



Original Article

# In silico analysis of *GATA4* variants demonstrates main contribution to congenital heart disease

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## Abstract

**Introduction:** Congenital heart disease (CHD) is the most common congenital abnormality and the main cause of infant mortality worldwide. Some of the mutations that occur in the *GATA4* gene region may result in different types of CHD. Here, we report our *in silico* analysis of gene variants to determine the effects of the *GATA4* gene on the development of CHD.

**Methods:** Online 1000 Genomes Project, ExAC, gnomAD, GO-ESP, TOPMed, Iranome, GME, ClinVar, and HGMD databases were drawn upon to collect information on all the reported *GATA4* variations. The functional importance of the genetic variants was assessed by using SIFT, MutationTaster, CADD, PolyPhen-2, PROVEAN, and GERP prediction tools. Thereafter, network analysis of the *GATA4* protein via STRING, normal/mutant protein structure prediction via HOPE and I-TASSER, and phylogenetic assessment of the *GATA4* sequence alignment via ClustalW were performed.

**Results:** The most frequent variant was c.874T>C (45.58%), which was reported in Germany. Ventricular septal defect was the most frequent type of CHD. Out of all the reported variants of *GATA4*, 38 variants were pathogenic. A high level of pathogenicity was shown for p.Gly221Arg (CADD score=31), which was further analyzed.

**Conclusion:** The *GATA4* gene plays a significant role in CHD; we, therefore, suggest that it be accorded priority in CHD genetic screening.

## Introduction

Congenital heart disease (CHD) is the most common congenital malformation and a significant cause of childhood mortality with an estimated prevalence of 1% of infants born each year.<sup>1,2</sup> Cardiovascular abnormalities are reported in approximately 29% of dead infants. CHD can be caused by variants in different genes whose roles have evolved. The number of genes and variants thereof involved in the CHD pathogenesis has increased, and an accurate determination of the molecular mechanisms of CHD remains particularly challenging due to genetic heterogeneity and incomplete penetrance.<sup>3</sup> Also extremely complex is the differential diagnosis of CHD in that it is a multifactorial disease encompassing both genetic predisposition and environmental components.<sup>4</sup> Thus, it is vitally important to identify disease-causing genetic variants.<sup>5</sup> Some CHD-associated genes encode transcription factors such as *GATA4*, *NKX2-5*, and *TBX5*, and a number of gene variants identified in these genes have been associated with cardiac structure and functional impairment.<sup>1</sup> *GATA*-binding factor 4 (*GATA4*)

(OMIM: 600576) is one of the 6-member *GATA* family of transcription factors: *GATA1*, *GATA2*, *GATA3*, *GATA4*, *GATA5*, and *GATA6*. Amongst *GATA*-binding proteins, *GATA1-3* are expressed in hematopoietic stem cells as significant regulators, whereas *GATA4-6* are expressed in different mesoderm- and endoderm-derived tissues such as the heart, the lung, the gonad, the gut, and the liver.<sup>6</sup> Variants in the *GATA4*, *GATA5*, and *GATA6* genes have been found in patients with various types of CHD.<sup>7-9</sup> *GATA* proteins comprise 2 conserved zinc finger domains (ZNI and ZNII), which cover various aspects of functions including DNA attachment, *GATA4* preservation, and protein-protein and the target DNA sequence interactions. The *GATA4* gene consists of 7 exons located on chromosome 8p23.1-p22. The gene encodes one of the earliest-expressed transcription factors with 442 amino acids and is imperative for normal cardiogenesis. *GATA4* is significantly expressed in embryonic development, with the expression continuing in the adult myocardium.<sup>10-12</sup> A rise has been reported in the number of patients with CHD who reach adulthood.<sup>13</sup> This transcription factor



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contains 2 transcriptional activation domains (TAD1 and TAD2); 2 zinc finger domains: 1 at the c-terminal region (CZF) and the other at the n-terminal region (NZF); and 1 nuclear localization signal domain (NLS).<sup>14</sup> Variants in the *GATA4* gene are highly associated with different types of CHD,<sup>15</sup> including tetralogy of Fallot, ventricular septal defect, atrial septal defect, atrioventricular septal defect, patent ductus arteriosus, dilated cardiomyopathy, and pulmonary valve stenosis.<sup>14,16-21</sup>

The current literature lacks *in silico* analysis on the variants of the *GATA4* transcription factor and their critical role in the different levels of cardiovascular development. Accordingly, for the first time, we aimed to conduct a comprehensive *in silico* analysis of the effects of *GATA4* alterations associated with CHD.

### Materials and Methods

For the detection of genetic variants in the *GATA4* gene, the following methodology was utilized in the present study:

#### Data Collection

The amino acid sequence of the human *GATA4* gene was obtained from the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>), based on the human genome assembly GRCh37. Accordingly, the Human Gene Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk/ac/index.php>), as a strongly reliable database, was employed to identify alterations in the *GATA4* gene.<sup>22</sup> Concurrently, all pathogenic/likely pathogenic alterations reported in public access databases were identified. The databases were ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>),<sup>23</sup> dbSNP (the NCBI database of genetic variation; <https://www.ncbi.nlm.nih.gov/snp/>), GeneCards (the human gene database; <https://www.genecards.org/>),<sup>24</sup> ExAC (the exome aggregation consortium; <http://exac.broadinstitute.org/>),<sup>25</sup> the 1000 Genomes Project (<https://www.internationalgenome.org/>),<sup>26</sup> gnomAD (the Genome Aggregation Database; <http://gnomad.broadinstitute.org/>),<sup>27</sup> GO-ESP (NHLBI "Grand Opportunity" Exome Sequencing Project; <http://evs.gs.washington.edu/EVS/>),<sup>28</sup> TOPMed (Trans-Omics for Precision Medicine; <https://www.nhlbiwgs.org/>),<sup>29</sup> Iranome (<http://www.iranome.ir/>),<sup>30</sup> and the Greater Middle East (GME) Variome Project (<http://igm.ucsd.edu/gme/>).<sup>31</sup> Moreover, extensive research was carried out through computerized search of PubMed, Scopus, Google Scholar, ScienceDirect, MalaCards (the human disease database), and ResearchGate databases by using the following terms: *GATA4* variants, the clinical importance of the *GATA4* gene, *GATA4*-related disorders, CHD, the pathophysiology of CHD, and the incidence of CHD.

#### Frequency

The frequencies of the selected variants were determined using the aforementioned databases. Furthermore, the

number of participants and individuals having variations in the studied populations was reported.

### Computational Methods

Given its increasing importance and use to determine the possible effects of genetic variants, computational analysis was employed in the present study. The variants of the *GATA4* gene and their correlations with the molecular pathogenesis of CHD were further explored by predicting the pathogenicity/tolerance of the variants through the following bioinformatics tools: SIFT (Sorting Intolerant from Tolerant; [https://sift.bii.a-star.edu.sg/www/SIFT\\_seq\\_submit2.html](https://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html)),<sup>32</sup> PolyPhen-2 (Polymorphism Phenotyping, version 2; <http://genetics.bwh.harvard.edu/pph2/>),<sup>33</sup> PROVEAN (Protein Variation Effect Analyzer, version 1.1.3; [http://provean.jcvi.org/seq\\_submit.php](http://provean.jcvi.org/seq_submit.php)),<sup>34</sup> CADD (Combined Annotation-Dependent Depletion; <https://cadd.gs.washington.edu/>),<sup>35</sup> MutationTaster (<http://www.mutationtaster.org/>),<sup>36</sup> and GERP (Genomic Evolutionary Rate Profiling; <http://mendel.stanford.edu/SidowLab/downloads/gerp/>).<sup>37</sup> All these bioinformatics tools are capable of distinguishing pathogenic from nonpathogenic alterations. Protein sequences in the FASTA format (NM\_002052.5), the positions and substitutions of amino acids, and the positions of chromosomes were used as input data. A SIFT score of 0.05 or less is regarded as deleterious, and a SIFT score of greater than 0.05 is considered to signify a tolerated variant.<sup>32</sup> PolyPhen-2 results are shown with qualitative levels as benign, possibly damaging, and probably damaging. PolyPhen-2 prediction outputs have a numerical score range of 0 to 1. The cutoff score considered for PolyPhen-2 is 0.5, and variants with scores equal to or greater than 0.5 are predicted to be deleterious.<sup>33,38</sup> The cutoff score for PROVEAN is -2.5, and variants equal to or greater than -2.5 are assigned as deleterious.<sup>34</sup> Also calculated in the current investigation was the CADD score. All genomic features used to calculate the CADD score via a machine-learning model are summarized into a Phred score with a cutoff point of 20. Disease-causing variants display a high Phred score (>20), whereas a low score (<20) signifies less pathogenicity.<sup>35,39</sup> MutationTaster, which was applied for all the detected variants in the present study, considers an alteration to be a polymorphism if it is reported as a single-nucleotide polymorphism (SNP) in the HapMap data and the 1000 Genomes Project. Thus, any alteration that could result in premature termination codon and ultimately lead to nonsense-mediated mRNA decay is considered a disease-causing variant. GERP is an evolutionary measurement tool whose results are based on multi-species sequence alignment by comparison with neutral expectation. GERP scores show a reduction in the number of substitutions. Positive scores indicate a substitution deficit, while negative scores show that a site is probably evolving neutrally.<sup>40</sup>

