RESEARCH ARTICLE

Whole-exome sequencing analysis reveals co-segregation of a *COL20A1* missense mutation in a Pakistani family with striate palmoplantar keratoderma

Muhammad Ismail Khan[1] · Soyeon Choi[2] · Muhammad Zahid[1] · Habib Ahmad[1] · Roshan Ali[3] · Musharraf Jelani[4] · Changsoo Kang[2]

Abstract

Palmoplantar keratoderma (PPK) is a rare group of excessive skin disorder characterized by thickness over the palms and soles. The striate palmoplantar keratoderma (PPKS) is a form in which hyperkeratotic lesions are restricted to the pressure regions extending longitudinally in the length of each finger to the palm. Dominantly inherited mutations in genes including desmoglein 1, desmoplakin and keratin 1 have been suggested as genetic causes of PPKS. In this study, we investigated a three-generation Pakistani family segregating PPKS phenotype in autosomal dominant fashion to identify genetic cause in this family. We have performed whole-exome and Sanger sequencing followed by in silico bioinformatics analysis to pinpoint candidate mutation associated with PPK. Revealed a novel heterozygous mutation (NM_020882.2, *COL20A1* c. 392C > G; p.Ser131Cys) in the loop region close to fibronectin type III-1 domain of the c ollagen 20 α1. This variant was not found in our in-house 219 ethnically matched Pakistani unaffected controls and showed minor allele frequency of 3.4×10^{-5} in Exome Aggregation Consortium database containing exome data of 59,464 worldwide individuals. It was assigned as "pathogenic" by in silico prediction tools. Previously, association of mutation in the *COL14A1*, one of the paralogous gene of *COL20A1*, with PPK was reported in the study with a Chinese family. Our study proposes *COL20A1* gene as another potential candidate gene for PPKS which expand the spectrum of collagen proteins in the pathogenicity of PPK.

Keywords Palmoplantar keratoderma · Exome sequencing · COL20A1 · Mutations · PPKS · Pakistani family

Muhammad Ismail Khan and Soyeon Choi have contributed equally to this work.

✉ Musharraf Jelani
  mjelani@kau.edu.sa

✉ Changsoo Kang
  ckang@sungshin.ac.kr

[1] Department of Zoology, Islamia College University, Peshawar, Pakistan

[2] Department of Biology, Sungshin Women's University, Seoul, Republic of Korea

[3] Department of Molecular Biology and Genetics, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan

[4] Department of Genetic Medicine, Faculty of Medicine and Princess Al-Jawhara Albrahim Center of Excellence in Research of Hereditary Disorders, King Abdulaziz University, Jeddah 21589, Saudi Arabia

Introduction

Palmoplantar keratoderma (PPK) belongs to a group of common skin disorders characterized by excessive thickening of the epidermis over the palms and soles of the human body (Hennies et al. 1995). This disorder can be classified into hereditary and acquired forms (Lucker et al. 1994), and multiple family-based studied revealed that genetic factors are one of the causes increasing susceptibility to hereditary PPK. The feature of hyperkeratosis may be isolated (the sole dominant clinical feature) or it may be associated with other ectodermal abnormalities or extra cutaneous manifestations.

The striate (PPKS) is a form in which hyperkeratotic lesions are restricted to the pressure regions extending longitudinally in the length of each finger to the palm. It was revealed that mutations in three genes including *desmoglein* (*DSG1*), *desmoplakin* (*DSP*) and *keratin-1* (*KRT1*) increased susceptibility to the PPKS (Armstrong et al. 1999; Rickman et al. 1999; Whittock et al. 2002).

In punctate type of PPK (PPPK), numerous hyperkeratotic papules are distributed irregularly on the palms and soles, and mutations in the *alpha and gamma adaptin binding protein* (*AAGAB*) gene and *collagen 14 α1* (*COL14A1*) have been reported to be genetic causes of punctate type 1 and type 2 PPK, respectively (Giehl et al. 2012; Guo et al. 2012). All of genetic mutations mentioned above co-segregated with the disorders in dominant manner.

