In Silico Functional Assessment of COL4A3, COL4A4, and COL4A5 SNPs in Alport Syndrome*

Beyza Rümeysa ERGİNAL GEÇ**, Tuğba KAMAN***, Mesut KARAHAN****

Abstract

Aim: Alport syndrome is a rare genetic disorder characterized by hematuria, proteinuria, progressive renal failure, and, in some cases, hearing and visual impairment. This study aims to identify and prioritize deleterious missense single-nucleotide polymorphisms (SNPs) in the *COL4A3, COL4A4*, and *COL4A5* genes associated with Alport syndrome and to evaluate their effects on protein stability and three-dimensional structure using an integrated multi-tool *in silico* approach.

Method: SNP data and protein amino acid sequences were obtained from the NCBI dbSNP and UniProt databases; these data were used as input in *in silico* analyses performed using various bioinformatics tools, including SIFT, PolyPhen-2, SNPs&GO, PANTHER, PROVEAN, SNAP2, Mutation Assessor, I-Mutant 2.0, MUpro, and Project HOPE, to evaluate the potential structural and functional effects on COL4A3, COL4A4, and COL4A5 proteins. Only variants predicted as deleterious by all applied tools were selected for further analysis.

Results: A large number of SNPs were initially identified for *COL4A3, COL4A4,* and *COL4A5* genes; however, multi-tool consensus analysis identified 12, 14, and 146 deleterious missense variants in *COL4A3, COL4A4*, and *COL4A5,* respectively. Protein stability analyses produced divergent results: I-Mutant 2.0 predicted increased stability for 160 of 172 variants, whereas MUpro predicted decreased stability for 147 variants. In addition, a substantial number of deleterious variants involved glycine substitutions, which are likely to affect protein flexibility, folding, and intermolecular interactions, potentially impairing the structural integrity of the glomerular basement membrane.

Conclusion: This study represents a comprehensive consensus-based *in silico* evaluation of deleterious missense SNPs in COL4A3, COL4A4, and COL4A5 genes. The findings highlight the potential role of glycine substitutions in disease pathogenesis and provide an important basis for future experimental studies.

Keywords: Alport syndrome, single nucleotide polymorphism, bioinformatics, *COL4A3, COL4A4, COL4A5*.

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Alport Sendromunda COL4A3, COL4A4 ve COL4A5 SNP'lerinin İn Silico Fonksiyonel Değerlendirilmesi

Öz

Amaç: Alport sendromu, hematüri, proteinüri, ilerleyici böbrek yetmezliği ve bazı olgularda işitme ve görme bozuklukları ile karakterize nadir görülen bir genetik hastalıktır. Bu çalışma, Alport sendromuyla ilişkili *COL4A3, COL4A*4 ve *COL4A5* genlerindeki olası patojenik yanlış anlamlı tek nükleotid polimorfizmlerini (SNP'leri) belirlemek ve önceliklendirmek ve bu SNP'lerin protein stabilitesi ve üç boyutlu yapısı üzerindeki etkilerini çoklu in silico yaklaşım kullanarak değerlendirmektir.

Yöntem: SNP verileri ve protein amino asit dizileri NCBI dbSNP ve UniProt veri tabanlarından elde edildi; bu veriler, COL4A3, COL4A4 ve COL4A5 proteinleri üzerindeki potansiyel yapısal ve fonksiyonel etkileri değerlendirmek amacıyla SIFT, PolyPhen-2, SNPs&GO, PANTHER, PROVEAN, SNAP2, Mutation Assessor, I-Mutant 2.0, MUpro ve Project HOPE gibi çeşitli biyoenformatik araçları kullanılarak *in silico* analizler yapıldı. Kullanılan tüm araçlar tarafından zararlı olarak tahmin edilen varyantlar ileri analizler için seçildi.

Bulgular: Başlangıçta *COL4A3, COL4A4* ve *COL4A5* genlerinde çok sayıda SNP tespit edildi, ancak çoklu araçlara dayalı konsensüs analizi sonucunda sırasıyla 12, 14 ve 146 zararlı missense varyant tespit edildi. Protein stabilitesi analizleri farklı sonuçlar ortaya koymuştur: I-Mutant 2.0, 172 varyantın 160'ında stabilite artışı ön görürken MUpro, 147 varyantta stabilite azalması öngörmüştür. Ayrıca, zararlı varyantların önemli bir kısmının glisin substitüsyonlarını içerdiği ve bu değişimlerin protein esnekliği, katlanması ve moleküller arası etkileşimleri etkileyerek glomerüler bazal membranın yapısal bütünlüğünü bozabileceği belirlenmiştir.

Sonuç: Bu çalışma, *COL4A3, COL4A4* ve *COL4A5* genlerindeki zararlı missense SNP'lerin konsensüs temelli kapsamlı bir *in silico* değerlendirmesini sunmaktadır. Elde edilen bulgular, özellikle glisin substitüsyonlarının hastalık patogenezindeki olası rolünü ortaya koymakta ve gelecekte yapılacak deneysel çalışmalar için önemli bir temel sağlamaktadır.

Anahtar Sözcükler: Alport sendromu, tek nükleotid polimorfizmi, biyoenformatik, *COL4A3, COL4A4, COL4A5*.

Introduction

Alport syndrome is a genetic kidney disorder characterized by blood in the urine, progressive kidney failure, hearing loss, and eye abnormalities. It is the second most common inherited cause of kidney failure after autosomal dominant polycystic kidney disease. In 1927, Arthur C. Alport reported a series of cases involving patients with familial hereditary nephritis. Although not the first to describe the condition, he was the first to establish the association between the renal manifestations and sensorineural hearing loss. Additionally, he observed that the disorder could be accompanied by ocular abnormalities. Following his death, the characteristic clinical features of renal disease, hearing impairment, and visual disturbances became known as Alport syndrome[1].

Alport syndrome results from mutations in the *COL4A3, COL4A4,* and *COL4A5* genes, which encode the α3, α4, and α5 chains of type IV collagen, an essential structural component of the glomerular basement membrane (GBM) in the kidney. To date, over 500 distinct mutations affecting these genes have been identified, with the majority exhibiting X-linked or autosomal recessive patterns of inheritance[2,3].