### GATA4 Network Analysis

The functional association between 2 proteins is the primary purpose of the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database. This web-based tool expresses the interaction of proteins in a particular biological function.<sup>41</sup> STRING (version 11.0; <https://string-db.org/>) is used to recognize the known and predicted interactions between the GATA4 protein and other related proteins in a cell.<sup>42</sup>

### Prediction of Normal and Mutant Protein Structures

Structural and functional differences between wild-type and mutated GATA4 were anticipated by using HOPE (Have [y]Our Protein Explained; <https://www3.cmbi.umcn.nl/hope/input/>) and<sup>43</sup> I-TASSER (Iterative Threading ASSEmblY Refinement; <https://zhanglab.ccmb.med.umich.edu/I-TASSER/>).<sup>44-46</sup> The objective was to analyze a pathogenic variant with a high CADD score. HOPE shows the 3D structural and functional effects of a point mutation in human proteins. The input for this tool is the amino acid sequence of the GATA4 protein and the specific amino acid alteration of the variant.<sup>43</sup> The I-TASSER server predicts secondary structures and 3D models through various alignment methods. The accuracy of the formed models is evaluated based on a confidence score (C-score). Predicted models with a C-score of greater than -1.5 are considered to possess a correct topology. I-TASSER predicts the template modeling score (Tm-score) and the root mean square deviation (RMSD). The TM-score ranges between 0 and 1, with higher values specifying better structural models.<sup>47</sup>

### Phylogenetic Analysis

GATA4 protein sequences from 5 different organisms, namely *Homo sapiens* (humans), *Canis lupus familiaris* (dogs), *Rattus norvegicus* (rats), *Gallus gallus domesticus* (chickens), and *Xenopus laevis* (African clawed frogs), were retrieved from UniProt (the Universal Protein Resource; <https://www.uniprot.org/>). Afterward, all the GATA4 protein sequences were aligned via the multiple sequence alignment program ClustalW (version 1.83; <https://www.genome.jp/tools-bin/clustalw>). Thereafter, a phylogenetic tree was built by using ClustalW via the neighbor-joining method. As a result of the multiple sequence alignment, the tree showed scores that represented a sequence distance measure. These values determine the length of the branches, with the length showing the distance between the sequences.

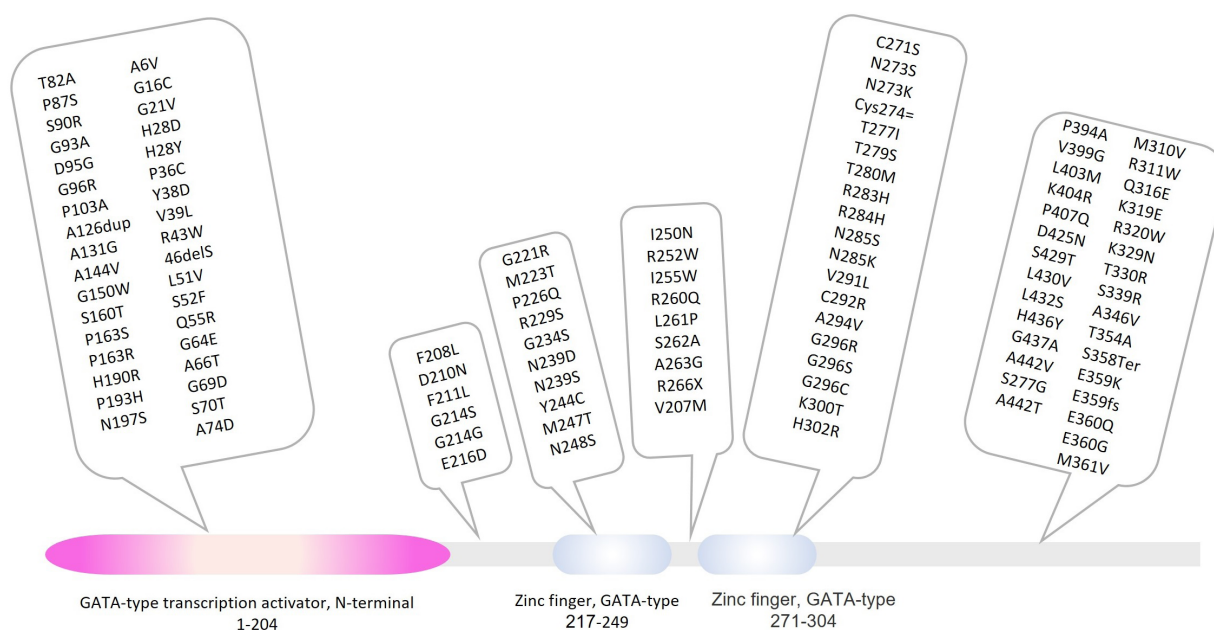
### Results

#### Literature Analysis

Using online databases and publications, we succeeded in finding 110 reported variations in the GATA4 gene. We also determined the frequency of the gene variants from online resources. The data are depicted in Table 1. The distributions of the reported variants in the different regions of the GATA4 gene are presented in Figure 1.

#### Frequency of the Variants

A wide range of GATA4 variants has been reported in different countries such as Japan, Australia, the United States, Brazil, Egypt, India, Germany, Lebanon, France, Iran, Italy, and especially China. Precise data on the reported variations and the phenotype condition of the



**Figure 1.** The image shows the distribution of the variations of the GATA4 gene. The figure demonstrates the 3 main parts of the GATA4 protein, the GATA-type transcription activator, and the zinc finger regions. The numbers beneath the protein structure represent the number of amino acids in the GATA4 amino acid sequence. The reported variations are categorized based on their locations.