The prevalence of affected individuals may be underestimated in cases when mildly affected individuals do not seek specialized medical care or they might be diagnosed incorrectly (Schiller et al. 2014). Isolated PPK patients (n = 36) have been observed without associated anomalies of skin or appendages including ichthyosis, ectodermal dysplasia and epidermolysis bullosa (Has and Technau-Hafsi 2016).

Recently, genetic studies using whole-exome sequencing (WES) analysis is a promising approach to identify genetic causes of hereditary PPK.

In this study, we performed whole-exome sequencing of a consanguineous Pakistani family presenting clinical features of PPKS in order to pinpoint a causative mutation for it.

Materials and methods

Study subjects

A consanguineous family affected with PPKs (Fig. 1) was ascertained from a remote village of Khyber Pakhtunkhwa province, Pakistan. The disease was assumed to segregate in autosomal dominant form in this family. Informed consent form was taken from the family elders or the legal guardians of the affected individuals under the ages of 18. The study was approved by the institutional review board for medical genetics research and ethics, King Abdulaziz University, Jeddah, Saudi Arabia under project.

Whole-exome and Sanger sequencing

Peripheral blood (10 ml) was drawn from five affected and eight unaffected family members after informed consents were obtained, and genomic DNAs were isolated using QIAamp DNA Blood Max kit (Qiagen, Hilden, Germany). Concentration of gDNAs were measured by PicoGreen™ assay according to the manufacturer's instructions (Promega, Madison, WI, USA). Whole-exomes of five individuals were captured using SureSelect V5 kit (Agilent Technologies, Santa Clara, CA, USA), and were sequenced as 100-bp
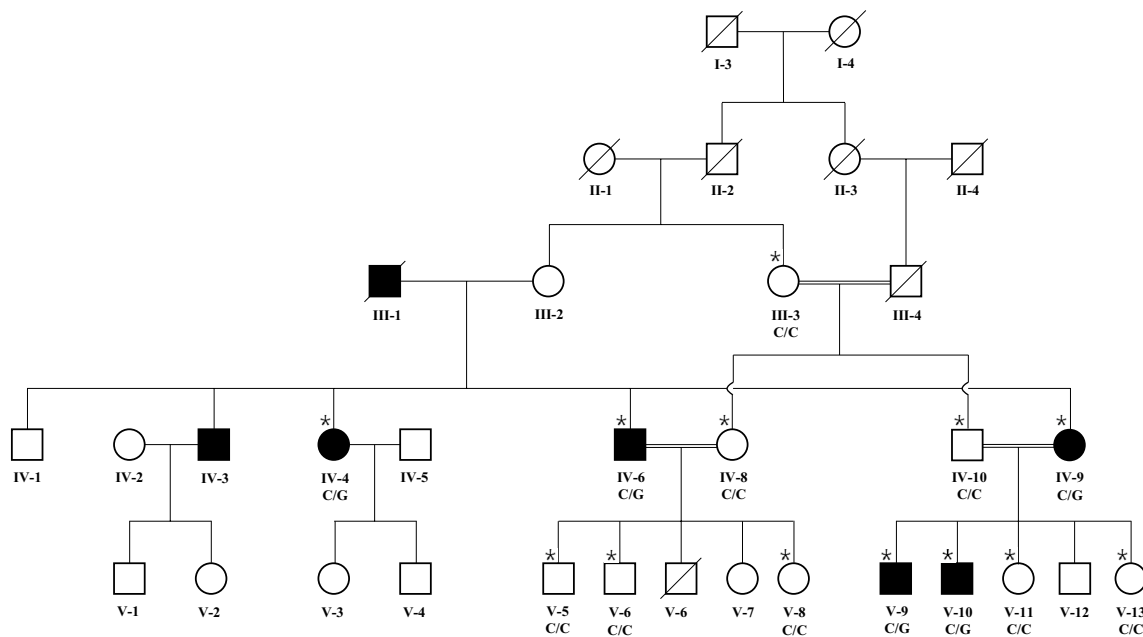