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The *COL4A5* gene is located on the X chromosome. Hemizygous males carrying pathogenic variants in *COL4A5* are typically more severely affected. However, heterozygous female carriers may exhibit a spectrum of clinical manifestations, ranging from isolated hematuria to progressive renal decline and end-stage renal disease (ESRD)[3,4]. Loss of any one of the type IV collagen α chains disrupts the formation of the α3α4α5 heterotrimer, which is essential for the structural integrity of the glomerular basement membrane (GBM). The clinical phenotype and disease course of males with autosomal recessive Alport syndrome due to biallelic pathogenic variants in COL4A3 or COL4A4 are usually very similar to those of hemizygous males with X-linked Alport syndrome due to pathogenic COL4A5 variants. In contrast, due to X-chromosome inactivation, female carriers of pathogenic COL4A5 variants may have a higher risk of developing ESRD than heterozygous carriers of pathogenic COL4A3 or COL4A4 variants associated with autosomal recessive Alport syndrome[5].

A detailed physical examination with a family history should be performed for diagnosis. Laboratory evaluation should encompass urinalysis, urine microscopy, and assessment of renal function. Individuals with Alport syndrome commonly present with hematuria, proteinuria, edema, hypertension, and progressive deterioration of kidney function, which may culminate in end-stage renal disease (ESRD). As the disease advances, clinical parameters typically worsen, marked by increasing levels of proteinuria, rising blood pressure, declining glomerular filtration rate (GFR), and eventual development of ESRD. The onset of ESRD most frequently occurs between the ages of 16 and 35 years[6].

Due to abnormalities in type IV collagen in the inner ear, individuals with Alport syndrome frequently develop bilateral sensorineural hearing loss in late childhood. High-frequency hearing loss is typically the initial manifestation, emerging in late infancy or early adolescence, preceding the onset of kidney failure[7].

Patients with Alport syndrome may exhibit various ocular symptoms. Affected individuals may experience anterior lenticonus, a cone-shaped lens that leads to aberrant refraction and reduced visual acuity. Cataracts, dystrophy, corneal damage, and aberrant pigment changes in the retina with yellow or white spots (spot and speckle retinopathy) are further problems[8-11].

Early diagnosis of Alport syndrome is critical, as, depending on the stage of the disease at which treatment is initiated, pharmacological inhibition of the renin-angiotensin-aldosterone system (RAAS) can reduce the progression to end-stage renal disease (ESRD)[3,12].

The renin-angiotensin-aldosterone system plays a key role in regulating blood flow in the kidneys, ensuring proper salt and water balance, and influencing tissue development within renal structures.

Activation of the renin-angiotensin-aldosterone system (RAAS) contributes to both systemic and glomerular capillary hypertension, leading to hemodynamic injury in the vascular endothelium and glomeruli. Recognizing the role of this system is important, as

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blocking its activity can help slow the progression of chronic kidney disease in patients with proteinuria.

Although current treatments do not offer a cure, they can substantially slow the progression to kidney failure, especially when started before any decline in glomerular filtration rate (GFR)[12]. Since early intervention can modify the course of Alport nephropathy, timely diagnosis of Alport syndrome is critically important.

Genes Associated with Alport Syndrome

The *COL4A5* gene encodes one of the six subunits of type IV collagen, which is a key structural component of basement membranes. Mutations in this gene are associated with X-linked Alport syndrome, also referred to as hereditary nephritis[13].

The *COL4A4* gene encodes one of the six subunits of type IV collagen, a major structural component of basement membranes. However, this specific subunit is expressed only in certain types of basement membranes. Mutations in COL4A4 are associated with autosomal recessive Alport syndrome (type II) as well as with benign familial hematuria, also known as thin basement membrane disease[14].

The *COL4A3* gene encodes one of the alpha subunits of type IV collagen, a multimeric protein that serves as a key structural component of basement membranes. Type IV collagen is composed of three alpha chains that form a triple helix structure. These chains are encoded by six distinct genes (*COL4A1 to COL4A6*), each contributing to the assembly of specific α-chain combinations. Mutations in *COL4A3* are associated with the autosomal recessive form of Alport syndrome[15].

Polymorphism

Genetic polymorphisms arise from variations in genetic material among individuals and populations. Variants with a population frequency greater than 1% are generally referred to as polymorphisms, whereas those with lower frequencies are considered rare variants.[16]

Single Nucleotide Polymorphism

Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome. The human genome is estimated to contain approximately 3–10 million SNPs. SNPs that directly affect protein structure and function are typically located in coding regions of DNA. However, SNPs located in regulatory regions may also alter gene expression and contribute to phenotypic variation or disease susceptibility. In contrast, SNPs that do not significantly affect gene function or phenotype are referred to as neutral SNPs[17].

SNPs serve as valuable genetic markers for identifying disease-associated genes. Single-nucleotide polymorphisms (SNPs) located within a gene or its regulatory regions may directly influence disease by altering gene function. They are also useful markers for

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tracing the inheritance of disease-related genes within families. Therefore, further research is essential to uncover SNPs linked to complex conditions such as cardiovascular disease, diabetes, and cancer[18].

There are several databases that the public can access for SNPs. SNPs are especially significant when they arise in coding regions, as non-synonymous variants can induce alterations in the amino acid sequence. SNPs are likely to play an important role in the functional diversity of encoded proteins in human populations and are associated with many diseases. SNPs can influence gene regulation by altering protein function, affecting transcriptional and translational processes, and modifying protein stability or solubility[19,20].

Databases for Single Nucleotide Polymorphism Analysis

Due to the large number of genetic polymorphisms in the human genome, extensive research is required to identify disease susceptibility and support personalized drug design[21]. However, to facilitate these studies, numerous computational methods are used to identify potentially important variants before performing in vitro or in vivo testing. Therefore, *in silico* approaches represent effective bioinformatics tools to distinguish harmful SNPs from neutral ones using certain algorithms[22].

The overall effect of polymorphism, including functional and structural changes, can be analyzed through various databases. Consequently, bioinformatics tools play a crucial role in identifying and prioritizing potentially deleterious SNPs, as experimental validation of every variant is impractical. As a preliminary study, these bioinformatics approaches offer an effective filtering strategy to assess whether the identified SNPs are deleterious or neutral[23].

Given the genetic heterogeneity of Alport syndrome, identifying high-confidence deleterious variants in COL4A3, COL4A4, and COL4A5 genes is of critical importance. In this context, this study provides a comprehensive consensus-based prioritization of deleterious missense variants (i.e., variants predicted as deleterious by all applied *in silico* tools), aiming to improve the reliability of variant prioritization and to support future genetic and experimental studies in Alport syndrome.

Material and Methods

Type of Research

This study is an *in silico* analysis of the COL4A3, COL4A4, and COL4A5 genes associated with Alport syndrome using a variety of publicly available bioinformatics tools. *In silico* analyses can be used to predict deleterious SNPs in the COL4A3, COL4A4, and COL4A5 genes associated with Alport syndrome, enabling the selection of target SNPs for genotyping in disease association studies. Therefore, the findings of this study may facilitate future experimental validation studies.