**Table 1.** Reported frequency of the variants in online databases

DNA Change	Genomic Placement on Chromosome 8	1000Genome	ExAC	GnomAD	GO-ESP	TOPMED	Iranome	GME
c.17C>T	11565838	0.0002	0.00002	0.00003	-	-	-	-
c.46G>T	1156586	0.0002	-	0.00003	-	0.000016	-	-
c.62G>T	11565883	0.0002	0.00004	0.00002588	-	0.000024	-	-
c.82C>G	11565903	-	-	-	-	0.000008	-	-
c.82C>T	11565903	-	-	-	-	0.000008	-	-
c.106C>T	11565927	-	-	-	-	-	-	-
c.112T>G	11565933	-	-	-	-	-	-	-
c.115G>T	11565936	-	0.000007541	0.000007541	-	-	-	-
c.127C>T	11565948	-	0	0.00006	-	0.000056	-	-
c.136-138delTCC	11565957	-	-	-	-	-	-	-
c.151C>G	11565972	-	-	-	-	-	-	-
c.155C>T	11565976	-	-	-	-	-	-	-
c.164A>G	11565985	-	-	-	-	-	-	-
c.191G>A	11566012	-	-	-	-	0.000008	-	-
c.196G>A	11566017	-	-	-	-	0.000032	-	-
c.206G>A	11566027	-	-	-	-	-	-	-
c.209G>C	11566030	-	-	-	-	-	-	-
c.221C>A	11566042	-	0.00003625	0.00003625	-	0.000032	-	-
c.244A>G	11566065	-	-	0.00006	-	0.000032	-	-
c.259C>T	11566080	-	-	-	-	-	-	-
c.270C>A	11566091	-	-	-	-	-	-	-
c.278G>C	11566099	-	-	-	-	0.000016	-	-
c.284A>G	11566105	-	-	-	-	-	-	-
c.286G>A	11566107	-	-	-	-	-	-	-
c.307C>G	11566128	-	-	-	-	-	-	-
c.357_359CGC	11566175-11566176	-	-	-	-	-	-	-
c.392C>G	11566213	-	-	0.00003	-	0.000303	-	-
c.431C>T	11566252	-	-	0.0001441	-	0.000016	-	-
c.448G>T	11566269	-	-	-	-	-	-	-
c.479G>C	11566300	-	-	-	-	-	-	-
c.487C>T	11566308	0.0002	0.0002	0.00003	-	0.000175	-	-
c.488C>G	11566309	-	0.0002	0.00003	-	0.000008	-	-
c.569A>G	11566390	-	-	-	-	-	-	-
c.578C>A	11566399	-	-	-	-	-	-	-
c.590A>G	11566411	-	-	-	-	-	-	-
c.620C>T	11561728	0.2017	-	0.16213	-	0.174583	-	-
c.622T>C	11606433	-	-	-	-	-	-	-
c.628G>A	11606439	-	0.00003	-	0.00008	0.000008	-	-
c.631T>C	11606442	-	-	-	-	-	-	-
c.640G>A	11606451	-	-	-	-	-	-	-
c.640G>A	11606451	-	-	-	-	-	-	-
c.648C>G	11606459	-	-	-	-	-	-	-
c.661G>A	11606472	-	-	-	-	-	-	-
c.668 T>C	11606479	-	-	-	-	-	-	-
c.677C>A	11606488	-	-	-	-	-	-	-
c.687G>T	11606498	-	-	-	-	-	-	-

Table 1. Continued.

c.700G>A	11606511	-	-	-	-	-	-	-
c.715A>G	11606526	-	-	-	-	-	-	-
c.716A>G	11606527	-	-	-	-	-	-	-
c.731A>G	11606542	-	-	-	-	-	-	-
c.740T>C	11606551	-	-	-	-	-	-	-
c.743A>G	11606554	-	0.000016	0.000008	-	-	-	-
c.749T>A	11606560	-	-	-	-	-	-	-
c.754C>T	11606565	-	-	-	-	-	-	-
c.764T>C	11606575	-	-	-	-	-	-	-
c.779G>A	11606590	-	-	0.00003	-	0.000008	-	-
c.782T>C	11606593	-	-	-	-	-	-	-
c.783T>G	11606594	-	-	-	-	-	-	-
c.788C>G	11607624	-	-	-	-	-	-	-
c.796C>T	11607632	-	-	-	-	-	-	-
c.799G>A	11607635	0.0004	0.000306	0.00029	-	0.000231	-	-
c.812G>C	11607648	-	-	-	-	-	-	-
c.818A>G	11607654	-	-	0.00003	-	-	-	-
c.819C>A	11607655	-	-	-	-	-	-	-
c.822C>T	11607658	0.0018	0.002243	0.00258	0.00308	0.003297	-	-
c.830 C>T	11607666	-	-	0.000004	-	0.000008	-	-
c.835A>T	11607671	-	-	-	-	-	-	-
c.839C>T	11607675	-	-	-	-	-	-	-
c.848G>A	11607684	-	-	-	-	-	-	-
c.851G>A	11607687	-	-	-	-	-	-	-
c.854A>G	11607690	-	-	-	-	-	-	-
c.855T>C	11607691	-	-	-	-	-	-	-
c.871G>C	11607707	-	-	-	-	-	-	-
c.874T>C	11607710	-	-	-	-	-	-	-
c.881C>T	11607717	-	-	-	-	-	-	-
c.886G>C	11607722	-	-	-	-	-	-	-
c.886G>A	11607722	-	-	-	-	-	-	-
c.886G>T	11607722	-	-	-	-	-	-	-
c.899A>C	11607735	-	-	-	-	-	-	-
c.905A>G	11607741	-	-	-	-	-	-	-
c.928A>G	11612573	-	-	-	-	0.000008	-	-
c.931C>T	11612576	-	-	-	-	-	-	-
c.946C>G	11612591	-	0.000008	-	-	-	-	-
c.955A>G	11612600	-	-	-	-	-	-	-
c.958C>T	11612603	-	-	-	-	0.000008	-	-
c.989C>G	11612634	-	-	-	-	-	-	-
c.1017C>A	11614463	-	-	-	-	-	-	-
c.1037C>T	11614483	0.00060	0.00178	0.00124	0.00238	0.00149	-	-
c.1060G>A	11614503	-	-	-	-	-	-	-
c.1074delC	11614520	-	-	-	-	-	-	-
c.1075delG	11614521	-	-	-	-	-	-	-
c.1078G>C	11614524	-	0.000074	-	0.00008	0.000135	-	-
c.1079A>G	11614525	-	-	-	-	-	-	-



Table 1. Continued.

c.1081A>G	11614527	-	-	-	-	-	-	-
c.1129A>G	11614575	0.04293	0.09621	0.10632	0.10057	0.08186	-	0.133198
c.1180C>A	11615835	0.0064	0.002522	0.00003	-	0.000080	0.0025	0.003524
c.1196T>G	11615851	-	-	-	-	-	-	-
c.1207C>A	11615862	-	-	-	-	-	-	-
c.1211A>G	11615866	-	-	-	-	-	-	-
c.1220C>A	11615875	0.0012	-	0.00010	0.00113	0.000247	0.000625	0.00503
c.1273G>A	11615928	0.0034	0.002117	0.00003	-	0.000239	0.01188	0.002517
c.1286G>C	11615941	-	-	-	-	-	-	-
c.1288C>G	11615943	-	-	-	-	-	-	-
c.1295T>C	11615950	-	-	-	-	-	-	-
c.1306C>T	11615961	-	-	-	-	-	-	-
c.1310G>C	11615965	-	-	-	-	-	-	-
c.1324G>A	11615979	-	-	-	-	0.000008	-	-
c.1325C>T	11615980	-	-	-	0.00008	0.000104	-	-

individuals studied in different countries are depicted in Table 2. Genetic alterations in c.1129A>G were reported in 3 countries: China (0.33%), Germany (23%), and Australia (19.04%), with the highest frequency in Germany. Additionally, c.874T>C (45.58%), which was reported in Germany, represented the highest frequency among all the reported variations.

### Bioinformatics

The results of the identification and analysis of the variations via online prediction tools are shown in Table 3. Out of the 110 substitutions identified, PROVEAN predicted 55 variations to be deleterious and 50 variations to be neutral. (Five variations were not available.) SIFT predicted 62 alterations to be damaging and 33 variations to be tolerated. (Fourteen variations were not available.) PolyPhen-2 defined 25 variations as benign, 18 as possibly damaging, and 59 as probably damaging. (Eight variations were not available.) MutationTaster predicted 82 disease-causing variations and 11 polymorphisms. (Seventeen variations were not available.) The maximum CADD score (Phred score = 53) was shown by c.796C>T R266X, indicating high pathogenicity, while c.196G>A, A66T showed the lowest CADD score (Phred score = 0.009). As a result, among the 110 substitutions, 38 were predicted to be deleterious by PROVEAN, SIFT, PolyPhen-2, and MutationTaster.

In this study, c.1075G>A indicated the highest GERP score (5.83), which represents 4.83 fewer substitutions than was expected. No negative GERP scores were reported for these variations.

### Protein-Protein Interaction Network Analysis

As is illustrated in Figure 2, STRING, version 11.0, demonstrated that 11 proteins (GATA4, NKX2-5, MEF2C, ZFPM2, TBX5, BMP4, SRF, BMP2, HAND2, NPPA,

and HEY2) and 41 edges (protein-protein associations) grouped to create a protein network.

