

Original research

CYP4V2 mutation screening in an Iranian Bietti crystalline dystrophy pedigree and evidence for clustering of CYP4V2 mutations

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Received 11 November 2018; revised 26 December 2018; accepted 16 January 2019

Available online 2 March 2019

Abstract

Purpose: To report the genetic analysis of an Iranian Bietti crystalline dystrophy (BCD)-affected family, and to review previously reported mutations in the gene and assess the distribution of affected amino acids in the encoded protein.

Methods: The eleven exons of *CYP4V2* were sequenced in the DNA of the proband of the Iranian BCD family. The putative disease-causing variation was screened in all affected and non-affected members. BCD causing *CYP4V2* mutations previously reported in the literature were compiled, and positions of amino acids affected by nonsense and missense mutations were mapped onto the primary structure of the *CYP4V2* protein.

Results: C.1219G > T in *CYP4V2* that causes p.Glu407* was identified as cause of BCD in the Iranian family. The mutation segregated with disease status. Clinical presentations were similar among affected members, except that one patient presented with retinal macular hole. Twelve nonsense and 47 missense mutations in *CYP4V2* were compiled. Inspection of distribution of amino acids affected by the mutations suggested non-random distribution and clustering of affected amino acids in nine regions of the protein, including regions that contain the heme binding site, the metal binding site, and a region between these binding sites. The most C-terminus proximal nonsense mutation affected position 482.

Conclusions: This study presents results of the genetic analysis of an Iranian BCD family. Protein regions affected by mutations within the nine mutation clusters include regions well conserved among orthologous proteins and human CYP4 proteins, some of which are associated with known functions. The findings may serve to identify reasonable candidate gene region targets for gene editing therapy approaches.

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Keywords: p.Glu407*; Bietti crystalline dystrophy; BCD; *CYP4V2*; *CYP4V2* mutations

Introduction

In 1937, the Italian ophthalmologist GB Bietti first described shared eye presentations in three individuals; the condition later came to be known as Bietti crystalline dystrophy (BCD; MIM210370).¹ Clinical diagnosis of BCD is fairly straightforward. This retinopathy is characterized by presence of numerous very small yellow–white spots at the posterior pole of the fundus, and sometimes also in the corneal

The authors declare absence of any competing, commercial, or conflicts of interests, regarding this publication.

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Peer review under responsibility of the Iranian Society of Ophthalmology.

<https://doi.org/10.1016/j.joco.2019.01.007>

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limbus.^{2–4} Atrophy of the retinal pigmented epithelium (RPE) and choriocapillaris, pigment clumping, and choroidal sclerosis ensue. Affected individuals experience decreased vision, night blindness, and constriction of visual fields.⁵ Disease progression is usually slow, with legal blindness being the end stage presentation. Onset of symptoms is usually between the third and fifth decades of life. Although lipid inclusions similar to those seen in the retina are present in lymphocytes and skin fibroblasts of some BCD patients, clinical anomalies seem to be limited to the eye.^{5,6} Recent advances using in vitro models of BCD may shed light on underlying features of BCD etiology and possibly treatment protocols.⁷ BCD patients from several parts of the world have been reported, but most cases have been from East Asian populations particularly China and Japan.^{1,8} Among European countries, the largest patient cohort described was from Italy.⁹ In a cross-sectional study performed on 121 index retinitis pigmentosa (RP) patients, prevalence of BCD based on clinical diagnosis was 2.5% (3/121) of all RP index cases, and 10% (3/31) of autosomal recessive non-syndromic RP index cases.¹⁰ Therefore, although BCD is generally considered a rare disorder, it may be under-diagnosed because correct diagnosis in the cross-sectional study sometimes requires careful clinical examination.