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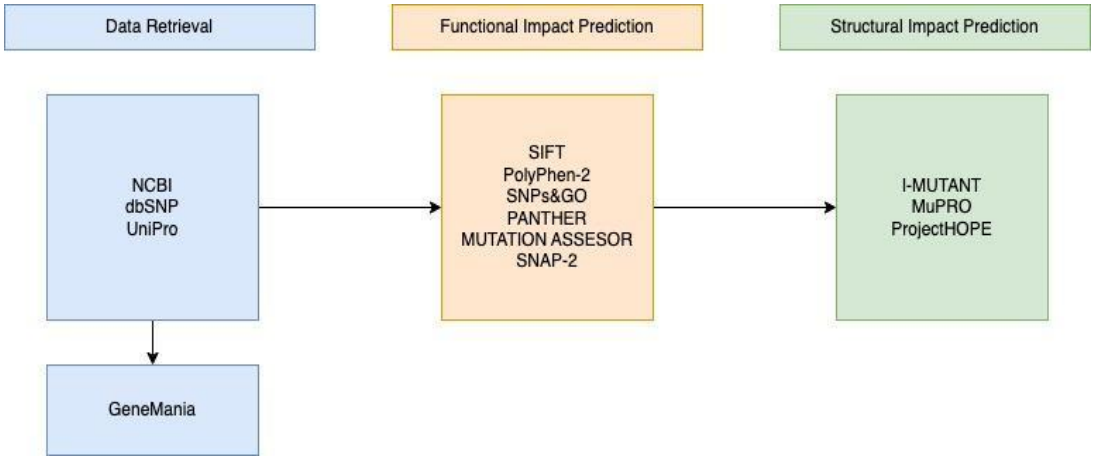
Workflow Chart

Figure 1. The *in silico* tools used, and the analysis workflow are presented as a schematic diagram.



Data Mining

The NCBI dbSNP database was used to access SNPs in the *COL4A3, COL4A4,* and *COL4A5* genes in January 2021. SNPs in these three genes were selected for analysis. The amino acid sequences of the encoded proteins were obtained from the UniProt database[24].

Bioinformatics Data Analysis

SIFT (Sorting Intolerant From Tolerant; accessed in February 2021), PolyPhen-2 (Prediction of functional effects of human nsSNPs; accessed in February 2021), SNPs&GO (Predicting human disease-related mutations in proteins with functional annotations; accessed in April 2021), PANTHER (Protein Analysis Through Evolutionary Relationships; accessed in July 2021), PROVEAN (Protein Variation Effect Analyzer; accessed in July 2021), Mutation Assessor (accessed in August 2021), and SNAP2 (SNP effect predictor; accessed in August 2021) bioinformatics tools were used to evaluate the effects of SNPs on protein structure and function.

SIFT predicts the functional impact of amino acid substitutions by analyzing sequence homology and the physicochemical characteristics of amino acids. Variants with SIFT scores ≤0.05 were classified as deleterious, whereas those with scores >0.05 were classified as tolerated[25,26].

PolyPhen-2 assesses the structural and functional consequences of amino acid alterations in specific proteins. Variants classified as “possibly damaging” or “probably damaging” were considered potentially deleterious[27].

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SNPs&GO integrates functional annotation data to evaluate the likelihood of a single nucleotide polymorphism being disease-associated. Variants with a disease probability >0.5 were classified as disease-associated.

PROVEAN estimates the potential deleterious effects of amino acid substitutions on protein function. Variants with PROVEAN scores ≤−2.5 were considered deleterious, whereas scores >−2.5 were considered neutral[28].

PANTHER evaluates the probable impact of missense variants on the functional integrity of target proteins. Variants classified as “probably damaging” or “possibly damaging” were considered potentially deleterious, whereas variants classified as “probably benign” were considered non-deleterious.

Mutation Assessor classifies variants according to their predicted functional impact as neutral, low, medium, or high; variants classified as having medium or high functional impact were considered potentially deleterious. SNAP2 predictions with scores >0 were considered to have an effect, whereas scores ≤0 were considered neutral[29].

Only variants consistently predicted to be deleterious or functionally damaging by all seven functional prediction tools were included in the consensus deleterious variant set and subjected to subsequent protein stability and structural analyses.

The effects of these consensus deleterious variants on protein stability were evaluated using I-Mutant 2.0 and MUpro (accessed in August 2021). MUpro analyses were performed using the protein sequence as input. MUpro provides two distinct prediction outputs: an estimated ΔΔG value generated using a support vector machine (SVM) regression model and a prediction of the direction of stability change generated using a separate classification model. Therefore, the predicted ΔΔG value should not be interpreted as the confidence score of the stability classification. Since these outputs are generated using different prediction methodologies, they may not always be concordant[30,31].

Three-dimensional structural interpretation of amino acid substitutions was performed using the Project HOPE tool (accessed in August 2021). Unless otherwise specified, the default parameters provided by the respective web tools were used[32].

Gene–gene interactions

In addition, co-expression patterns and gene localization were analyzed using the GeneMANIA tool[33].