### Differences Between the Wild-Type GATA4 Protein and the Mutant Model

In this study, the effects of the predicted disease-causing p.Gly221Arg variant in GATA4 with the CADD Phred score of 31 were further analyzed. The variant, p.Gly221Arg, with a high level of pathogenicity is a heterozygous missense variant in the conserved N-terminal zinc finger of GATA4.<sup>74</sup> HOPE results showed the alteration of glycine to arginine at position 221 (G221R, CADD Phred = 31). The size, charge, and hydrophobicity value of the 2 residues, as well as the differences between them, are presented in Figure 3A. The mutant residue showed a larger size, with a positive charge, while the wild-type protein charge was neutral. Furthermore, arginine was more hydrophobic than was glycine. These differences in amino acid features could affect the zinc finger site of the protein and its function. Accordingly, this change in the GATA4 sequence might result in the conformation of the protein and exert negative influences on the structure of the protein in this specific residue (Figure 3B). I-TASSER produced 3D structures of GATA4 in 5 models with different C-scores. A model with a C-score of -0.5, an estimated TM-score of 0.65, and an estimated RMSD of 8.2 Å was selected. Hence, the findings proved that the solubility of the mutant protein was similar to that of the wild-type one, with a score of 3 (Figure 3C).

### GATA4 Protein Sequence Alignment and the Phylogenetic Tree

According to the phylogenetic tree generated by ClustalW, the human GATA4 protein had the closest homology with that of *Canis lupus familiaris* (dogs). Further, the most distant orthologue was *Xenopus laevis* (African clawed

**Table 2.** Frequency of the variants in different populations

DNA Change	Condition <sup>1</sup>	Population (Frequency)	CHD Type	References
c.17C>T	-	China (0.2%)	VSD	48
c.46G>T	-	China (0.62%)	AF	49
c.62G>T	Uncertain significance	China (1%)	ASD	50, 51
c.82C>G	-	China (0.62%)	AF	49
c.82C>T	-	China (2%)	VSD	52
c.106C>T	-	China (0.45%)	ASD	53
c.112T>G	-	China (0.66%)	AF	10
c.115G>T	-	China (0.45%)	DCM	11
c.127C>T	Uncertain significance	China (0.62%)	VSD	54
c.136-138delTCC	-	China (0.2%)	VSD	48
c.151C>G	-	China (1.92%)	TOF	55
c.155C>T	Pathogenic	Japan (6.25%)	ASD	56
c.164A>G	-	China (0.43%)	VSD	57
c.191G>A	-	China (0.38%)	VSD, CTD	58, 59
c.196G>A	-	China (0.29%)	VSD, PDA, TOF	59-61
c.206G>A	-	Australia (0.28%)	VSD	62
c.209G>C	-	China (0.76%)	AF	63
c.221C>A	-	China (0.26%)	PS	61
c.259C>T	-	China (0.55%)	ASD	50
c.270C>A	-	China (0.83%)	CHD	64
c.278G>C	-	America (0.15%)	ASD	65
c.284A>G	-	China (0.83%)	CHD	64
c.286G>A	-	China (0.43%)	VSD	57
c.307C>G	-	China (0.66%)	AF	10
c.357-359CGC	Pathogenic	China (0.2%)	VSD	48
c.392C>G	Uncertain significance	Brazil (3.12%)	AVSD	66
c.431C>T	-	Japan (0.9%)	PA, ASD	67
C.448G>T	-	China (0.26%)	TOF	61
c.479G>C	-	China (0.76%)	AF	63
c.487C>T	Pathogenic; Uncertain significance	China (0.31%) America (0.93%)	AVSD, VSD, SA+SV, TOF, TGA VSD, PS, TOF, VSD	48, 59, 61, 68, 69
c.488C>G	Uncertain significance	Australia (0.28%)	VSD	62
c.569A>G	-	China (0.45%)	ASD	53
c.578C>A	-	Egypt (9.09%)	VSD, VSD, ASD	70
c.590A>G	-	China (0.43%)	VSD	57
c.620C>T	-	India (3%)	ASD, VSD	71
c.622T>C	-	Germany (1.47%)	VSD	72
c.628G>A	Uncertain significance	China (0.26%)	AVSD	61
c.631T>C	-	Germany (2.9%)	VSD, AVSD	72
c.640G>A	-	Germany (1.47%)	VSD	72
c.640G>A	-	India (1%)	ASD	71
c.648C>G	-	Lebanon (1.66%)	TOF	73
c.661G>A	Pathogenic	France (family-based)	CHD	74
c.668T>C	-	Germany (1.47%)	VSD	72
c.677C>A	-	China (0.45%)	DCM	11
c.687G>T	-	Germany (4.41%)	VSD	72
c.700G>A	-	Germany (1.47%)	AVSD	72

Table 2. Continued.

c.715A<G	-	Germany (1.47%)	VSD	72
c.716A>G	-	Germany (1.47%)	VSD	72
c.731A>G	-	Germany (2.94%)	VSD	72
c.740T>C	Uncertain significance	Germany (1.04%)	AF	75
c.743A>G	-	Germany (2.94%)	ASD, AVSD	72
c.749T>A	-	China (0.26%)	VSD	61
c.754C>T	-	Germany (1.47%)	AVSD	72
c.764T>C	-	Germany (1.47%)	ASD	72
c.779G>A	-	Germany (1.47%)	VSD	72
c.782T>C	-	Germany (2.94%)	VSD, ASD	72
c.783T>G	-	China (0.45%)	ASD	53
c.788C>G	-	China (0.44%)	VSD	76
c.796C>T	-	Germany (2.94%)	ASD, AVSD	72
c.799G>A	-	China (0.58%)	ASD, CTD	59, 77
c.812G>C	-	China (0.9%)	DCM	78
c.818 A>G	-	Germany (1.47%)	AVSD	72
c.819C>A	-	Iran (1)	VSD, ASD	79
c.822C>T	Benign; Likely benign; Uncertain significance	Germany (0.97%) Australia (0.28%)	ASD, ASD, DCM, TOF, VSD	62, 80
c.830C>T	-	Germany (1.47%)	AVSD	72
c.835A>T	-	China (0.45%)	DCM	11
c.839C>T	Uncertain significance	China (13.33%)	AVSD, ASD	81
c.848G>A	-	Germany (1.47%)	AVSD	72
c.851G>A	-	France (0.3%)	ASD	82
c.854A>G	-	China (1.92%)	TOF	55
c.855T>C	-	Germany (1.47%)	AVSD	72
c.871G>C	-	China (0.66%)	DCM	82
c.874T>C	-	Germany (45.58%)	ASD,VSD, AVSD	72
c.881C>T	-	Germany (1.47%) Iran(1)	ASD, CHD	72, 79
c.886G>C	Pathogenic	China (0.47%)	VSD	83
c.886G>A	Pathogenic	America(Family-based) Italy(family-based)	ASD, PVS	84, 85
c.886G>T	Pathogenic	America (0.93%)	ASD	68
c.899A>C	-	China (Family-based)	ASD	14
c.905A>G	-	Germany (1.47%)	AVSD	72
c.928A>G	Pathogenic	China (Family-based)	ASD	86
c.931C>T	-	China(Family-based)	TOF, VSD, ASD, PDA	87
c.946C>G	Pathogenic	America (0.31%)	ASD	65
c.958C>T	Likely pathogenic; Uncertain significance	Italy (family-based)	ASD	88
c.989C>G	Uncertain significant	Japan (0.39%)	PTA,ASD	89
c.1017C>A	-	Japan (0.39%)	PA,VSD	89
c.1037C>T	-	America (0.93%)	ASD	68
c.1060G>A	-	China (1.17%)	ASD	77
c.1074delC	-	Japan(family-based)	ASD	68
c.1075G>A	Pathogenic	China (0.41%)	VSD	48
c.1075delG	Pathogenic	Japan(family-based)	ASD	56
c.1079A>G	-	China (0.26%)	VSD	61
c.1081A>G	-	Germany (1.47%)	VSD	90



Table 2. Continued.

c.1129A>G	-	China (0.33%) Germany (23.9%) Australia (19.04%) Iran (45%)	ASD, VSD, AVSD, TOF, PA	17, 59, 62, 80, 91
c.1180C>A	-	India (1.62%)	AVSD,VSD	92
c.1196T>G	-	China (0.45%)	VSD	53
c.1207C>A	-	America (0.93%)	ASD	68
c.1211A>G	-	China (0.43%)	VSD	57
c.1220C>A	Benign; Uncertain significance	China (0.59%) Iran (Family-based)	ASD, AVSD, VSD, TOF, VSD	17, 48, 59, 69, 77, 93
c.1273G>A	Uncertain significance, Pathogenic	America (0.16%)	PA, PS, ASD, TOF, AVSD	65
c.1286G>C	-	China (0.2%)	VSD	48
c.1288C>G	-	Germany (2.94%)	ASD	90
c.1295T>C	-	India (0.32%)	PDA	92
c.1306C>T	-	China (8%)	CSDs	60
c.1310G>C	-	America (1.28%)	BAV	94
c.1324G>A	-	Germany (1.47%)	VSD	90
c.1325C>T	Pathogenic	China (0.34%)	VSD	48