BCD incidence in affected families suggested a monogenic autosomal recessive inheritance mode. Genetic analysis led to identification of *CYP4V2* that encodes cytochrome P450, family 4, subfamily V, polypeptide 2, as its causative gene.^{11,12} The many genetic studies that have been done since the discovery of the gene confirm that BCD is an autosomal recessive disorder and that mutations in *CYP4V2* account for disease status in virtually all affected individuals genetically analyzed.^{9,13–23} *CYP4V2* is highly expressed in the RPE, and it is thought that genetic defects in the encoded protein prevent degradation of lipid substrates which then accumulate in the eyes of patients.^{24,25} However, the exact molecular mechanism underlying *CYP4V2* involvement in crystal accumulation and RPE atrophy remains unclear.

Clinical and genetic studies on BCD have mostly been performed on patients from China and Japan. Here, we report a study on an inbred Iranian pedigree (BCD-100) with four BCD-affected individuals distributed in two generations (Fig. 1A). The clinical features of the proband of pedigree were previously reported.²⁶ To the best of our knowledge, this is the first report of results of genetic analysis on BCD patients from Iran. In addition to results on the Iranian family, we present review on all published mutation screening studies that demonstrates clustering of mutations in the encoded protein.

Methods

This research was performed in accordance with the Declaration of Helsinki and with approval of the Ethics Board of the University of Tehran and the Ophthalmic Research Center of Shahid Beheshti University of Medical Sciences. Participants consented to participate in the study.

Clinical evaluations

The proband of BCD-100 and all his affected and available non-affected family members were recruited²⁶ (Fig. 1A). There was no related past medical, surgical, or drug history in the pedigree, except for one patient who had undergone pars plana deep vitrectomy for macular hole in his left eye. Complete ocular examination including central visual acuity (VA) with Snellen chart, slit-lamp biomicroscopy, applanation tonometry, and dilated funduscopy was performed on available pedigree members. BCD diagnosis was based on observation of small sparkling yellowish crystals in the posterior pole, atrophy of retinal pigment epithelium, and sclerosis of the choriocapillaris. Color fundus photography, optical coherence tomography (OCT), electroretinography (ERG), and electro-oculography (EOG) were done for the proband of the pedigree.

Genetic analysis

DNA was isolated from leukocytes of the proband and seven other available members of pedigree BCD-100 by a standard phenol–chloroform protocol. The eleven exons of *CYP4V2* in the DNA of the proband were amplified by polymerase chain reactions (PCR). Sequences of primers are available upon request. The amplicons were sequenced using the dideoxy nucleotide terminator chemistry and an ABI Prism 3700 instrument (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). *CYP4V2* reference sequences used were NC_000004.11, NM_207352.3, and NP_997235. Upon identification of a putative disease causing mutation, the variation was screened in other pedigree members by direct sequencing. It was also sought in the newly available Iranome database (www.iranome.ir) that contains exome sequence data on 800 healthy Iranians.

In addition to screening of the Iranian pedigree, a review of the literature was performed on all previously reported BCD causing mutations in *CYP4V2*. Relevant articles were sought in Google Scholar using search words *CYP4V2*, BCD, and Bietti Crystalline Dystrophy, and also in the Human Genome Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk/ac/index.php>). Results of mutation screenings were recorded, and the mutations were mapped onto the primary structure of the encoded protein. Distribution of the mutations along the length of the protein were noted with respect to known functional domains and landmarks of the protein, with respect to conserved regions in the orthologous bird (*Gallus gallus*; NP_001001879.1) and fish (*Kryptolebias marmoratus*, XP_017263932) proteins, and also with respect to two other human CYP4 cytochromes (CYP4A22, NP_001010969.2 and CYP4B1, NP_001306092.1). For identification of conserved regions, the amino acid sequences were aligned using the ClustalW software (European Bioinformatics Institute, Hinxton, UK; <http://www.ebi.ac.uk/clustalw>). The human sequence was considered a prototype of the mammalian *CYP4V2*