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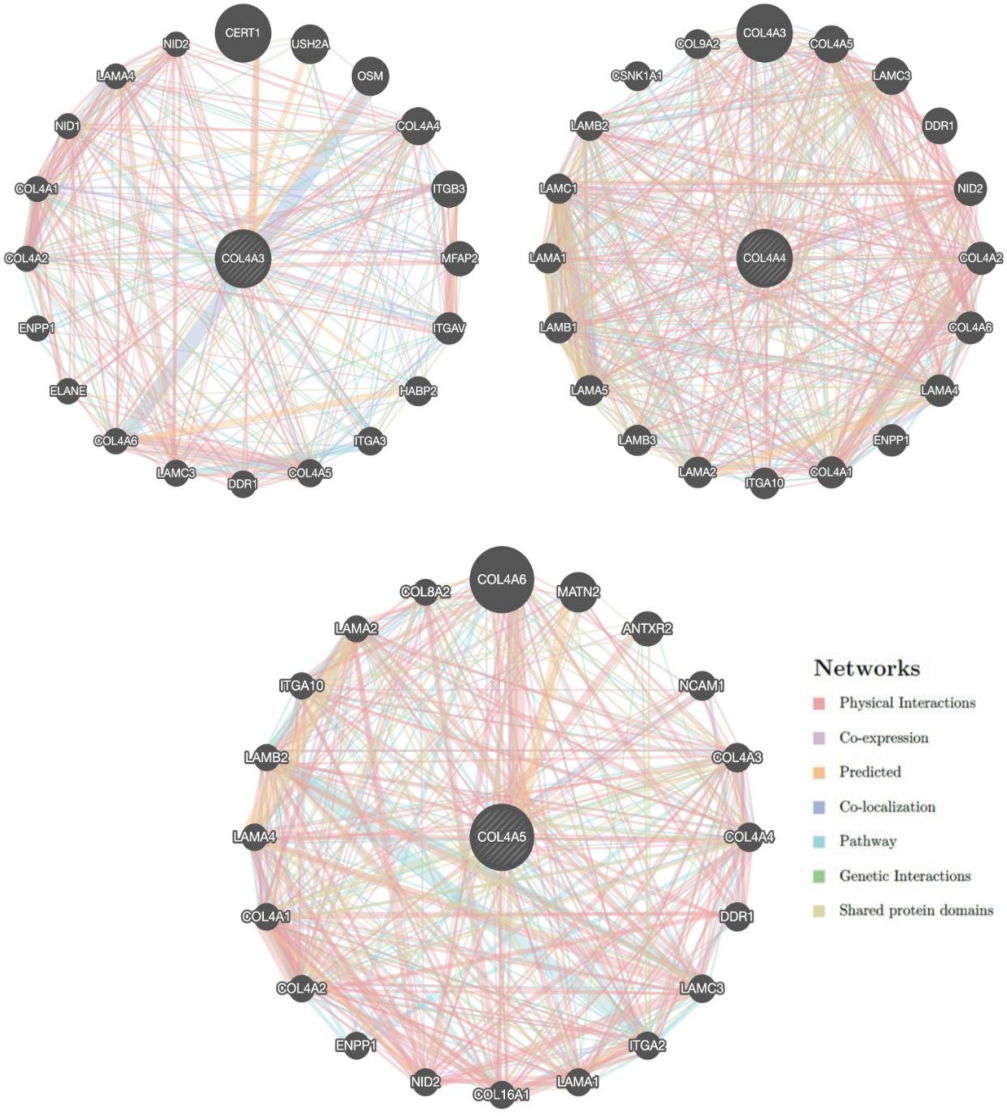
Figure 2. Gene-gene interactions of COL4A3, COL4A4, and COL4A5.



Results

The amino acid substitutions identified in each gene were analyzed using SIFT, SNPs&GO, PolyPhen-2, PANTHER, PROVEAN, SNAP2, and Mutation Assessor. A summary table of all analyzed genes and the corresponding results obtained from each database was prepared (Table 1). The variants associated with the disease in these databases were then analyzed with I-Mutant and MUpro software tools, and their effects on protein stabilization were investigated.

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Table 1. List of deleterious SNPs identified in COL4A3, COL4A4, and COL4A5 genes.

COL4A3	COL4A4	COL4A5						
rs375290088	rs377511303	rs104886043	rs104886080	rs104886111	rs104886139	rs104886173	rs104886210	rs104886245
rs374787000	rs374815903	rs104886052	rs104886082	rs104886112	rs104886140	rs104886174	rs104886211	rs104886246
rs372237167	rs371191979	rs104886055	rs104886083	rs104886115	rs104886141	rs104886176	rs104886212	rs104886247
rs371185804	rs373150214	rs104886056	rs104886084	rs104886116	rs104886142	rs104886177	rs104886214	rs104886248
rs368351146	rs374340855	rs104886057	rs104886085	rs104886117	rs104886143	rs104886179	rs104886215	rs104886250
rs375040636	rs371817534	rs104886059	rs104886086	rs104886118	rs104886144	rs104886180	rs104886218	rs104886251
rs267606745	rs372606845	rs104886060	rs104886088	rs104886119	rs104886145	rs104886182	rs104886219	rs104886252
rs267599232	rs370474706	rs104886061	rs104886091	rs104886120	rs104886147	rs104886183	rs104886221	rs104886253
rs199956740	rs371172166	rs104886062	rs104886092	rs104886121	rs104886150	rs104886184	rs104886223	rs104886255
rs190598500	rs371915593	rs104886063	rs104886093	rs104886122	rs104886151	rs104886185	rs104886224	rs104886257
rs121912827	rs370917166	rs104886066	rs104886096	rs104886125	rs104886153	rs104886186	rs104886225	rs104886261
rs121912826	rs267599231	rs104886067	rs104886097	rs104886126	rs104886157	rs104886188	rs104886228	rs104886263
	rs267599228	rs104886068	rs104886098	rs104886127	rs104886158	rs104886189	rs104886229	rs104886264
	rs202242354	rs104886069	rs104886099	rs104886129	rs104886160	rs104886190	rs104886231	rs104886266
		rs104886070	rs104886100	rs104886130	rs104886161	rs104886191	rs104886232	rs104886267
		rs104886073	rs104886101	rs104886133	rs104886163	rs104886195	rs104886235	rs104886269
		rs104886074	rs104886102	rs104886134	rs104886165	rs104886196	rs104886236	rs104886272
		rs104886075	rs104886103	rs104886135	rs104886166	rs104886199	rs104886237	rs104886273
		rs104886076	rs104886105	rs104886136	rs104886168	rs104886201	rs104886240	rs104886274
		rs104886078	rs104886107	rs104886137	rs104886171	rs104886202	rs104886242	rs201220208
		rs104886079	rs104886108	rs104886138	rs104886172	rs104886209	rs104886244	

It should be noted that the ΔΔG values and the Increase/Decrease classifications reported by MUpro are generated by distinct prediction models and may therefore not always be concordant.

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Table 2. The effect of SNPs predicted to be deleterious by all software tools used on protein stabilization was determined with I-Mutant and MUpro.