Abbreviation: AVSD, atrioventricular septal defect; ASD, atrial septal defects; CDH, congenital diaphragmatic hernia; CTD, conotruncal heart defects; CHD, congenital heart disease; CSDS, cardiac septal defects; DCM, dilated cardiomyopathy; DORV, double-outlet right ventricle; DILV, double-inlet left ventricle; LVHT, left ventricular hypertrabeculation; LVNC, left ventricular noncompaction; PA, pulmonary atresia; PA + IVS, pulmonary atresia with interventricular septum; PVS, pulmonary valve stenosis; SA+SV, single atrium with single ventricle; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; TGA, transposition of the great arteries; VSD, ventricular septal defect; PTA, persistent truncus arteriosus; BAV, bicuspid aortic valve; AF, atrial fibrillation; PDA, patent ductus arteriosus; PS, pulmonary stenosis

<sup>1</sup> According to ClinVar

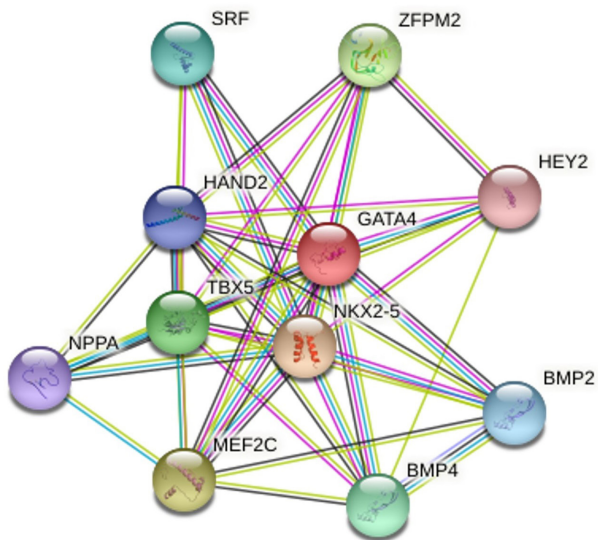
frogs) (Figure 4A). The results of the multiple-alignment sequencing of the species are illustrated in Figure 4B.

## Discussion

CHD is the most frequent congenital abnormality and the major cause of infant mortality the world over. *GATA4*, a transcription factor with 2 zinc finger domains, has been reported to play an essential role in embryogenesis and cardiac development.<sup>90</sup> The *GATA4* gene is reported to modulate heart hypertrophy in adults.<sup>95</sup> The number of studies seeking to explicate the correlation between *GATA4* variants and CHD occurrence is on the rise. Indeed, recent studies have identified several novel variants in the *GATA4* gene with potential roles in CHD development.<sup>17</sup>

CHD is very heterogeneous, and the etiology of the majority of cases remains greatly unknown. Both genetic and environmental factors contribute to CHD.<sup>96</sup> Therefore, the elucidation of the pathogenesis and differential diagnosis of the disease requires the identification of not only the disease-causing or susceptibility genes but also new genetic variants associated with the different types of CHD. Research has linked several genes to CHD, with *NKX2-5*, *TBX5*, and *GATA4* comprising the most studied transcription factor genes.<sup>15</sup> These genes interact during embryonic development, and they are involved in the regulation of cardiogenesis and embryonic heart development.<sup>97</sup> Protein-protein interactions between transcription factors play a vital role in biological systems. The results concerning *GATA4* protein interactions,

generated by STRING, showed that 11 proteins (*GATA4*, *NKX2-5*, *MEF2C*, *ZFPM2*, *TBX5*, *BMP4*, *SRF*, *BMP2*, *HAND2*, *NPPA*, and *HEY2*) grouped in a network. *GATA4* and *NKX2-5* transcription factors are critical to cardiomyocyte hypertrophy; thus, single-point variants could create an imbalance in the interaction between these proteins.<sup>12</sup> *GATA4* has been shown to interact with *HAND2* to modulate the transcription of the downstream gene by binding to the conserved GATA-binding sites of the *HAND2* promoter.<sup>98</sup> *NKX2-5*, as a central regulator of many aspects of heart development, interacts with *SRF* and *GATA4* to promote the expression of the cardiac sarcomeric protein gene.<sup>99</sup> Mutations in the *ZFPM2* gene, which encodes the FOG2 protein (a transcription regulator of the GATA family members), disrupt the interaction with *GATA4* or the nucleosome remodeling and deacetylation (NuRD) complex and, thus, lead to CHD.<sup>100-103</sup> Loss-of-function mutation in the *MEF2C* gene, which encodes a transcription factor required for normal cardiovascular development, is associated with increased vulnerability to CHD in humans.<sup>104</sup> *MEF2C*, *TBX5*, and *GATA4* can induce cardiomyocyte differentiation and directly reprogram endogenous cardiac fibroblasts into functional cardiomyocytes.<sup>105</sup> Remarkably, *BMP2* and *BMP4* are vital for cardiogenesis in that they induce the expression of *NKX2-5* and *GATA4* transcription factors. These 2 genes play a significant role during the initial induction of cardiogenesis. Nevertheless, no association between *BMP2* and *BMP4* genetic variations (rs1049007, rs235768, and rs17563) and the risk of CHD



**Figure 2:** The image presents the STRING protein-protein interaction analysis. The network contains 11 nodes and 41 edges. The edges are represented with various colors, with each color indicating protein-protein associations. The GATA4 protein and its functional interactions with 11 other proteins display possible effects on each other.

*NKX2-5*: Homeobox protein *NKX2-5*, *MEF2C*: Myocyte-specific enhancer factor 2C, *ZFP2*: Zinc finger protein *ZFP2*, *TBX5*: T-box transcription factor, *BMP4*: Bone morphogenetic protein 4, *SRF*: Serum response factor, *BMP2*: Bone morphogenetic protein 2, *HAND2*: Heart- and neural crest derivatives-expressed protein 2, *NPPA*: Natriuretic peptides A, *HEY2*: Hairy/enhancer-of-split related with YRPW motif protein 2.

was reported by Li FF et al.<sup>106</sup> Variations in the *NPPA* gene, which encodes the ANP precursor, are correlated with hypertension, stroke, coronary artery disease, and heart failure.<sup>107</sup> The *HEY2* transcription factor plays an important function in mammalian heart development.

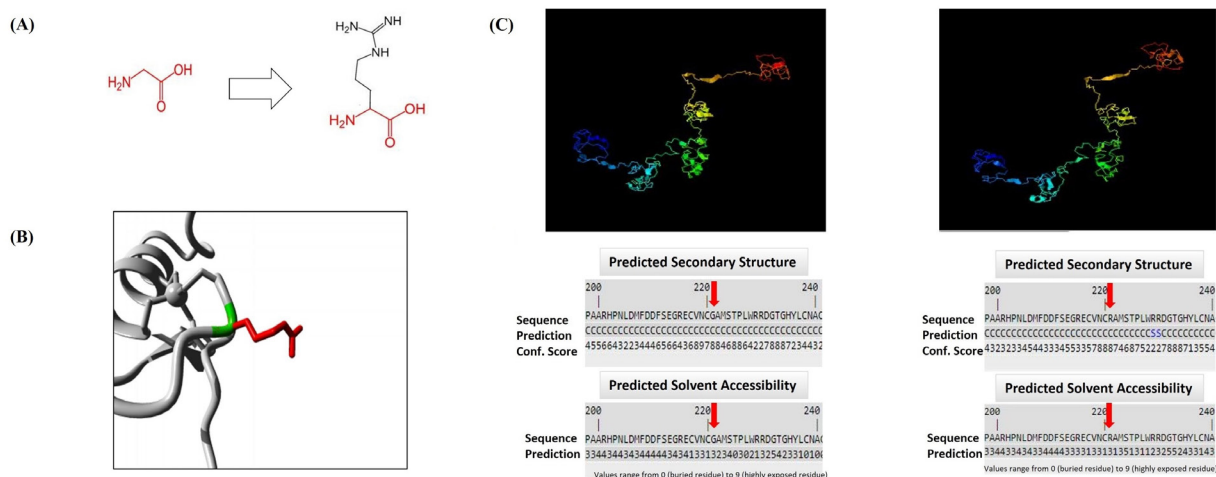
Three non-synonymous variations, namely c.286A>G (p.Thr96Ala), c.293A>C (p.Asp98Ala), and c.299T>C (p.Leu100Ser), were reported to affect the second helix of *HEY2* in the diseased cardiac tissues of 2 cases with atrioventricular septal defect, suggesting its possible function in the regulation of ventricular septation in humans.<sup>108</sup> Somatic mutations were identified in *NKX2-5* and its molecular partners, *TBX5* and *GATA4*, as well as the transcription factor *HEY2*, in formalin-fixed tissues taken from a collection of hearts with atrial septal defect,<sup>109</sup> ventricular septal defect, and atrioventricular canal defect.<sup>90, 108, 110-112</sup>