Fig. 1 Pedigree of the Pakistani family affected by striate palmoplantar keratoderma (PPKS). The black-filled symbols denote affected individuals whose symptoms are present over their palms and soles. In this family, PPKS is inherited as dominant mode. Blood samples from the individuals marked with asterisk were available for whole-exome sequencing and Sanger sequencing. Each normal individual was homozygous for the normal C allele (serine) at the 392th nucleotide position on *COL20A1*; on the contrary, all the affected individuals were heterozygous for the G allele (cysteine) which replaces serine with cystein at the amino position of 131th of the COL20A1 protein

paired-end reads on an Illumina HiSeq2000 machine (Illumina, San Diego, CA, USA). The genotype of candidate variants was confirmed in all family members by Sanger sequencing.

Bioinformatic analysis

To screen candidate variants which are likely to cause hyperkeratosis in the family, we utilized several bioinformatic tools and followed a series of criteria to filter out other variants less related to the disease. Using SeqMan NGen (Lasergene Genomic Suite v.12, DNASTAR, Madison, WI, USA), we aligned the sequenced reads in FASTQ file format to hg 19 (GRCh37, NCBI). Arraystar v. 12 (Rockvile, MD, USA) annotated normal and variant alleles based on dbSNP 142 (UCSC).

Annotated normal and variant alleles were sorted out to screen the candidate mutations triggering hyperkeratosis, following a set of criteria. (1) We hypothesized that striate palmoplantar keratoderma inherits as an autosomal dominant mode of inheritance in this family; that is, affected individuals (IV-4, -6, -9, V-9 and -10) are heterozygous for the minor allele; unaffected parents (IV-8, -10, V-5, -6, -8 -11 and -13) are homozygous for the major allele. (2) Variants showing a minor allele frequency > 0.003 in the ExAc browser (http://exac.broadinstitute.org) were excluded. (3) Heterozygous variants in our in-house exome sequence data obtained from 45 normal unrelated Pakistani individuals were also excluded. (4) Synonymous and deep intronic variants other than those present at splice junctions were excluded. The remaining candidate variants were further analyzed in silico to predicted their pathogenic effect using PolyPhen-2, PROVEAN, and MutationTaster softwares (Adzhubei et al. 2010; Choi et al. 2012; Schwarz et al. 2014).

Three-dimensional protein modeling

No X-ray crystal structure was available for the *N*-terminal of the protein where the mutation of interest had occurred. As no suitable template was available for the *N*-terminal region of the protein; therefore, we generated ab-initio three dimensional protein model using online Robetta Server (Bystroff et al. 2000) and Quark (Xu and Zhang 2011). The model was predicted only for the segment 23–165 (leaving the signal peptide i.e. 1–22) due to the limitation of the servers. The predicted models were assessed using the program Verify3D (Bowie et al. 1991), PROCHECK, WHAT_CHECK (Hooft et al. 1996), Errat (Colovos and Yeates 1993), and Prove (Pontius et al. 1996). The Ramachandran plot (Ramachandran et al. 1963) was analyzed to find residues in forbidden regions. The selected model was refined using ModRefiner (Xu and Zhang 2011). the effect of Serine to Cystein amino acid at position 131 using PDB_Hydro (Azuara et al. 2006) was assessed in the predicted wild type and mutant models. The structural difference was assessed using Biovia Discovery studio visualizer.