COL4A3					COL4A4				
SNP ID	**I Mutant**	**RI**	**MUpro**	**ddG**	**SNP ID**	**I Mutant**	**RI**	**MUpro**	**ddG**
rs375290088	Inc.	5	Dec.	-0.132	rs377511303	Inc.	4	Dec.	-0.084
rs374787000	Inc.	3	Dec.	-0.580	rs374815903	Inc.	3	Dec.	-0.494
rs372237167	Inc.	2	Dec.	-0.262	rs371191979	Inc.	2	Dec.	-0.241
rs371185804	Inc.	5	Dec.	-0.272	rs373150214	Inc.	6	Dec.	-0.227
rs368351146	Inc.	2	Dec.	-0.604	rs374340855	Inc.	5	Dec.	-0.586
rs375040636	Inc.	4	Inc.	0.072	rs371817534	Inc.	5	Dec.	-0.843
rs267606745	Dec.	0	Dec.	-0.525	rs372606845	Inc.	4	Inc.	0.040
rs267599232	Inc.	5	Dec.	-0.278	rs370474706	Inc.	4	Dec.	-0.227
rs199956740	Inc.	3	Dec.	-0.202	rs371172166	Inc.	4	Dec.	-0.603
rs190598500	Inc.	4	Dec.	-0.247	rs371915593	Inc.	2	Dec.	-0.253
rs121912827	Inc.	6	Dec.	-0.186	rs370917166	Inc.	2	Dec.	-0.746
rs121912826	Inc.	4	Dec.	-0.586	rs267599231	Inc.	2	Dec.	-0.901
					rs267599228	Inc.	5	Dec.	-0.112
					rs202242354	Inc.	2	Dec.	-10.171
COL4A5									
SNP ID	**I Mutant**	**RI**	**MUpro**	**ddG**	**SNP ID**	**I Mutant**	**RI**	**MUpro**	**ddG**
rs104886043	Inc.	3	Inc.	0.002	rs104886153	Dec.	0	Dec.	-0.201
rs104886052	Inc.	5	Dec.	-0.512	rs104886157	Inc.	0	Dec.	-1.310
rs104886055	Inc.	3	Dec.	-0.451	rs104886158	Inc.	3	Dec.	-0.507
rs104886056	Inc.	4	Dec.	-0.320	rs104886160	Inc.	5	Dec.	-0.145
rs104886057	Inc.	6	Dec.	-0.058	rs104886161	Inc.	4	Dec.	-0.754
rs104886059	Inc.	4	Dec.	-0.297	rs104886163	Inc.	5	Dec.	-0.131
rs104886060	Inc.	4	Dec.	-0.449	rs104886165	Inc.	5	Dec.	-0.058
rs104886061	Inc.	4	Dec.	-0.677	rs104886166	Inc.	4	Dec.	-0.348
rs104886062	Inc.	5	Dec.	-0.031	rs104886168	Inc.	7	Inc.	0.046
rs104886063	Inc.	5	Dec.	-0.647	rs104886171	Inc.	4	Dec.	-0.304

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rs104886066	Inc.	6	Inc.	0.028	rs104886172	Inc.	4	Dec.	-0.357
rs104886067	Inc.	5	Dec.	-0.358	rs104886173	Inc.	2	Dec.	-0.689
rs104886068	Inc.	6	Dec.	-0.058	rs104886174	Inc.	3	Dec.	-12.704
rs104886069	Inc.	4	Dec.	-0.598	rs104886176	Inc.	5	Dec.	-0.252
rs104886070	Inc.	2	Dec.	-0.526	rs104886177	Inc.	2	Dec.	-0.085
rs104886073	Inc.	3	Dec.	-0.129	rs104886179	Inc.	6	Dec.	-0.635
rs104886074	Inc.	5	Dec.	-0.182	rs104886180	Inc.	7	Inc.	0.179
rs104886075	Inc.	2	Dec.	-0.800	rs104886182	Inc.	5	Dec.	-0.598
rs104886076	Inc.	5	Dec.	-0.283	rs104886183	Inc.	5	Dec.	-0.344
rs104886078	Inc.	3	Inc.	0.051	rs104886184	Inc.	4	Dec.	-0.294
rs104886079	Inc.	1	Dec.	-0.250	rs104886185	Inc.	4	Dec.	-0.193
rs104886080	Inc.	2	Dec.	-10.300	rs104886186	Inc.	4	Dec.	-0.407
rs104886082	Inc.	2	Dec.	-0.627	rs104886188	Inc.	5	Dec.	-0.278
rs104886083	Inc.	3	Inc.	0.030	rs104886189	Inc.	3	Dec.	-0.090
rs104886084	Inc.	4	Dec.	-0.430	rs104886190	Inc.	3	Dec.	-0.241
rs104886085	Inc.	3	Dec.	-0.497	rs104886191	Inc.	5	Dec.	-0.535
rs104886086	Inc.	2	Dec.	-0.461	rs104886195	Inc.	5	Inc.	0.007
rs104886088	Inc.	4	Dec.	-0.573	rs104886196	Inc.	4	Dec.	-0.336
rs104886091	Inc.	3	Dec.	-0.356	rs104886199	Inc.	4	Inc.	0.001
rs104886092	Inc.	2	Dec.	-0.247	rs104886201	Inc.	4	Dec.	-0.155
rs104886093	Inc.	2	Dec.	-0.430	rs104886202	Inc.	6	Inc.	0.010
rs104886096	Inc.	4	Inc.	0.089	rs104886209	Inc.	3	Dec.	-0.329
rs104886097	Inc.	6	Dec.	-0.160	rs104886210	Inc.	4	Dec.	-11.172
rs104886098	Inc.	5	Dec.	-0.379	rs104886211	Inc.	5	Inc.	0.070
rs104886099	Inc.	4	Inc.	0.226	rs104886212	Inc.	4	Inc.	0.1361
rs104886100	Inc.	4	Dec.	-0.293	rs104886214	Inc.	5	Dec.	-0.420
rs104886101	Dec.	1	Dec.	-0.554	rs104886215	Inc.	7	Inc.	0.107
rs104886102	Dec.	1	Dec.	-0.339	rs104886218	Dec.	2	Dec.	-0.416
rs104886103	Inc.	1	Dec.	-0.065	rs104886219	Dec.	1	Dec.	-1.233
rs104886105	Inc.	3	Dec.	-0.205	rs104886221	Dec.	0	Dec.	-15.651