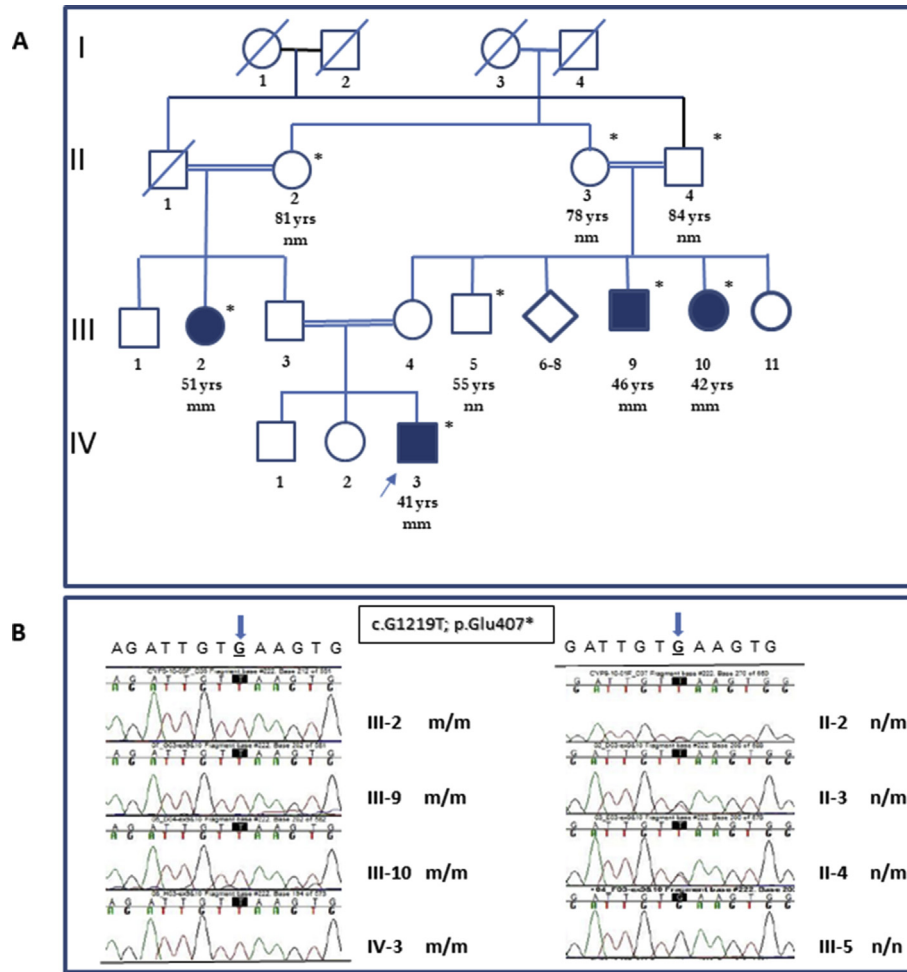


Fig. 1. Iranian Bietti crystalline dystrophy (BCD) pedigree (BCD-100) with p.Glu407* mutation in *CYP4V2*. A. *CYP4V2* genotypes of individuals tested are presented. Arrow, proband; *, individuals available for clinical examination and genetic analysis; filled circles and squares, BCD affected; unfilled circles and squares, asymptomatic at time of examination; m, mutated *CYP4V2* allele; n, wild-type *CYP4V2* allele. B. DNA sequence chromatograms showing the homozygous and heterozygous c.1219G > T genotypes of *CYP4V2*, and the wild type genotype.

sequence, as variation among orthologous proteins from three mammalian species were minimal (not shown).

