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Original article

3'UTR SNPs and Haplotypes in the *GATA4* Gene Contribute to the Genetic Risk of Congenital Heart Disease

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ABSTRACT

Introduction and objectives: Single-nucleotide polymorphisms within a microRNA binding site can have different effects on gene expression, influencing the risk of disease. This study aimed to evaluate the association between single-nucleotide polymorphisms and haplotypes in the 3'UTR of the *GATA4* gene and congenital heart disease risk.

Methods: Bioinformatics algorithms were used to analyze single-nucleotide polymorphisms in putative microRNA-binding sites of *GATA4* 3'UTR and to calculate the difference in free energy of hybridization (ΔFE, kcal/mol) for each wild-type vs the variant allele.

Results: The study population comprised 146 Caucasian patients (73 males; 6.68 ± 7.79 years) and a 265 healthy newborn participants (147 males). The sum of all |ΔFE| was considered to predict the biological importance of single-nucleotide polymorphisms binding more microRNAs. Next, the 4 polymorphisms (+1158 C > T, +1256 A > T, +1355 G > A, +1521 C > G) with the highest predicted $|\Delta FE_{tot}|$ (9.91, 14.85, 11.03, 21.66 kcal/mol, respectively) were genotyped in a case-control study (146 patients and 250 controls). Applying a correction for multiple testing only the +1158 T allele was found to be associated with a reduced risk showing significant difference between patients and controls. Haplotype analysis showed that the T-T-G-C haplotype (more uncommon in congenital heart diseases than in controls) was associated with a significantly decreased risk (P = .03), while the rare C-A-A-C haplotype, which was very uncommon in controls (0.3%) compared with the disease (2.4%), was associated with a 4-fold increased risk of disease (P = .04).

Conclusions: Common variants in 3'UTR of the *GATA4* gene jointly interact, affecting the congenital heart disease susceptibility, probably by altering microRNA posttranscriptional regulation.

Los polimorfismos de nucleótido único y los haplotipos de la región 3'UTR del gen *GATA4* contribuyen al riesgo genético de cardiopatía congénita

Palabras clave:
Haplotipo
Polimorfismos de nucleótido único
3'UTR
GATA4
Cardiopatía congénita

RESUMEN

Introducción y objetivos: Los polimorfismos de nucleótido único situados en un lugar de unión de microácidos ribonucleicos (miARN) pueden tener diferentes efectos en la expresión génica, y ello puede influir en el riesgo de enfermedad. Este estudio tiene como objetivo evaluar la asociación existente entre los polimorfismos de nucleótido único y los haplotipos presentes en la región 3'UTR del gen *GATA4* y el riesgo de cardiopatía congénita.

Métodos: Se utilizaron algoritmos de bioinformática para analizar los polimorfismos de nucleótido único en los presuntos lugares de unión de miARN en la región 3'UTR del gen *GATA4* y para calcular la diferencia de energía de hibridación libre (ΔFE, kcal/mol) para cada alelo de tipo natural *(wild-type)* en comparación con cada variante alélica.

Resultados: Formaron la población de estudio 146 pacientes caucásicos (73 varones; edad, 6,68 ± 7,79 años) y 265 recién nacidos sanos (147 varones). Se consideró que la suma de todos los ΔFE predecía la importancia biológica de los polimorfismos de nucleótido único al unirse a más miARN. A continuación se determinó el genotipo de los 4 polimorfismos (+1158 C > T, + 1256 A > T, + 1355 G > A, +1521 C > G) que tenían el valor predicho de ΔFE total más alto (9,91, 14,85, 11,03 y 21,66 kcal/mol respectivamente) en un estudio de casos y controles (146 pacientes y 250 controles). Al aplicar una corrección por multiplicidad de pruebas, tan solo el alelo +1158 T mostró una diferencia significativa entre los pacientes y los controles. El análisis de los haplotipos puso de manifiesto que el haplotipo T-T-G-C (más infrecuente en los pacientes con cardiopatías congénitas que en los controles) se asociaba a una disminución del riesgo significativa (p = 0,03), mientras que el haplotipo muy infrecuente C-A-A-C, que se daba de manera muy poco común en los controles

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(0,3%) en comparación con los pacientes con la enfermedad (2,4%), se asociaba a un aumento de 4 veces en el riesgo de enfermedad (p = 0,04).
Conclusiones: Las variantes frecuentes de la región 3'UTR del gen *GATA4* interaccionan de manera conjunta y con ello afectan a la susceptibilidad a la cardiopatía congénita, probablemente mediante la alteración de la regulación postranscripcional de los miARN.