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rs104886107	Inc.	6	Dec.	-0.265	rs104886223	Dec.	5	Dec.	-0.385
rs104886108	Inc.	7	Dec.	-1.061	rs104886224	Inc.	3	Dec.	-0.145
rs104886111	Inc.	3	Dec.	-0.516	rs104886225	Inc.	3	Dec.	-0.110
rs104886112	Inc.	6	Inc.	0.273	rs104886228	Inc.	4	Dec.	-0.454
rs104886115	Inc.	4	Inc.	0.200	rs104886229	Inc.	4	Dec.	-0.335
rs104886116	Inc.	2	Dec.	-0.387	rs104886231	Inc.	5	Dec.	-0.206
rs104886117	Inc.	3	Dec.	-0.568	rs104886232	Inc.	4	Dec.	-0.095
rs104886118	Inc.	4	Dec.	-0.082	rs104886235	Inc.	6	Inc.	0.005
rs104886119	Inc.	2	Dec.	-0.671	rs104886236	Inc.	4	Dec.	-0.745
rs104886120	Inc.	5	Dec.	-0.048	rs104886237	Inc.	3	Dec.	-0.178
rs104886121	Inc.	3	Inc.	0.046	rs104886240	Inc.	5	Dec.	-0.735
rs104886122	Inc.	3	Dec.	-0.339	rs104886242	Inc.	4	Dec.	-0.099
rs104886125	Inc.	2	Dec.	-0.066	rs104886244	Inc.	5	Dec.	-0.304
rs104886126	Dec.	1	Dec.	-0.448	rs104886245	Inc.	5	Dec.	-0.799
rs104886127	Dec.	1	Dec.	-0.426	rs104886246	Inc.	4	Dec.	-0.160
rs104886129	Inc.	0	Dec.	-0.803	rs104886247	Inc.	6	Dec.	-0.049
rs104886130	Inc.	8	Dec.	-0.109	rs104886248	Inc.	5	Dec.	-0.077
rs104886133	Inc.	3	Dec.	-0.336	rs104886250	Inc.	6	Dec.	-0.144
rs104886134	Inc.	3	Dec.	-0.245	rs104886251	Inc.	3	Dec.	-0.068
rs104886135	Inc.	4	Dec.	0.411	rs104886252	Inc.	5	Dec.	-0.178
rs104886136	Inc.	3	Dec.	-0.625	rs104886253	Inc.	5	Dec.	-0.542
rs104886137	Inc.	7	Dec.	-0.437	rs104886255	Inc.	4	Dec.	-0.072
rs104886138	Inc.	4	Dec.	-0.033	rs104886257	Inc.	6	Dec.	-0.557
rs104886139	Inc.	6	Dec.	-0.274	rs104886261	Inc.	3	Inc.	0.124
rs104886140	Inc.	4	Dec.	-0.212	rs104886263	Inc.	5	Dec.	-0.314
rs104886141	Inc.	4	Dec.	-0.494	rs104886264	Inc.	7	Dec.	-0.292
rs104886142	Inc.	4	Dec.	-0.116	rs104886266	Inc.	4	Dec.	-0.561
rs104886143	Inc.	7	Dec.	-0.622	rs104886267	Dec.	1	Inc.	0.177
rs104886144	Inc.	5	Dec.	-0.332	rs104886269	Inc.	6	Inc.	0.335
rs104886145	Inc.	4	Dec.	-0.201	rs104886272	Inc.	6	Dec.	-0.408

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rs104886147	Inc.	3	Dec.	-0.368	rs104886273	Inc.	5	Inc.	0.046
rs104886150	Inc.	2	Dec.	-0.901	rs104886274	Inc.	4	Inc.	0.068
rs104886151	Inc.	4	Dec.	-0.159	rs201220208	Dec.	9	Dec.	-0.982

Inc.: Increase, **Dec.**: Decrease

Modeling SNPs with Project HOPE

Each amino acid has different charge, size, and hydrophobic values. For this reason, amino acid changes due to single-nucleotide polymorphisms create differences in charge, size, and hydrophobicity between wild type and mutant type. Glycine substitutions were observed in a substantial proportion of variants across the three analyzed genes. Detailed structural information for all glycine substitutions could not be fully retrieved using the Project HOPE tool.

Glycine substitutions were frequently observed among the variants consistently predicted to be deleterious in this study. A total of 192 amino acid substitutions were identified, of which 189 (98.4%) involved glycine residues. The remaining substitutions included two cysteine-to-phenylalanine and one proline-to-threonine substitutions.

Since a single rsID can be associated with multiple amino acid substitutions, the total number of amino acid substitutions does not necessarily correspond to the number of unique consensus deleterious SNPs. Therefore, Table 3 summarizes amino acid substitutions rather than unique rsIDs.

The distribution of glycine substitutions across the three analyzed genes is summarized in Table 3.

Table 3. Distribution of glycine substitutions among the identified amino acid substitutions in COL4A3, COL4A4, and COL4A5.

		COL4A3	COL4A4	COL4A5	TOTAL
Glycine	Glutamic Acid	3	2	21	26
Glycine	Aspartic Acid	1	2	32	35
Glycine	Serine	1	0	19	20
Glycine	Arginine	5	6	40	51
Glycine	Cysteine	0	0	10	10
Glycine	Valine	2	2	31	35
Glycine	Alanine	0	1	9	10
Glycine	Tryptophan	0	0	2	2

Discussion

Amino acid substitutions caused by missense SNPs can significantly alter the structural and functional properties of proteins, including charge distribution, hydrophobicity,

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stability, and folding[34]. These changes have the potential to affect protein function and have serious biological repercussions. Substitutions in specific key domains have the potential to interfere with three-dimensional conformation and protein–protein interactions, thereby contributing to disease development[35].

The identification of single-nucleotide polymorphisms (SNPs) is crucial for the early diagnosis of hereditary diseases. Early genetic diagnosis of Alport syndrome may enable timely initiation of treatment, which can delay the progression of kidney disease and the onset of kidney failure.

Clinical research suggests that renal deterioration can be effectively attenuated and long-term patient outcomes improved through early diagnosis followed by the timely initiation of angiotensin-converting enzyme inhibitor (ACEI) therapy[12].

Genetic testing is recommended to support the diagnosis of Alport syndrome. In individuals with suspected Alport syndrome, next-generation sequencing (NGS)-based customized gene panels enable the simultaneous analysis of COL4A3, COL4A4, and COL4A5 and facilitate the identification of potentially disease-associated variants.

In silico analyses are particularly valuable for the functional interpretation and prioritization of genetic variants identified through NGS. By predicting the potential functional and structural effects of these variants, in silico tools can help distinguish potentially deleterious variants from those less likely to affect protein function. Therefore, integrating in silico prediction tools with NGS data may facilitate the prioritization of relevant candidate variants for subsequent experimental and clinical evaluation.

In this study, SNPs in the COL4A3, COL4A4, and COL4A5 genes associated with Alport syndrome were comprehensively analyzed using SIFT, PROVEAN, Mutation Assessor, PolyPhen-2, SNAP2, I-Mutant 2.0, MUpro, Project HOPE, and GeneMANIA to evaluate their potential effects on protein function, stability, and structure. This consensus-based approach provides a stringent framework for variant prioritization by selecting only those variants consistently predicted to be deleterious across all in silico prediction tools, thereby increasing confidence in identifying potentially deleterious variants.

