analizado en esta primera fase.

PALABRAS CLAVE

Codón de parada; Fibrilina; Nosología de Ghent; Síndrome de Marfan; análisis bioinformático

ABSTRACT

Background: Marfan syndrome (MFS) is an autosomal dominant disorder, caused by mutations in the gene encoding fibrillin-1, it is a glycoprotein component of elastic fibers that has important structural and regulatory functions in the extracellular matrix, which leads to dysregulation of transforming growth factor-beta (TGF-β) signaling, which affects the skeletal, ocular, and cardiovascular systems of connective tissue. These affected patients have a shortened life expectancy, largely dependent on cardiovascular complications. Its good prognosis will depend on an efficient diagnosis, treatment and the knowledge of the patient of his disease.

Objectives: To identify the polymorphisms that influence the expression of the phenotype and quality of life of an individual with Marfan Syndrome.

Materials and Methods: For this we take the result of the molecular study carried out by the patient, using the Sanger sequencing method, which was processed and its performance characteristics were determined by the Jhon Welsh Cardiovascular Diagnostic Laboratory (Baylor College of Medicine (BCM) Houston Texas). This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), which allowed the sequences of the study result to be achieved. The results of the sequencing were subjected to a bioinformatic analysis to relate the polymorphisms found in the patient with Marfan Syndrome, using tools such as: Homology modeling, which consists of aligning the amino acid sequence of the protein with sequences of proteins whose structure is already known, based on observations where proteins with similar functions have similar structures (homologous proteins), these proteins are used whose structure is already known to fold the problem protein, the proteins used as templates are Obtained from the public database as PDB. In some cases, the problem protein presents mutations that are of our interest and need to be studied and analyzed, for this, additional steps were carried out in the modeling of the protein to add these mutations and thus verify how they affect the protein itself. These mutations previously identified with techniques such as sequencing and alignment were then reproduced in our three-dimensional structure.

Results: When performing the analysis of the results obtained, it shows us a couple of mutations not reported and which were not related to the disease. We have a mutation changing a Serine for a Glycine (S713G) it is evident that it does not occur within any domain, it is in a loop, where we have a change of a hydrophobic amino acid for another hydrophobic amino acid and the appearance of a premature stop codon due to the mutation of a glutamic acid (E2097X). The appearance of this stop codon would leave the protein incomplete, leaving 774 amino acids outside, this cut occurs in a TB-type domain and would leave out 10 domains of different types (TB, EGF_CA, vWFA and cEGF), this finding is what most affects the phenotype of the patient, as it is a multi-domain protein. Because it is a protein of 2871 amino acids very long for its analysis of fragments by segments of between 200-400 amino acids and these were modeled using the Modeller software and for areas where Modeller presented problems the Swiss Model web server was used, in this way they were modeled the various segments of the protein. Having all these segments, each one was joined using Chimera software, until the protein was completed.

Conclusions: It was found that when aligning the two complete structures, the wildtype with the mutated one, the RMSD is 0.034 Å, an imperceptible change, while in the loop of the S713G mutation we have an RMSD of 0.700 Å, which is a tiny change However, future studies are pending to evaluate with molecular dynamics if this mutation at this point affects the structure of the protein over time, the result of the protein with a rather complex, large, elongated structure that is very difficult to analyze.
For now, the main mutation that would affect the protein would be the premature stop codon, which may explain the phenotypic traits of the patient analyzed in this first phase.

KEYWORDS
Stop codon; Fibrillin; Ghent nosology; Marfan syndrome; bioinformatic analysis

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