Abbreviations

3'UTR: 3' untranslated region
CHD: congenital heart disease
PCR: polymerase chain reaction
RNA: ribonucleic acid
SNP: single nucleotide polymorphism
ΔFE: difference in free energy of hybridization

INTRODUCTION

Congenital heart disease (CHD) is the most prevalent of all birth defects (between 75 and 90 per 10 000 for live births in the last 20 years) and is the leading cause of death from congenital malformations in the neonatal period and during the first year of life.[1] CHD comprises a heterogeneous group of cardiac defects that arise during fetal development. To date, the molecular mechanisms involved in such abnormal cardiogenesis remain largely unknown. Genetic and epigenetic variations are recognized as the predominant cause of CHD, although the identification of precise alterations has proven challenging, principally because CHD is a complex process.[2]

Genes involved in transcriptional controls, known as transcription factors, have been identified as major players in cardiac development.[3,4] In particular, the transcription factor *GATA4* is suggested to be crucial for normal heart specification and development.[5,6] As for the other transcription factors, a long list of mutations in the *GATA4* gene has been identified in CHD patients, but the contribution of each of these mutations to disease risk, especially for sporadic forms, is very low and not well-defined.[2,7,8] Recently, experimental studies showed that miRNAs (nonprotein-coding small molecules of ribonucleic acid ~20–22 nucleotides) may modulate cardiogenesis by altering the expression of critical cardiac regulatory proteins.[7,9,10] Accordingly, data from our group indicated that common single nucleotide polymorphism (SNPs) in the 3'UTR of *GATA4* gene altered the miRNA-mRNA binding, dysregulating *GATA4* gene expression.[11] The purpose of the present work was to expand the analysis of 3'UTR of the *GATA4* gene by analyzing selected SNPs and related haplotypes in this region in order to confirm its major role in modulating CHD risk.

METHODS

Study Population

The study population comprised a group of 146 Caucasian patients (73 males; 6.68 ± 7.79 years), who were diagnosed with an isolated, nonsyndromic CHD and a control group of 265 healthy newborn participants (147 males). A sample of venous blood was collected from adult participants, whereas a cord blood sample was obtained from newborns (both CHDs and controls). This study was conducted with informed consent of all participants or their parents and was approved by the local Ethics Research Committee.

Genotyping

Genomic DNA was isolated from blood using standard procedures according to the manufacturer's instructions (QIAGEN BioRobot EZ1 System). The 3'UTR sequence was amplified by polymerase chain reaction (PCR) using specific primers as previously described.[12] The PCR products were used for PCR sequencing reactions by using the CEQ DTCS Quick Start Kit. After purification, the sequencing reaction products were analyzed with a CEQ 8800 capillary sequence (Beckman Coulter, Germany), according to the manufacturer's protocol. Resulting sequences were analyzed by using CEQ 8800 software packages and aligned against a reference sequence obtained from Gene Bank BLAST (Basic Local Alignment Search Tool).

Single Nucleotide Polymorphism Selection

The 10 common genetic variants located in the 3'UTR region of the *GATA4* gene observed in our population were analyzed for putative miRNA-binding sites using bioinformatics algorithms in order to calculate the difference in free energy of hybridization (ΔFE, kcal/mol) for each wild-type vs variant allele, as previously described.[11,13] Briefly, MicroSNiPer[14] was used to predict the impact of each SNP on putative miRNA targets. Highly stable miRNA/target duplexes are represented as having a very low minimum free energy (kcal/mol) that has been calculated for both the common and the variant alleles by RNACofold program,[15] from the Vienna RNA package (version 1.8.5). The difference in the free energies between the 2 alleles was computed as "variation of FE" (ΔFE). The sum of all |ΔFE| (|ΔFEtot|) was calculated to predict the biological importance of SNPs binding more miRNAs.

Statistical Analysis

Single-locus tests of association between SNPs allele frequencies and case-control status were carried out via standard unpaired Student's *t* test and chi-square analysis, using StatView statistical package, version 5.0.1 (Abacus Concepts, Berkeley, California). Logistic regression analysis was used to estimate odds ratio (OR) and 95% confidence interval (95%CI) for the association between CHD and the presence of the polymorphism. In this analysis, a Bonferroni correction for (4 genotypes) multiple testing was performed to evaluate statistical significance at an adjusted *P*-value threshold ($P = .05/4 \leq .0125$).