Results

Clinical characteristics of subjects

Affected individuals in the Pakistani family were clinically diagnosed as striate type of PPK. Both males and females were affected. All the affected individuals had mild epidermal thickening over the pressure areas of palms and fingers (Fig. 2a, b); however, soles were not affected. One (V-10) of the affected individuals had intellectual disability, global developmental delay and language impairment and his brain magnetic resonance imaging showed delayed myelination;
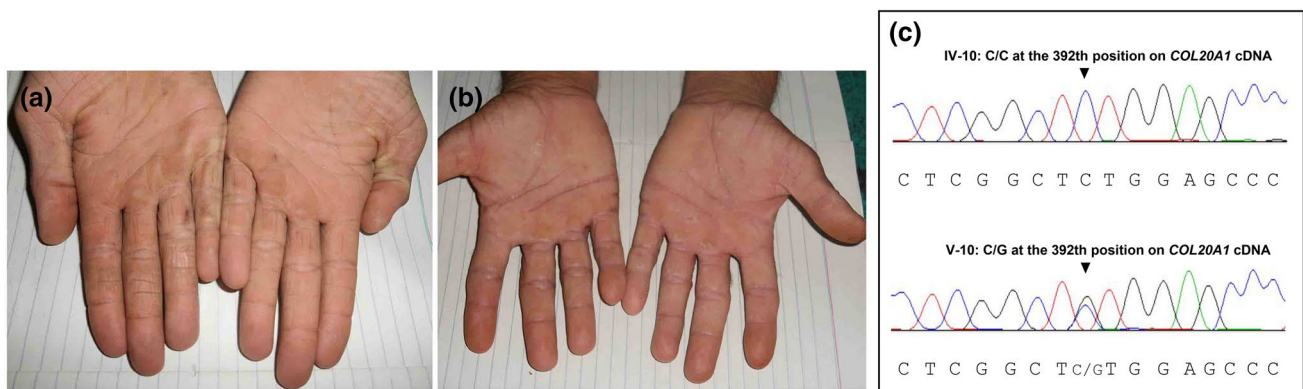

Fig. 2 Clinical presentation of patients showing keratoderma over the palms. Note the specifically the yellowish appearance at the pressure areas and figures in patients IV-6 (**a**) and V-9 (**b**). **c** illustrates Sanger sequencing results of the normal individual (IV-10) and the PPKS patient (V-10) who is a son of the former. Including IV-10, the genotypes at the mutant loci (*COL20A1* c.392 C > G) of all normal individuals are homozygous for the C allele; those of the affected are heterozygous C and G alleles. (Color figure online)

however, these neurological features were not found in other PPK patients of the family.

Whole-exome sequencing and bioinformatic analysis

Among 13 genomic DNA samples available, whole exomes of five samples (the affecteds: IV-6 and V-9, the unaffecteds: IV-8, V-5 and -8) were sequenced. On average, the total number of bases identified in the reads was 9.2 Gb. It was 96.73% of bases that acquired phred score over 20. The mean coverage of the target regions was 100.

Including all the synonymous as well as non-synonymous variations, the five individuals have 46,138 exonic variants that met the first quality control criteria, which required that minimum Q call should be above 20 and minimum depth coverage be above ten. Of these, 4137 variants were heterozygous, and 336 variants remained after excluding the 3801variants which were found in 45 unrelated in-house Pakistani controls. Further exclusion of variants that show minor allele frequencies higher than 0.003 and that do not change amino acid sequence, 20 missense variants remained (Table 1). To narrow down to causative mutation, we further performed genotyping of all the 20 variants in the eight other family members (III-3, IV-4, -9, -10, V-6, -10, -11, -13) by Sanger sequencing, and

found that 19 variants except COL20A1 p.Ser131Cys were carried by at least one normal individuals in the family. Therefore, we could pinpoint that the COL20A1 variant replacing serine with cysteine at 131th amino acid position of the *COL20A1* gene (NM_020882.2, c.392C > G) at chromosome 20 as the candidate mutation. Concurrently, this variant was not found in the Sanger sequencing results healthy 219 normal Pakistani individuals. This gene is known to encode collagen type XX alpha 1 chain, however, there has been no human disease associated with disruptions in the normal function of this protein.

In silico analysis to simulate pathogenicity of the *COL20A1* p.Ser131Cys mutation suggested that this mutation was predicted to be "disease causing" by PolyPhen-2; PROVEAN, however mutation taster predicted it to be polymorphism.