Results

Clinical evaluations

Four members of BCD-100 (III-2, III-9, III-10, and IV-3) were diagnosed to have BCD. Affected individuals reported night blindness and progressive visual loss as early symptoms of the disease. Corrected distance visual acuity at time of diagnosis (at ages 41–51 years) ranged from finger count to 20/25 (Table 1). Slit-lamp examination revealed normal anterior segment without any corneal limbal crystal deposition. Fundus examination showed bilateral numerous yellowish-white dot shaped crystalline deposits distributed in the posterior pole and mid-periphery, pigment deposits, diffuse RPE, and choriocapillaris atrophy (Fig. 2A, B). OCT depicted intraretinal hyper-reflective spots with loss of RPE

and photoreceptor layers (Fig. 2C). A closed macular hole was evident in the proband's left eye (Fig. 2D). ERG (Fig. 2E) and EOG (Fig. 2F) responses were severely decreased which documented severe chorioretinal degeneration. All ophthalmic examinations were normal in four other healthy BCD-100 members aged 55–84 years old (II-2, II-3, II-4, and III-5).

Genetic analysis

The BCD-100 pedigree suggested an autosomal recessive mode of disease inheritance (Fig. 1A). Sequencing of the exons of *CYP4V2* in the proband showed two sequence variations, c.367C > G that causes p.Leu22Val, and c.1219G > T that causes p.Glu407* (Fig. 1B). Both were present in the homozygous state. C.1219G > T in exon 9 that causes p.Glu407* was considered to be the disease-causing mutation. The variation that causes p.Leu22Val is a common polymorphisms and thus unlikely to be associated with disease

Table 1
 Characteristics of Bietti crystalline dystrophy (BCD) patients from BCD-100 pedigree.

Patient	Sex	Age at examination (years)	Age of disease onset	Initial visual symptoms	Corneal crystalline deposits	Corrected distance visual acuity	CYP4V2 genotype
III-2	F	51	4th decade	Nyctalopia	Absent	OD: CF 2 m OS: CF 2 m	mm
III-9	M	46	4th decade	Nyctalopia	Absent	OD: 20/30 OS: 20/30	mm
III-10	F	42	4th decade	Nyctalopia	Absent	OD: 20/200 OS: 20/80	mm
IV-3	M	41	4th decade	Nyctalopia	Absent	OD: 20/25 OS: 20/30	mm

F: Female; M: Male; mm: Homozygous for the mutated allele; CF: Counting fingers.