The *GATA4* missense variation (p.G221R), on which we focused in the present study, was identified in three 46, XY DSD patients from a family of French origin. The in vitro assays in that investigation demonstrated the failure of the p.G221R mutant protein to bind to *FOG2*, which is required for gonad formation. Furthermore, the mutant protein failed to transactivate the anti-Müllerian hormone promoter.<sup>74</sup>

Some variants of *GATA4* investigated in the present study have been previously analyzed for genotype-phenotype correlations. These investigations evaluated families manifesting those variations associated with different CHD types.

Lourenço D et al.<sup>74</sup> reported the G221R variant in 5 members of a family with cardiac anomalies including atrial septal defect, tetralogy of Fallot, and congenital cyanotic heart disease.

In a study conducted by Garg V et al.,<sup>84</sup> the c.886G>A (G296S) variation of *GATA4* was stated in 13 affected members with atrial septal defect in a family with 5 generations. The authors also reported the E359del variation of *GATA4* in 5 members of another family with



**Figure 3:** A) The image presents the schematic structure of a normal amino acid on the left (glycine) and a mutant one on the right (arginine) at position 221 of the *GATA4* protein. The red parts show the similar parts of the amino acids (the backbone), and the black part shows the unique part of the amino acids (the side chain). This picture illustrates the structural differences between the 2 amino acids. The G221R alteration is shown by HOPE. B) A photograph generated by HOPE shows that the G221R variation affects the structure of the *GATA4* protein. The green color shows the wild-type residue (glycine), and the red color represents the mutant residue (arginine). C) I-TASSER shows the secondary and 3D structure, as well as the predicted solvent accessibility, of the normal (left) and G221R mutant (right) of the *GATA4* protein.

Table 3. In silico analysis of GATA4 variations

DNA Change	Protein Change	Variant Type	dbSNP	HGMD	CADD <sup>1</sup>	MutationTaster	PolyPhen <sup>2</sup> (Score)	PROVEAN <sup>3</sup>	SIFT <sup>4</sup> (Score)	GERP
c.17C>T	A6V	Missense	rs199922907	CM086821	24.48	DC	PRD (0.986)	NE	DE (0.01)	NA
c.46G>T	G16C	Missense	rs533331682	CM117802	23	POLYMORPHISM	PRD (1.000)	NE	TO (0.1)	NA
c.62G>T	G21V	Missense	rs202213149	CM107596	24.4	DC	PRD (0.972)	NE	DE (0.02)	NA
c.82C>G	H28D	Missense	rs1406275331	CM117803	25	DC	PRD (0.993)	DE	DE (0)	NA
c.82C>T	H28Y	Missense	rs1406275331	CM0910178	24	DC	PRD (0.993)	DE	DE (0)	NA
c.106C>T	P36C	Missense	-	CM1313746	25.2	DC	PRD (1)	DE	DE (0)	NA
c.112T>G	Y38D	Missense	-	CM123513	26	DC	PRD (0.998)	DE	DE (0)	NA
c.115G>T	V39L	Missense	rs1139241	CM147377	24	DC	PRD (0.958)	NE	DE (0)	NA
c.127C>T	R43W	Missense	rs387906770	CM119519	25	DC	PRD (1)	DE	DE (0)	NA
c.136 e138delITCC	46delS	deletion	-	-	-	-	-	NE	-	NA
c.151C>G	L51V	Missense	-	CM1312064	23.6	DC	PRD (0.977)	NE	DE (0.01)	NA
c.155C>T	S52F	Missense	rs104894074	CM1312064	25.6	DC	PRD (0.975)	DE	DE (0)	NA
c.164A>G	Q55R	Missense	-	CM125062	22.9	DC	POD (0.586)	NE	DE (0.01)	NA
c.191G>A	G64E	Missense	rs1249347695	CM107237	11.21	POLYMORPHISM	BENIGN (0.392)	NE	TO (0.99)	NA
c.196G>A	A66T	Missense	rs1139244	CM1010269	0.009	DC	BENIGN (0)	NE	TO (0.58)	NA
c.206G>A	G69D	Missense	-	CM109056	-	POLYMORPHISM	BENIGN (0.157)	NE	TO (0.46)	NA
c.209G>C	S70T	Missense	-	CM115165	10.71	DC	BENIGN (0.001)	NE	TO (0.46)	NA
c.221C>A	A74D	Missense	rs1258064099	CM1010265	19.96	POLYMORPHISM	PRD (0.997)	NE	TO (0.14)	NA
c.244A>G	T82A	Missense	rs961114777	-	12.10	POLYMORPHISM	BENIGN (0)	-	TO (0.38)	NA
c.259C>T	P87S	Missense	-	CM107597	-	-	PRD (0.977)	NE	TO (0.14)	NA
c.270C>A	S90R	Missense	-	CM104917	-	-	BENIGN (0.440)	NE	DE (0.04)	NA
c.278G>C	G93A	Missense	rs56191129	CM076206	19.85	DC	POD (0.943)	NE	TO(0.09)	NA
c.284A>G	D95G	Missense	-	CM104918	-	-	BENIGN (0)	NE	TO (0.06)	NA
c.286G>A	G96R	Missense	-	CM1213107	-	POLYMORPHISM	BENIGN (0.012)	NE	TO (0.06)	NA
c.307C>G	P103A	Missense	-	CM123514	19.50	DC	BENIGN (0.001)	NE	TO (0.7)	NA
c.357_359CGC	A126dup	Duplication	rs1182566703	-	-	-	-	NE	-	NA
c.392C>G	A131G	Missense	rs1013984246	-	18.4	POLYMORPHISM	BENIGN (0.002)	NE	TO (0.66)	NA
c.431C>T	A144V	Missense	rs1308945507	CM161974	14.22	POLYMORPHISM	POD (0.727)	NE	TO (0.16)	NA
c.448G>T	G150W	Missense	rs1024075653	CM1010266	26.0	DC	PRD (0.997)	DE	DE (0)	NA
c.479G>C	S160T	Missense	rs1358565879	CM115166	23.5	DC	POD (0.891)	NE	TO (0.35)	NA
c.487C>T	P163S	Missense	rs387906769	CM076201	22.1	DC	POD (0.669)	NE	NA	NA
c.488C>G	P163R	Missense	rs540578824	CM109057	25.4	DC	PRD (0.973)	DE	TO (0.42)	NA

Table 3. Continued.