In summary, the present study identified the following:

A total of 1125 SNPs were identified for COL4A3. A substantial proportion of SNPs were excluded based on multi-tool consensus analysis. A total of 12 SNPs were consistently predicted to be deleterious across all prediction tools.

A total of 1338 SNPs were identified for COL4A4. A total of 14 SNPs were consistently predicted to be deleterious across all prediction tools.

A total of 1342 SNPs were identified for COL4A5, of which 146 amino acid changes were consistently predicted to be deleterious.

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The deleterious SNPs identified in this study were consistent with previously reported case studies[36-39].

A prominent finding of this study is the high frequency of glycine substitutions among deleterious variants. Collagen is characterized by repeating Gly-X-Y motifs, in which every third amino acid is glycine. This structure enables the formation of a tight triple helix, as glycine's minimal side chain allows the three chains to pack closely together. Mutations affecting glycine residues can disrupt the integrity of the triple helix by delaying folding and reducing structural stability. Such alterations are especially detrimental to type IV collagen, which forms a major component of the glomerular basement membrane, and are known to cause inherited disorders such as Alport syndrome[40,41]. The following structural interpretations of the identified amino acid substitutions are based on the biochemical properties of the amino acids and previously published experimental evidence, rather than solely on Project HOPE predictions.

According to the analysis results, 26 glycine-glutamic acid and 35 glycine-aspartic acid substitutions were identified. Glycine is neutral, whereas glutamic acid and aspartic acid are negatively charged; therefore, these substitutions may alter local electrostatic interactions and affect protein stability[42].

20 glycine-serine, 35 glycine-valine, 10 glycine-alanine, and 2 glycine-tryptophan substitutions were identified. These substitutions involve replacement of glycine by amino acids with different side-chain sizes and physicochemical properties and may therefore affect structural flexibility, triple-helix formation, and protein stability[43,44].

51 glycine-arginine substitutions were identified. Glycine is neutral, whereas arginine carries a positive charge. This substitution may alter the local electrostatic environment and affect protein structure and interactions[45].

10 glycine-cysteine substitutions were identified. Cysteine has different physicochemical properties compared with glycine; therefore, glycine-to-cysteine substitutions may lead to functional and structural changes in proteins[46].

Apart from glycine substitutions, 2 cysteine-to-phenylalanine and 1 proline-to-threonine substitutions were identified. Cysteine residues can contribute to protein stability through disulfide bond formation, whereas proline has a distinctive role in determining local protein conformation. Therefore, substitutions involving these residues may influence local protein structure and stability[47,48].

In light of these findings, this study identified potentially deleterious amino acid substitutions associated with SNPs in Alport syndrome-related genes. These findings provide preliminary insights that may contribute to future genetic and experimental studies.

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Conclusion

In this study, SNPs in the *COL4A3, COL4A4*, and *COL4A5* genes associated with Alport syndrome were evaluated using multiple in silico prediction tools, and variants consistently predicted to be deleterious across all applied tools were identified.

Protein stability analyses did not produce a uniform consensus between I-Mutant 2.0 and MUpro. I-Mutant 2.0 predominantly predicted increased stability, whereas MUpro predominantly predicted decreased stability. Therefore, the direction of the stability changes cannot be conclusively determined based solely on these computational predictions.

Glycine substitutions accounted for 189 of the 192 identified amino acid substitutions (98.4%). Given the structural importance of glycine in collagen, these substitutions may affect protein conformation and proper folding.

Our findings showed that COL4A5 contained the highest number of potentially deleterious variants. The higher number of deleterious variants identified in COL4A5 is consistent with the prominent role of COL4A5 variants in the genetic basis of Alport syndrome reported in the literature[49].

The identification and prioritization of these variants may provide useful information for future genotyping, genetic screening, and experimental studies. These findings provide a prioritized set of potentially deleterious variants for further genetic, experimental, and clinical evaluation. However, further computational, experimental, and clinical studies are required to validate the actual effects of these variants and determine their potential relevance to genetic screening and disease characterization.

This study was derived from the master's thesis of Beyza Rümeysa Erginal Geç, entitled 'Analysis of Some Genes Associated with Alport Syndrome Using Bioinformatics Tools', conducted in the Department of Molecular Biology.

REFERENCES

1. Williamson DA. Alport's syndrome of hereditary nephritis with deafness. *Lancet*. 1961;2(7216):1321-1323. doi: 10.1016/S0140-6736(61)90899-6.

2. Gubler MC, Levy M, Broyer M, et al. Alport's syndrome: A report of 58 cases and a review of the literature. *Am J Med*. 1981;71(4):493-505. doi: 10.1016/0002-9343(81)90571-4.

3. Temme J, Peters F, Lange K, et al. Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-linked and autosomal recessive Alport mutations. *Kidney Int*. 2012;81(8):779-783. doi: 10.1038/ki.2011.452.

4. Gross O, Weber M. From the molecular genetics of Alport's syndrome to principles of organo-protection in chronic renal diseases. *Med Klin (Munich)*. 2005;100(12):826-831.

5. Migeon BR. X inactivation, female mosaicism, and sex differences in renal diseases. *J Am Soc Nephrol*. 2008;19(11):2052-2059. doi: 10.1681/ASN.2008020198.

6. Kashtan CE. Alport syndrome: Achieving early diagnosis and treatment. *Am J Kidney Dis*. 2021;77(2):272-279. doi: 10.1053/j.ajkd.2020.03.026.

7. Izzedine H, Tankere F, Launay-Vacher V, Deray G. Ear and kidney syndromes: Molecular versus clinical approach. *Kidney Int*. 2004;65(4):1290-1302.

8. Shaw EA, Colville D, Wang YY, et al. Characterization of the peripheral retinopathy in X-linked and autosomal recessive Alport syndrome. *Nephrol Dial Transplant*. 2007;22(1):104-108. doi: 10.1093/ndt/gfl607.

9. Byrne MC, Budisavljevic MN, Fan Z, et al. Renal transplant in patients with Alport's syndrome. *Am J Kidney Dis*. 2002;39(4):769-775. doi: 10.1053/ajkd.2002.31997.

10. Perrin D, Jungers P, Grünfeld JP, et al. Perimacular changes in Alport's syndrome. *Am J Ophthalmol*. 1980;90(1):1-7. doi: 10.1016/s0002-9394(14)75069-7.

11. Shah SN, Weinberg DV. Giant macular hole in Alport syndrome. *Ophthalmic Genet*. 2010;31(2):89-91. doi: 10.3109/13816811003767128.

12. Gross O, Licht C, Anders HJ, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int*. 2012;81(5):494-501. doi: 10.1038/ki.2011.407.