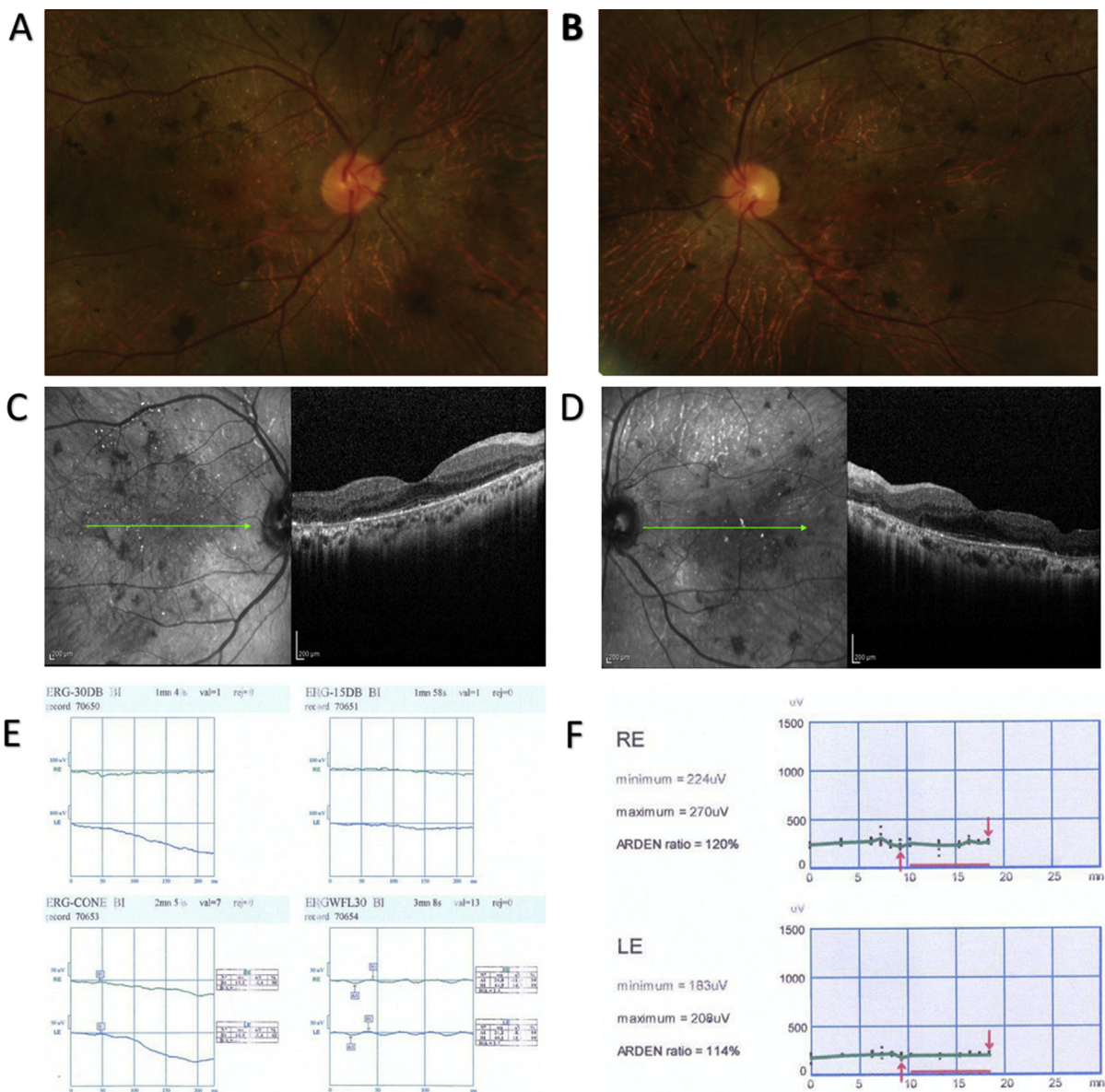


Fig. 2. Images that evidence Bietti crystalline dystrophy (BCD) in proband (IV-3) of BCD-100. A, B. Color fundus photographs show bilateral yellowish crystalline deposits in the posterior pole, retinal pigment epithelium (RPE) clumping, with RPE and choriocapillaris atrophy. C. Optical coherence tomography (OCT) of the right eye illustrates intraretinal hyper-reflective spots related to crystalline deposits within the neuroretinal layers, disturbed RPE-photoreceptor outer/inner segment layers, thinning of the foveal region, and perifoveal external limiting membrane loss. D. Closed macular hole is seen in the OCT of the left eye. E. Electroretinography (ERG) shows severely extinguished dark adapted and light adapted responses in both eyes. F. Electrooculography (EOG) of both eyes depicts decreased voltages and abnormal ARDEN ratio.

status (rs1055138; minimum allele frequency (MAF) = 0.409, <https://www.ncbi.nlm.nih.gov/projects/SNP>). The variation has previously been reported not to be cause of BCD.¹⁵ The PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>) and SIFT (<http://sift.jcvi.org/>) bioinformatics tools predict that the variation is not deleterious. C.1219G > T in exon 9 that causes p.Glu407* was considered to be the disease-causing mutation. Sequencing of eight pedigree members showed that all four clinically-diagnosed BCD patients were homozygous carriers of the c.1219G > T mutation and that the unaffected individuals were heterozygous carriers of the mutation or homozygous carriers of the wild type allele (Fig. 1B). Segregation of the p.Glu407*-causing variation with disease status supports its role in disease etiology. The c.1219G > T variation was not observed in the exome sequences of 800 healthy Iranians reported at the Iranome site. It was also not observed in in-house exome data of at least 50 Iranians without ocular diseases. The p.Glu407* mutation is expected to affect protein function because it will cause premature translational termination and deletion of 119 amino acids from the C-terminus of the encoded protein. The PROVEAN bioinformatics tool (provean.jcvi.org) predicts that the amino acid change will deleteriously affect protein function.