Analysis of the three-dimensional structural modeling of both the normal and mutated COL20A1 proteins showed that two forms have almost identical topology except some minor changes. Both the wild-type and mutation COL20A1 consist of seven beta strands at *N*-terminal region, two small alpha helices and loop regions. The mutated amino acid position of 131 resides in the loop region. The mutation influenced the surface atom distributions as shown in the surface view of the models (Fig. 3).

Table 1 The list of candidate variations causing hyperkeratosis in the Pakistani family

Gene name	Chromosome	Amino acid change	Mutant allele frequency in ExAC[a]	In silico simulation of protein function		
				PROVEAN	Mutation taster	Polyphen-2
RAPGEF2	Chr 04	p. E285G	Not reported	Damaging	Disease causing	Probably damaging
ZNF705G	Chr 08	p. A292V	Not reported	Damaging	Polymorphism	Probably damaging
PDGFRL	Chr 08	p. G94D	Not reported	Damaging	Disease causing	N/A
KIF20B	Chr 10	p. K1372E	Not reported	Damaging	Disease causing	Probably damaging
KIF20B	Chr 10	p. L397I	Not reported	Damaging	Disease causing	Probably damaging
PPP6R2	Chr 22	p. G931R	Not reported	Tolerated	Polymorphism	N/A
SEMA3A	Chr 07	p. N693S	Not reported	Tolerated	Disease causing	Benign
DCTD	Chr 04	p. K85N	8.2×10^{-6}	Tolerated	Polymorphism	Benign
EXTL3	Chr 04	p. S407G	2.5×10^{-5}	Tolerated	Disease causing	Benign
COL20A1	Chr 20	p. S131C	3.4×10^{-5}	Damaging	Polymorphism	Possibly damaging
DLG5	Chr 10	N/A	5.8×10^{-5}	Tolerated	Disease causing	Probably damaging
ASPM	Chr 01	p. H542Y	6.6×10^{-5}	Tolerated	Polymorphism	Benign
NRP2	Chr 02	p. N354K	3.2×10^{-4}	Tolerated	Polymorphism	Benign
FAM69C	Chr 18	p. P305L	3.8×10^{-4}	Damaging	Disease causing	Possibly damaging
USP54	Chr 10	N/A	4.3×10^{-4}	Tolerated	Disease causing	N/A
SH3RF2	Chr 05	p. K343N	4.7×10^{-4}	Tolerated	Polymorphism	Benign
C17orf70	Chr 17	p. P158L	5.3×10^{-4}	Damaging	Polymorphism	Probably damaging
LZTS1	Chr 08	p. V497I	6.3×10^{-4}	Tolerated	Polymorphism	Benign
GSN	Chr 09	N/A	1.8×10^{-3}	Tolerated	Disease causing	Possibly damaging
DCHS2	Chr 04	p. K1348Q	2.5×10^{-3}	Tolerated	Polymorphism	N/A