13. GeneCards. COL4A5 gene. GeneCards. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL4A5. Accessed April 27, 2026.

14. GeneCards. COL4A4 gene. GeneCards. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL4A4. Accessed April 27, 2026.

15. GeneCards. COL4A3 gene. GeneCards. https://www.genecards.org/cgi-bin/carddisp.pl?gene=COL4A3. Accessed April 27, 2026.

16. Karki R, Pandya D, Elston RC, Ferlini C. Defining “mutation” and “polymorphism” in the era of personal genomics. *BMC Med Genomics*. 2015;8:37. doi:10.1186/s12920-015-0115-z.

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17. Ismail S, Essawi M. Genetic polymorphism studies in humans. *Middle East J Med Genet*. 2012;1(2):57-63. doi: 10.1097/01.MXE.0000415225.85003.47.

18. MedlinePlus. What are single nucleotide polymorphisms (SNPs)? MedlinePlus Genetics.https://medlineplus.gov/genetics/understanding/genomicresearch/snp/. Accessed April 27, 2026.

19. Lander ES. The new genomics: Global views of biology. *Science*. 1996;274(5287):536-539.

20. Dabhi B, Mistry KN. In silico analysis of SNP in human TNF-α gene. *Meta Gene*. 2014;2:586-595. doi: 10.1016/j.mgene.2014.07.005.

21. Cargill M, Altshuler D, Ireland J, et al. Characterization of SNPs in coding regions of human genes. *Nat Genet*. 1999;22(3):231-238. doi: 10.1038/10290.

22. Rozario LT, Sharker T, Nila TA. In silico analysis of deleterious SNPs of human MTUS1 gene. *PLoS One*. 2021;16(6):e0252932. doi: 10.1371/journal.pone.0252932.

23. Mah JT, Low ES, Lee E. In silico SNP analysis and bioinformatics tools. *Drug Discov Today*. 2011;16(17-18):800-809. doi: 10.1016/j.drudis.2011.07.005.

24. The UniProt Consortium. UniProt: The universal protein knowledgebase in 2021. *Nucleic Acids Res*. 2021;49(D1):D480-D489. doi:10.1093/nar/gkaa1100.

25. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4(7):1073-1081. doi: 10.1038/nprot.2009.86.

26. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. *Annu Rev Genomics Hum Genet*. 2006;7:61-80. doi: 10.1146/annurev.genom.7.080505.115630.

27. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7(4):248-249. doi: 10.1038/nmeth0410-248.

28. Choi Y, Chan AP. PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015;31(16):2745-2747. doi: 10.1093/bioinformatics/btv195.

29. Hecht M, Bromberg Y, Rost B. Better prediction of functional effects for sequence variants. *BMC Genomics*. 2015;16(Suppl 8):S1. doi: 10.1186/1471-2164-16-S8-S1.

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30. Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: Predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Res*. 2005;33(Web Server issue):W306-W310. doi: 10.1093/nar/gki375.

31. Cheng J, Randall A, Baldi P. Prediction of protein stability changes for single-site mutations using support vector machines. *Proteins*. 2006;62(4):1125-1132. doi: 10.1002/prot.20810.

32. Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics*. 2010;11:548. doi: 10.1186/1471-2105-11-548.

33. Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res*. 2010;38(Web Server issue):W214-W220. doi: 10.1093/nar/gkq537.

34. Wang Z, Moult J. SNPs, protein structure, and disease. *Hum Mutat*. 2001;17(4):263-270.

35. Yue P, Li Z, Moult J. Loss of protein structure stability in disease. *J Mol Biol*. 2005;353(2):459-473. doi: 10.1016/j.jmb.2005.08.020.

36. Zhou J, Hertz JM, Tryggvason K. Mutation in COL4A5 chain in Alport syndrome. *Am J Hum Genet*. 1992;50(6):1291-1300.

37. Knebelmann B, Deschenes G, Gros F, et al. Substitution of arginine for glycine 325 in COL4A5. *Am J Hum Genet*. 1992;51(1):135-142.

38. Renieri A, Seri M, Myers JC, et al. De novo mutation in COL4A5 gene. *Hum Mol Genet*. 1992;1(2):127-129. doi: 10.1093/hmg/1.2.127.

39. Choi M, Anistan YM, Eckardt KU, Gollasch M, Nickel P. Possible digenic disease in COL4A3 and COL4A5. *Nephron*. 2019;141(3):213-218. doi: 10.1159/000495764.

40. Kashtan CE, Fish AJ, Kleppel M, Yoshioka K, Michael AF. Nephritogenic antigens in Alport syndrome. *J Clin Invest*. 1986;78(4):1035-1044. doi: 10.1172/JCI112658.

41. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome and type IV collagen. *N Engl J Med*. 2003;348(25):2543-2556. doi: 10.1056/NEJMra022296.

42. Kuivaniemi H, Tromp G. Type III collagen: Structure and diseases. *Gene*. 2019;707:151-171. doi: 10.1016/j.gene.2019.05.003.

43. Shboul M, Sassi H, Jilani H, et al. Glycine to serine mutation in COL2A1 gene. *AIMS Mol Sci*. 2021;8(1):76-85. doi: 10.3934/molsci.2021004.

44. Nuytinck L, Tükel T, Kayserili H, Apak MY, De Paepe A. Glycine to tryptophan substitution in collagen. *J Med Genet*. 2000;37(5):371-375. doi: 10.1136/jmg.37.5.371.

45. Molnár J, Szakács G, Tusnády GE. Disease-associated mutations in transmembrane proteins. *PLoS One*. 2016;11(3):e0151760. doi: 10.1371/journal.pone.0151760.

46. Wilcox W, Scott L, Cohn D. An N-terminal glycine to cysteine mutation in the collagen COL1A1 gene produces moderately severe osteogenesis imperfecta. *Am J Hum Genet*. 1994;55(suppl 3).

47. MacArthur MW, Thornton JM. Influence of proline residues on protein conformation. *J Mol Biol*. 1991;218(2):397-412. doi: 10.1016/0022-2836(91)90721-H.

48. Creighton TE. Disulphide bonds and protein stability. *BioEssays*. 1988;8(2-3):57-63. doi: 10.1002/bies.950080204.

49. Yavas C, Ozdemir Ozgenturk N, Dogan M, et al. A deeper insight into COL4A3, COL4A4, and COL4A5 variants and genotype-phenotype correlation of a Turkish cohort with Alport syndrome. *Mol Syndromol*. 2024;15(1):1-13. doi: 10.1159/000533915.

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