Supplementary Table S1 lists 81 reported pathogenic mutations in *CYP4V2*, including 12 nonsense and 47 missense mutations that to the best of our knowledge include all nonsense and missense mutations that have been reported till now. Fig. 3 shows the distribution of the nonsense and missense mutations along the length of the encoded protein that contains 525 amino acids. It also schematically shows positions of the mutations with respect to known functional domains and landmarks of the protein. As nonsense mutations are likely to have profound effects on protein structure and function, these are mapped together and separately from the missense mutations. By a non-subjective eye inspection of the distribution of the missense mutations, we defined nine regions of mutation clustering (I-IX), each region containing two to 25 amino acids and 2 to 7 mutations (Fig. 3). Two regions (VI and VIII) include known functional landmarks in the protein and another (VII) is positioned close to and between the two landmarks. Region VI includes the heme binding site (p.Glu329) and region VIII includes the cytochrome p450 cysteine heme-iron ligand signature (p.Cys467). Region I is positioned within the transmembrane domain. It is evident that the p.Glu407* mutation found in the Iranian BCD-100 family is expected to cause deletion of Region VIII.

Table 2 more precisely shows the distribution of the mutation clusters described above with respect to conserved regions among orthologous sequences and with respect to other members of the human *CYP4* family. Amino acids in regions defined by mutation clustering are generally better conserved among the orthologous as compared to the paralogous human proteins. Regions defined by clusters VI, VII, and VIII are well conserved in both orthologous and paralogous sequences, and 14 of the 18 mutations reported in these regions affect totally conserved amino acids in both groups of *CYP4* proteins. Fourteen of the 18 reported mutations outside regions VI, VII,

and VIII affect fully conserved (nine amino acids) or semi-conserved (five) amino acids in orthologous proteins, whereas only four affect non-conserved amino acids. Only three of the mutations outside regions VI, VII, and VIII affect fully conserved (one amino acid) or semi-conserved (two amino acids) in paralogous proteins, whereas fifteen affect non-conserved amino acids. These figures suggest that at least some of the regions defined by mutations outside regions VI, VII, and VIII may have *CYP4V2* specific functions. P.Ser482* is the most C-terminus proximal nonsense mutation reported to date. Its position signifies that amino acids after position 482 at the C-terminus of the protein are important for protein structure and/or function.

Discussion

Reports of clinical presentations and genetic analysis of BCD patients from outside of China and Japan exist, but are few.^{9,10,14,16,22} To the best of our knowledge, only six unrelated BCD patients from the Middle East have been reported: three from Lebanon and three whose origins were designated as Arabic or the Middle East.^{14,16,22} Interestingly, two of the three Lebanese probands carried the same homozygous p.Ile111Thr mutation in *CYP4V2*, and the two described to be of Middle Eastern origin both had the same p.Gln450*-causing mutation.^{14,16} This suggests that, like China and Japan, different specific *CYP4V2* mutation with likelihood of a founder effect may be common among BCD patients of each of various populations. Verification of this would be of value for possible gene therapy intervention protocols. The BCD pedigree described here is the first to be reported from Iran. The p.Glu407*-causing mutation found has only recently been reported in a single patient of unknown origin.²² More Iranian BCD patients need to be genetically screened to assess the mutation spectrum in this population. As consanguineous marriages are common in Iran, and as BCD may be under-diagnosed, attentive clinical screenings may identify more patients. The clinical presentations of the four BCD affected individuals with the same p.Glu407* mutation were similar, except for difference in their visual acuity and occurrence of macular hole in one patient. The former may at least partly be due to differences in age at time of examination. Although macular hole formation has also been previously reported in a few BCD patients, strong evidence in favor of implicating *CYP4V2* for this condition is lacking.^{27,28}