c.569A>G	H190R	Missense	-	CMI1313747	24	DC	PRD (0.988)	DE	DE (0)	NA
c.578C>A	P193H	Missense	-	-	24.2	DC	POD (0.921)	NE	DE (0.05)	NA
c.590A>G	N197S	Missense	-	CM125063	15.18	POLYMORPHISM	BENIGN (0.009)	NE	TO (0.65)	NA
c.620C>T	-	5' UTR	rs61277615	-	8.145	-	-	-	-	NA
c.622T>C	F208L	Missense	--	-	20.6	DC	BENIGN (0.071)	NE	TO (1)	NA
c.628G>A	D210N	Missense	rs377673676	CM1010267	32	DC	PRD (0.996)	DE	NA	5.08
c.631T>C	F211L	Missense	-	-	22.4	DC	BENIGN (0.005)	NE	TO (0.47)	NA
c.640G>A	G214G	Synonymous	-	CM051488	24.3	-	-	NE	TO (1)	NA
c.640C>A	G214S	Missense	-	-	24.3	DC	POD (0.921)	DE	TO (0.442)	NA
c.648C>G	E216D	Missense	-	CM061008	-	-	PRD (0.999)	DE	DE (0)	NA
c.661C>A	G221R	Missense	rs398122402	CM110562	32	DC	PRD (0.999)	DE	DE (0)	NA
c.668T>C	M223T	Missense	-	-	23.6	DC	BENIGN (0.126)	DE	TO (0.32)	NA
c.677C>A	P226Q	Missense	-	CM147378	25.3	DC	PRD (1)	DE	DE (0)	NA
c.687C>T	R229S	Missense	-	-	24.2	DC	PRD (0.998)	DE	DE (0)	3.83
c.700G>A	G234S	Missense	-	-	28.1	DC	PRD (1)	DE	DE (0)	5.61
c.715A>G	N239D	Missense	-	-	27.3	DC	PRD (0.995)	DE	NA	NA
c.716A>G	N239S	Missense	-	-	25.8	DC	PRD (1)	DE	DE (0)	NA
c.731A>G	Y244C	Missense	-	-	28.9	DC	PRD (1)	DE	DE (0)	NA
c.740T>C	M247T	Missense	rs1131691325	CM104219	25.5	DC	POD (0.890)	DE	DE (0)	NA
c.743A>G	N248S	Missense	rs749360828	-	25.9	DC	PRD (0.994)	DE	DE (0)	NA
c.749T>A	I250N	Missense	-	CM1010268	27.8	DC	PRD (0.994)	DE	DE (0.01)	NA
c.754C>T	R252W	Missense	-	CM131318	31	DC	PRD (1)	DE	DE (0)	NA
c.764T>C	I255T	Missense	-	-	25	DC	POD (0.748)	DE	DE (0)	NA
c.779G>A	R260Q	Missense	rs1245034279	-	27.9	DC	PRD (0.979)	DE	DE (0.01)	NA
c.782T>C	L261P	Missense	-	-	26.2	DC	POD (0.653)	DE	DE (0)	NA
c.783T>G	S262A	Missense	-	CM1313748	-	-	BENIGN (0.255)	NE	TO (0.2)	NA
c.788C>G	A263G	Missense	-	CM128406	24.5	DC	BENIGN (0.449)	NE	DE (0.04)	NA
c.796C>T	R266X	Nonsense	-	--	53	DC	-	NA	NA	NA
c.799G>A	V267M	Missense	rs116781972	CM068343	24.9	DC	BENIGN (0.401)	NE	TO (0.09)	NA
c.812G>C	C271S	Missense	-	-	27.7	-	PRD (1)	DE	DE (0)	NA
c.818A>G	N273S	Missense	rs1340083717	-	25.9	DC	PRD (1)	DE	DE (0)	NA
c.819C>A	N273K	Missense	-	-	25.3	-	PRD (1)	DE	DE (0)	NA

Table 3. Continued.

c.822C>T	Cys274=	synonymous	rs55980825	-	11.33	DC	-	NE	-	4.51
c.830C>T	T277I	Missense	rs1236909953	-	27	DC	POD (0.770)	DE	DE (0)	NA
c.835A>T	T279S	Missense	-	-	26.3	-	PRD (0.999)	DE	DE (0)	NA
c.839C>T	T280M	Missense	rs387906771	CM104913.	29	DC	PRD (1)	DE	DE (0)	NA
c.848C>A	R283H	Missense	rs180765750	-	31	DC	PRD (1)	DE	DE (0)	NA
c.851C>A	R284H	Missense	-	CM160385	31	DC	PRD (1)	DE	DE (0)	NA
c.854A>G	N285S	Missense	-	CM1312065	24.4	DC	POD (0.858)	DE	DE (0.02)	NA
c.855T>C	N285K	Missense	-	CM051504	0.667	DC	PRD (0.999)	DE	DE (0)	NA
c.871G>C	V291L	Missense	-	CM141469	25.4	DC	PRD (0.997)	DE	DE (0)	NA
c.874T>C	C292R	Missense	-	CM051505	27	DC	PRD (1)	DE	DE (0)	NA
c.881C>T	A294V	Missense	-	CM051506	28.2	DC	PRD (1)	DE	DE (0)	NA
c.886G>C	G296R	Missense	rs104894073	CM114666	27.9	DC	PRD (1)	DE	NA	NA
c.C886A	G296S	Missense	rs104894073	CM031685	27.8	DC	PRD (1)	DE	NA	NA
c.886G>T	G296C	Missense	rs104894073	CM076203	29.2	DC	PRD (1)	DE	DE (0)	NA
c.899A>C	K300T	Missense	-	CM160006	24.6	DC	PRD (1)	DE	DE (0)	NA
c.905A>G	H302R	Missense	-	CM051507	24.6	DC	PRD (0.962)	DE	DE (0)	NA
c.928A>G	M310V	Missense	rs387906772	CM102095	26.4	DC	POD (0.934)	DE	DE (0)	NA
c.931C>T	R311W	Missense	-	-	-	DC	PRD (0.999)	DE	DE(0)	NA
c.946C>G	Q316E	Missense	rs56298569	CM076200	26.6	DC	PRD (0.996)	DE	DE (0)	NA
c.955A>G	K319E	Missense	-	CM140184	29.2	DC	PRD (0.991)	DE	DE (0.01)	NA
c.958C>T	R319W	Missense	rs1282433424	CM106844	33	DC	PRD (1)	DE	DE (0)	NA
c.989C>G	T330R	Missense	-	CM123286	23.5	DC	BENIGN (0.048)	DE	TO (0.14)	NA
c.1017C>A	S339R	Missense	rs1042942931	-	20.9	DC	BENIGN (0.93)	NE	DE (0.03)	NA
c.1037C>T	A346V	Missense	rs115372595	CM076205	14.12	POLYMORPHISM	BENIGN (0.112)	NE	TO (0.28)	3.93
c.1060G>A	T354A	Missense	-	CM107551	-	-	BENIGN (0)	NE	TO (0.21)	NA
c.1074delC	S358X	Nonsense	-	-	-	-	-	-	-	NA
c.1075G>A	E359K	Missense	rs368489876	CM086820	25.2	DC	PRD (1)	NE	NA	5.83
c.1075delG	E359fs	Deletion	rs1585703301	-	-	-	-	-	-	NA
c.1078G>C	E360Q	Missense	rs141808522	-	24.6	DC	PRD (0.985)	NE	NA	5.83
c.1079A>G	E360G	Missense	-	CM1010264	25.9	DC	PRD (0.985)	NE	TO (0.37)	NA
c.1081A>G	M361V	Missense	-	-	17.29	DC	POD (0.664)	NE	TO (0.49)	NA
c.1129A>G	S377G	Missense	rs3729856	CM164458	-	-	BENIGN (0)	NE	TO (0.56)	0.906
c.1180C>A	P394A	Missense	rs200319078	CM119355	17.05	POLYMORPHISM	BENIGN (0)	NE	TO (0.39)	2.670