[a]Allele frequency from Exome Aggregation Consortium database

(a) p.Ser131Cys
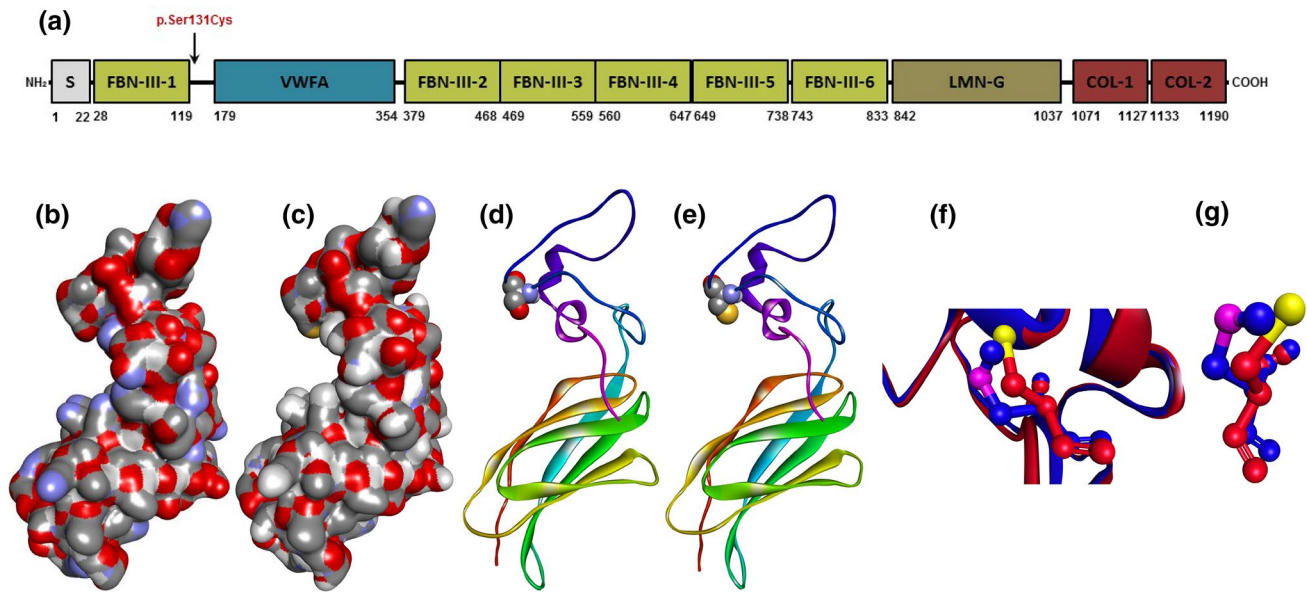
(b) **(c)** **(d)** **(e)** **(f)** **(g)**

Fig. 3 a Schematic diagram of COL20A1 protein and position of the p.Ser131Cys mutation. The domain information was obtained from ExPASy database (http://www.uniprot.org/uniprot/Q9P218). The 3D models of COL20A1 (residues 23–165). Surface was added with atom color (Carbon:gray, Oxygen:red, Nitrogen:blue, Slufer: yellow). normal protein (with Ser131) (**b** and **d**) and mutated protein (with Cys131) shown in (**c** and **e**). The residue number 131 is shown in CPK representation. **f** Superimposed mutated model (blue) over normal model (red). **g** Blue, Serine (with side chain Oxygen in purple), Red: Cysteine (with side chain Sulphur in yellow). (Color figure online)

Discussion

Clinically PPK can be classified into several subgroups, and there are 30 genes associated with the term "palmoplantar keratoderma" in NCBI (https://www.ncbi.nlm.nih.gov/gene). Recently, various clinical subtypes of PPK have been assigned to their causative genes based on their functions as structural proteins (keratins), cornified envelop (loricrin and transglutaminase) cell-to-cell adhesion (plakophilin, desmoplakin, desmoglein 1), cell-to-cell communication (connexins) and transmembrane signal transduction cathepsin C (Sakiyama and Kubo 2016; Stypczynska et al. 2016). Here we suggest, *COL20A1* p.Ser131Cys as a novel candidate mutation for PPKS phenotype identified through WES analysis. Previously its closest paralog, *collagen 14α1* (*COL14A1*, MIM 120324) gene has been reported to be the only collagen gene associated with PPK in a Chinese family (Guo et al. 2012).

The *COL20A1* spans 41,665 base pairs on chromosome 20q13.33 and encodes for 135.84 kD protein consisting of 1284 amino acids. According to the UniProtKB database COL20A1 has a signal peptide (1–22 aa) and a collagen alpha chain (23-1284 aa) with six domains of fibronectin type III (1–6), two domains of collagen like 1 and 2 and a laminin-G like domain (http://www.uniprot.org/uniprot/Q9P218). Our mutation p.Ser131Cys resides in the loop region after the fibronectin type III-1 domain (28–119 aa) (Fig. 3).

All our patients showed similar phenotype, although incomplete or age-dependent penetrance of dominant mutations has previously been reported in punctate palmoplantar keratoderma families (Guo et al. 2012; Martinez-Mir et al. 2003); however, we did not observe this phenomenon in our patients. Although there were additional neurological findings in one patient; however, they were not observed from other affected individuals in our family and thus the neurological phenotypes were not considered to be the results from COL20A1 p.Ser131Cys mutation.