The review of previously reported pathogenic *CYP4V2* mutations revealed a non-random distribution of the mutations in the primary structure of the encoded protein. Regions that include clusters of mutations are expected to be important with respect to structure and/or function of the protein. Nine clusters of missense mutations were defined. In support of the potential importance of the clusters, many mutations were positioned within regions well conserved in the primary structure of orthologous and paralogous sequences. In fact, the regions of two of the clusters (VI and VIII) include the heme and iron binding sites, and one region (VIII) may affect the

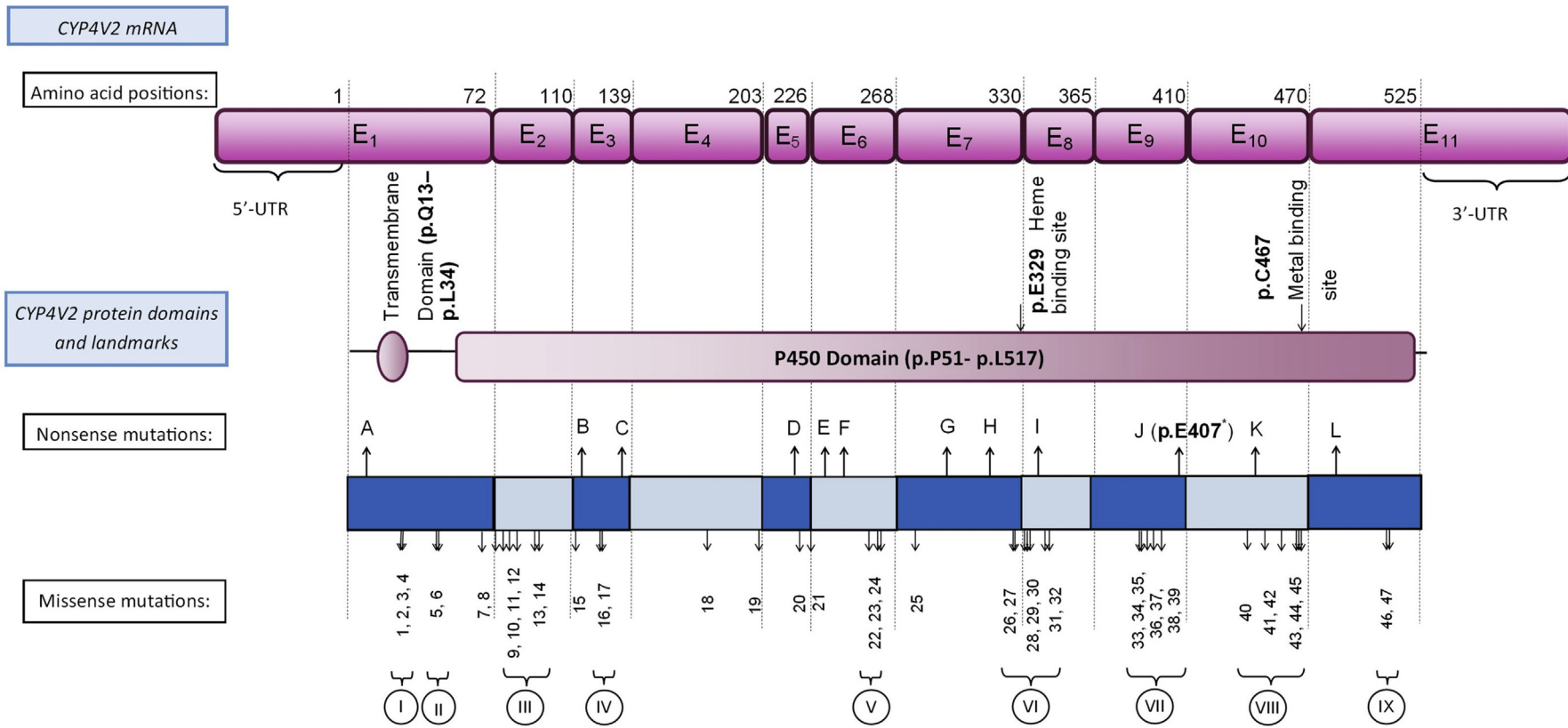


Fig. 3. Distribution of amino acids affected by reported nonsense and missense mutations along length of CYP4V2 protein. CYP4V2 mRNA (upper panel) and CYP4V2 protein (middle and lower panels), and correspondence between mRNA and protein are schematically shown. The lower panel shows the distribution of amino acids affected by nonsense (arrows pointing up) and missense (arrows pointing down) mutations. Clusters (I-IX) of positions of amino acids affected by missense mutations are also shown. Letters A–L refer to 12 nonsense mutations reported in the literature, and numbers 1–47 refer to 47 missense reported mutations (see Table S1).

Table 2
Positions of pathogenic CYP4V2 mutations with respect to conserved amino acids in orthologous and paralogous proteins.

Mutation cluster	Positions of bordering mutations	Exon	Conservation in human/ chicken/ fish	Conservation in CYP4 family CYP4V2/ CYP4B1/ CYP4F22
I	L22-G26	1	• • • LSLAG ITLLL VSILV : : : :	• • • LSLAG ----- LLFFL :
II	W44-Q45	1	• • WQ WW WF *	• • CR -- RQ
III	F73-G95	2	• • • • • FFQQIIEYTEEYRHMPLLLKLVWG FFKQLQQYADEFRKMPMFKLWLG FFRQIVDYTTSTRDVPFLFKLVWG **:*: :*: .:*.::**:*	• • • • • FFQQIIEYTEEYRHMPLLLKLVWG ----- LQDEKKVLDNMHHVLLVWMPVL :