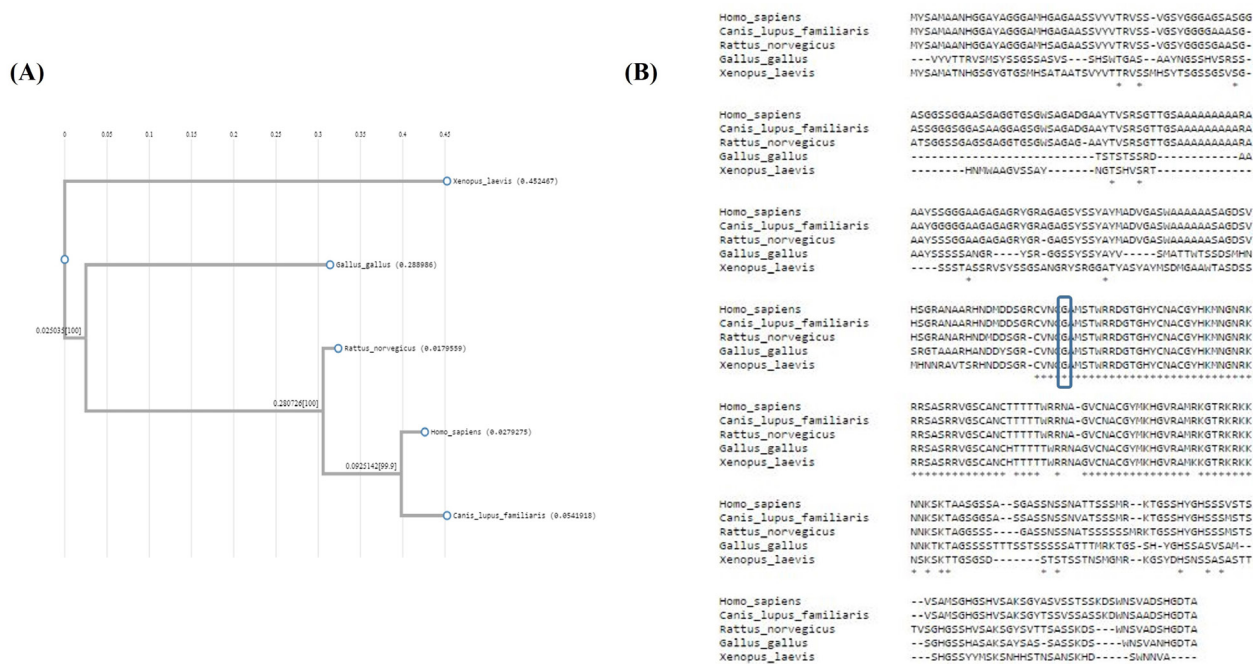
Table 3. Continued.

c.1196T>G	V399G	Missense	-	CM1313749	20.7	DC	BENIGN (0.131)	NE	TO (0.53)	NA
c.1207C>A	L403M	Missense	-	CM076202	25	DC	PRD (0.997)	NE	TO (0.06)	NA
c.1211A>G	K404R	Missense	-	CM125064	26.4	DC	PRD (0.996)	NE	DE (0)	NA
c.1220C>A	P407Q	Missense	rs115099192	CM086819	25.8	DC	POD (0.675)	DE	DE (0.04)	4.780
c.1273G>A	D425N	Missense	rs56208331	CM076207	29	DC	PRD (0.970)	DE	DE (0.01)	5.180
c.1286G>C	S429T	Missense	-	CM086818	23.18	DC	POD (0.646)	NE	DE (0.04)	NA
c.1288C>G	L430V	Missense	-	-	24.8	DC	PRD (0.990)	NE	DE (0)	NA
c.1295T>C	L432S	Missense	-	CM119354	27.98	DC	PRD (0.998)	DE	DE (0)	NA
c.1306C>T	H436Y	Missense	-	CM095707	26.08	DC	POD (0.851)	NE	DE (0)	NA
c.1310G>C	G437A	Missense	-	CM149081	23.5	DC	POD (0.787)	NE	DE (0)	NA
c.1324G>A	A442T	Missense	rs1270266865	-	26.8	DC	PRD (0.996)	NE	DE (0)	NA
c.1325C>T	A442V	Missense	rs146017816	-	27.1	DC	PRD (0.999)	NE	DE (0)	5.18

All GATA4 variants are reported based on the NCBI nucleotide (NM\_002052.5) and protein (NP\_002043.2) sequences (NC\_008177.2).

<sup>1</sup> CADD, Phred  $\leq 20$ : Neutral; Phred  $> 20$ : Damaging; <sup>2</sup> PolyPhen-2, score = 0-1.5; Benign; score = 0.15-0.85; Possibly damaging; score = 0.85-1; Probably damaging; <sup>3</sup> PROVEAN, score  $\leq -2.5$ : Deleterious; score  $> -2.5$ : Neutral; <sup>4</sup> SIFT, score  $\leq 0.05$ : Deleterious; score  $> 0.05$ : Tolerable; TO: Tolerable; DE: Deleterious; NE: natural; DC: Disease-causing; NA: Not available. PRD: Probably damaging; POD: Possibly damaging





**Figure 4: A)** The phylogenetic tree is constructed by CLUSTALW implementing the neighbor-joining method for GATA4 in *Homo sapiens* (humans), *Rattus norvegicus* (rats), *Canis lupus familiaris* (Dogs), *Xenopus laevis* (African clawed frogs), and *Gallus gallus domesticus* (chickens). The length of the horizontal lines shows the evolutionary distance between each organism based on the GATA4 protein sequence. **(B)** The results of the CLUSTALW multiple-sequence-alignment program show the conservation of the GATA4 G221 position among the different organisms. The identical (\*), conserved (:), and semiconserved (.) residues are specified. This position is highly conserved among the different species.

the autosomal dominant transmission of atrial septal defect in 4 generations, indicating *GATA4* as a genetic cause of atrial septal defect.

Sarkozy et al<sup>85</sup> detected the G296S variation of *GATA4* in 2 members of 1 family and 3 members of another family diagnosed with atrial septal defect.

Chen J et al<sup>14</sup> recognized the *GATA4* c.899A>C (K300T) substitution in 10 members of a family: 8 affected members with severe symptoms (7 patients with atrial septal defect and 1 patient with ventricular septal defect) and 2 unaffected members. The K300T substitution lessens the transcription of the *GATA4* target gene by harming the DNA-binding activity of *GATA4*.

Yu Chen et al<sup>86</sup> identified the c.928A>G (M310V) variant located in the NLS region of *GATA4* in all patients of a 3-generation family with atrial septal defect. The variant reduces the transcriptional activity of the *GATA4* protein and may disturb the interaction between *GATA4* and *TBX* or *NKX2-5*.

A genetic investigation conducted by E. D’Amato et al<sup>88</sup> reported the R319W variation in 3 members of a family: the proband and the proband’s sister, both diagnosed with atrial septal defect, and the proband’s father, who was considered not affected.

Rajagopal et al<sup>68</sup> studied 107 probands with cardiac abnormalities and identified the c.886G>T (G296C) variant in a proband with atrial septal defect and

pulmonary stenosis. They also reported the substitution in the proband’s father with persistent left superior vena cava to the coronary sinus. The G296S variation resulted in a reduction in *GATA4* DNA-binding activity and disrupted binding to the transcription factor *TBX5*. Also in their study, the c.1207C>A (L403M) variant was identified in a proband with a hypoplastic right ventricle and sinus venosus atrial septal defect. Their results also demonstrated the c.487C>T (P163S) and c.1037C>T (A346V) variants in probands with endocardial cushion defect. Additionally, a missense variation, c.931C>T (R311W), in *GATA4* was identified in a pedigree spanning 3 generations with 7 members diagnosed with CHD. All the affected members presented different cardiac phenotypes, including tetralogy of Fallot, ventricular septal defect, atrial septal defect, and patent ductus arteriosus, indicating that the same genetic alteration could lead to different subtypes of CHD.<sup>87</sup>

In the present study, we filtered the literature and online databases for the pathogenic variants of the *GATA4* gene. Our search yielded 210 variants; nonetheless, we excluded 100 of these variants due to a dearth of information and continued the study with 110 variations. After analyzing the frequency distributions of all the variants, we employed computational tools with different algorithms to predict the pathogenicity of the variants. As is shown in Table 3, our *in silico* analysis using MutationTaster, PolyPhen,

PROVEAN, and SIFT revealed 38 pathogenic genetic variations. Our findings may broaden the spectrum of the known *GATA4* genetic variations associated with different types of CHD.

### Conclusions

Several gene deficiencies could contribute to the pathogenesis of CHD. In this study, we drew upon different *in silico* predictive tools for the analysis of the variants of the *GATA4* gene. The most frequent variant was c.874T>C (45.58%), and the most frequent type of CHD was ventricular septal defect. Out of all the reported variants of *GATA4*, 38 variants were pathogenic. The p.Gly221Arg variant (CADD score=31) showed a high level of pathogenicity. All the identified pathogenic variations in *GATA4* could assist in the rapid identification and better understanding of the mechanisms underlying CHD.

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### Competing Interests

None declared.

### Ethical Approval

Not applicable.

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