The *COL14A1* gene, a closest paralog of *COL20A1*, has been known as the only collagen gene causing PPK phenotype in autosomal dominant fashion (Guo et al. 2012). The mutant *COL14A1* was suggested to alter the normal keratinocyte proliferation which could lead to the common features like hyperkeratosis in PPK patients (Guo et al. 2012). The COL14A1 is a member of fibril-associated collagen with an interrupted triple helix (FACIT) superfamily, interacting with the fibril surface and regulates fibrillogenesis (Ansorge et al. 2009). FACITs are a subgroup within the collagen family containing types IX, XII, and XIV collagens (Shaw and Olsen 1991), based on sequence homology collagen XX has also been included in this subgroup (Koch et al. 2001). The three-dimensional predicted protein structures of the mutant COL20A1 showed different atom

distribution on the surface. The amino acid serine at 131st position resides at the surface of the protein and Ser-to-Cys alteration might interfere the interactions between the monomers of collagen fibers. Oxygen in serine residue can form hydrogen bond with neighboring chain of collagen which can stabilize the structure; cysteine instead forms disulfide bridges. We suspect that the mutated cysteine residue may have effect on interaction with another collagen monomer as cysteines produce knots in collagens (Barth et al. 2003; Boulegue et al. 2008). Furthermore, the hydrophobic nature of cysteine (Nagano et al. 1999) as compared to hydrophilic serine can affect protein structure while interacting with the solvent water molecules.

Our genetic and in silico analyses suggest that *COL20A1* p.Ser131Cys is the genetic mutation underlying striate PPK in a consanguineous Pakistani family.

Compliance with ethical standards

Conflict of interest Muhammad Ismail Khan, Soyeon Choi, Muhammad Zahid, Habib Ahmad, Roshan Ali, Musharraf Jelani and Changsoo Kang declare that there is no conflict of interest on the contents of the manuscript.

Ethical approval Written informed consent was obtained from all study participants from this family. This study was approved by the Institutional Review Board Committees at King Abdulaziz University (Jeddah, Saudi Arabia) and Sungshin Women's University (Seoul, South Korea).

References

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249

Ansorge HL, Meng X, Zhang G, Veit G, Sun M, Klement JF, Beason DP, Soslowsky LJ, Koch M, Birk DE (2009) Type XIV collagen regulates fibrillogenesis: premature collagen fibril growth and tissue dysfunction in null mice. J Biol Chem 284:8427–8438

Armstrong DK, McKenna KE, Purkis PE, Green KJ, Eady RA, Leigh IM, Hughes AE (1999) Haploinsufficiency of desmoplakin causes a striate subtype of palmoplantar keratoderma. Hum Mol Genet 8:143–148

Azuara C, Lindahl E, Koehl P, Orland H, Delarue M (2006) PDB_Hydro: incorporating dipolar solvents with variable density in the Poisson-Boltzmann treatment of macromolecule electrostatics. Nucleic Acids Res 34:W38–W42

Barth D, Kyrieleis O, Frank S, Renner C, Moroder L (2003) The role of cystine knots in collagen folding and stability, part II. Conformational properties of (Pro-Hyp-Gly)n model trimers with N- and C-terminal collagen type III cystine knots. Chemistry 9:3703–3714

Boulegue C, Musiol HJ, Gotz MG, Renner C, Moroder L (2008) Natural and artificial cystine knots for assembly of homo- and heterotrimeric collagen models. Antioxid Redox Signal 10:113–125

Bowie JU, Luthy R, Eisenberg D (1991) A method to identify protein sequences that fold into a known three-dimensional structure. Science 253:164–170

Bystroff C, Thorsson V, Baker D (2000) HMMSTR: a hidden Markov model for local sequence-structure correlations in proteins. J Mol Biol 301:173–190

Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012) Predicting the functional effect of amino acid substitutions and indels. PLoS ONE 7:e46688

Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 2:1511–1519

Giehl KA, Eckstein GN, Pasternack SM, Praetzel-Wunder S, Ruzicka T, Lichtner P, Seidl K, Rogers M, Graf E, Langbein L et al (2012) Nonsense mutations in AAGAB cause punctate palmoplantar keratoderma type Buschke–Fischer–Brauer. Am J Hum Genet 91:754–759

Guo BR, Zhang X, Chen G, Zhang JG, Sun LD, Du WD, Zhang Q, Cui Y, Zhu J, Tang XF et al (2012) Exome sequencing identifies a COL14A1 mutation in a large Chinese pedigree with punctate palmoplantar keratoderma. J Med Genet 49:563–568

Has C, Technau-Hafsi K (2016) Palmoplantar keratodermas: clinical and genetic aspects. J Dtsch Dermatol Ges 14:123–139, quiz 140

Hennies HC, Kuster W, Mischke D, Reis A (1995) Localization of a locus for the striated form of palmoplantar keratoderma to chromosome 18q near the desmosomal cadherin gene cluster. Hum Mol Genet 4:1015–1020

Hooft RW, Vriend G, Sander C, Abola EE (1996) Errors in protein structures. Nature 381:272

Koch M, Foley JE, Hahn R, Zhou P, Burgeson RE, Gerecke DR, Gordon MK (2001) alpha 1(Xx) collagen, a new member of the collagen subfamily, fibril-associated collagens with interrupted triple helices. J Biol Chem 276:23120–23126

Lucker GP, Van de Kerkhof PC, Steijlen PM (1994) The hereditary palmoplantar keratoses: an updated review and classification. Br J Dermatol 131:1–14

Martinez-Mir A, Zlotogorski A, Londono D, Gordon D, Grunn A, Uribe E, Horev L, Ruiz IM, Davalos NO, Alayan O et al (2003) Identification of a locus for type I punctate palmoplantar keratoderma on chromosome 15q22-q24. J Med Genet 40:872–878

Nagano N, Ota M, Nishikawa K (1999) Strong hydrophobic nature of cysteine residues in proteins. FEBS Lett 458:69–71

Pontius J, Richelle J, Wodak SJ (1996) Deviations from standard atomic volumes as a quality measure for protein crystal structures. J Mol Biol 264:121–136

Ramachandran GN, Ramakrishnan C, Sasisekharan V (1963) Stereochemistry of polypeptide chain configurations. J Mol Biol 7:95–99

Rickman L, Simrak D, Stevens HP, Hunt DM, King IA, Bryant SP, Eady RA, Leigh IM, Arnemann J, Magee AI et al (1999) N-terminal deletion in a desmosomal cadherin causes the autosomal dominant skin disease striate palmoplantar keratoderma. Hum Mol Genet 8:971–976

Sakiyama T, Kubo A (2016) Hereditary palmoplantar keratoderma "clinical and genetic differential diagnosis". J Dermatol 43:264–274

Schiller S, Seebode C, Hennies HC, Giehl K, Emmert S (2014) Palmoplantar keratoderma (PPK): acquired and genetic causes of a not so rare disease. J Dtsch Dermatol Ges 12:781–788

Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014) MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods 11:361–362

Shaw LM, Olsen BR (1991) FACIT collagens: diverse molecular bridges in extracellular matrices. Trends Biochem Sci 16:191–194

Stypczynska E, Placek W, Zegarska B, Czajkowski R (2016) Keratinization disorders and genetic aspects in palmar and plantar keratodermas. Acta Dermatovenerol Croat 24:116–123

Whittock NV, Smith FJ, Wan H, Mallipeddi R, Griffiths WA, Dopping-Hepenstal P, Ashton GH, Eady RA, McLean WH, McGrath JA (2002) Frameshift mutation in the V2 domain of human keratin 1 results in striate palmoplantar keratoderma. J Invest Dermatol 118:838–844

Xu D, Zhang Y (2011) Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. Biophys J 101:2525–2534