IV	S122-M123	3	• • SM FL FA	• • SM -- LF
V	H254-Q259	6	• • • • HKSLQ HERNLK HDRTLK * . . . *	• • • • HKSLQ FLRACQ FRQACD . : : :
VI	D324-Y343	7-8	• • • • • DTFMFEGHDTTAAAINWSLY DTFMFEGHDTTAAAMNWVLY DTFMFEGHDTTAAAMNWTLH *****:*. ** *:	• • • • • DTFMFEGHDTTAAAINWSLY DTFMFEGHDTTTSGISWFLY DTFMFEGHDTTSSGISWMLF *****:*. ** *:
VII	K386-R400	9	• • • • • KETLRLFPSVPLFAR KEALRLFPSVPMFAR KESLRLFPSVPPYFAR **:*:***** **	• • • • • KETLRLFPSVPLFAR KESFRLYPPVPQVYR KESLRQYPPVTLVSR **:*:*. * . . . *
VIII	R443-C467	10	• • • • • RFFPENAGGRHPYAYVPFSAGPRNC RFFPENSKGRHPYAYVPFSAGPRNC RFFPQNSVGRPPYAYIPFSAGLRNC ****:*: ** *:*:*****	• • • • • RFFPENAGGRHPYAYVPFSAGPRNC RFSTENASKRHPFAMPFASAGPRNC RFDPDNPQQRSPYAVVPFSAGPRNC ** .:* . . * . * *:*:*****
IX	R508-P509	11	• • RP RP RP **	• • RP RS RT * .

Symbols above amino acids: •: Positions at which mutations have been reported. Symbols below amino acids: *: Positions which have a single, fully conserved residue; .: Positions which have conservation between groups of amino acids with strongly similar properties (semi-conserved amino acids); .: Positions which have conservation of amino acids with weakly similar properties. Designating of conservation made by CLUSTALW (<https://www.genome.jp/tools-bin/clustalw>).

appropriate positioning of the former two. In addition to the potential biochemical implications of the findings, the results of the review may serve to identify reasonable candidate gene region targets for gene editing therapy approaches including the CRISPR/Cas9 system.

Acknowledgments

We acknowledge the Ophthalmic Research Center of Shahid Beheshti University of Medical Sciences for funding this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.joco.2019.01.007>.

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