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ORIGINAL ARTICLE

A biallelic variant in IQCE predisposed to cause non-syndromic post-axial polydactyly type A

Muhammad Bilal[1*], Muhammad Raheel[2], Gul Hassan[3], Shah Zeb[4,5], Arif Mahmood[6], Zamrud Zehri[7], Hafiza Yasmin Manzoor[8], Muhammad Umair[9*]

ABSTRACT

Background: Polydactyly or hexadactyly is a familiar limb defect that either occurs as an isolated entity (non-syndromic) or is associated with severe (syndromic) morphological phenotypes. Generally, it appears due to a defect in the anteroposterior patterning during limb development.

Methods: Here, we present a proband having non-syndromic post-axial polydactyly (PAP) evaluated using whole exome sequencing followed by Sanger sequencing. Furthermore, 3D protein modeling was executed for the normal and mutated IQ domain-containing protein E (*IQCE*) gene.

Results: WES analysis revealed an already reported bi-allelic variant (c.395-1 G>A) in the *IQCE* gene, previously associated with PAP 7. Furthermore, 3D modeling revealed significant fluctuations in the IQCE protein secondary structure, thus affecting downstream signaling.

Conclusion: The work presented validated the significant role of the *IQCE* gene in the development and patterning of human limbs.

Keywords: PAPA, *IQCE*, reported variant, Pakistani population, 3D modeling, WES.

Introduction

Polydactyly is characterized as the presence of well-formed extra digits in upper or lower limbs (1). It can be an isolated deformity (non-syndromic) or associated with a complex progressive syndrome (syndromic). The syndromic condition exhibits severe phenotypic complications, including disorders such as Laurin-sand row syndrome, Acrocallosal syndrome, split-hand foot malformation, Bardet-Biedl Syndrome, and complex ciliatory diseases (2-5). Polydactyly is classified into three categories, which include postaxial polydactyly (PAP), pre-axial polydactyly and complex polydactyly. PAP and preaxial polydactyly is further divided into two subgroups: type A, with fully developed bone in the extra digit or type B, which is non-function in the form of the skin tag, with or without nail (6-8). PAP type A and B are the most prevalent type of polydactyly. To date, eleven genes have been associated with non-syndromic polydactyly [glioma-associated oncogene family zinc finger 1 (*GLI3*), *SHH, STKLD1*, *MIPOL1, GLI1, ZNF141,* IQ domain-containing protein E *(IQCE), FAM92A, KIAA0825*, *DACH1, PITX*] (6-13) (Tables 1 and 2). Abnormalities of human hands and feet occur frequently in the general population. The development of human limbs is regulated by a series of complex cellular pathways including hedgehog (HH), WNT, and bone morphogenetic proteins. Deficiency of any regulator in such pathways leads to diverse types of limb deformities and other syndromic

Correspondence to: Muhammad Bilal
*Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
Email: mbilal@bs.qau.edu.pk
Correspondence to: Muhammad Umair
*Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan.
Email: m_umair@umt.edu.pk
Full list of author information is available at the end of the article.
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skeletal deformities (14). Polydactyly is one of such deformities that results due to defects in anterior-posterior patterning of the limb development (14). In the era of advanced technologies, WES has been very successful in clinical exome analysis, solving many cases, and identifying novel candidate genes. WES has both low cost and quick meth for molecular analysis of genetic disorders (15). In the present study, we have investigated a proband exhibiting non-syndromic PAPA phenotype segregating in autosomal recessive mode. Using WES, we identified a previously reported bi-allelic variant in the intron five of the *IQCE* gene that might be associated with the polydactyly condition in our patient.

Subjects and Methods

For the present study, a family with an AR inheritance pattern was recruited from the Khyber Pakhtunkhwa province of Pakistan (Figure 1A). The proband (II-1) was evaluated by taking a medical history and performing biochemical tests at a local government hospital. Consent in written form was obtained from the participants for the genetic analysis, and the University of Management and Technology (UMT), Lahore, Pakistan Institutional Review Board approved the study in compliance with the Helsinki Declaration. Blood samples were collected and processed further for DNA extraction and quantification using standard methods (16,17). WES was performed using DNA from the proband (IV-1). WES and variants filtering steps were performed as described earlier (18-21). Standard-screening principles were used to search for different functional variants associated with the patient phenotype (22). The genes already reported in the Online Mendelian Inheritance in Man (OMIM) (Table 1) and literature (PUBMED) were given priority. Prioritized disease-causing variants were Sanger sequenced for segregation analysis (23,24). The pathogenic nature of the identified variant was calculated using different tools. ExAC, in-house 175 exomes and genomAD were searched to see if the variant is reported in the general population (25,26). Conservation of amino acid was determined using HomoloGene (National Center for Biotechnology Information). The partial amino acid sequence of IQCE, the encoding protein, was retrieved from the UniProt database with accession number P78357-1. The IQCE model was examined/evaluated and, after that, selected according to the obtained evaluation score provided by I-TASSER and MODELLER (27,28).

Results

Clinical examination

The proband (age 4 years) is a boy born to consanguineous parents that revealed bilateral PAP in hands and feet (Figure 1B). The extra digits were well-developed. Samples were attained from all the available family members. Hand/digit photographs were provided by the index (II-2). Hands and feet X-rays revealed underdeveloped carpals, metacarpals, and similarly underdeveloped tarsals and mete tarsals. No associated abnormality was observed, such as kidney stones, eye deformity, obesity, or hypogonadism. Syndactyly, facial dysmorphism and nail deformity was not observed. Physical examination demonstrated that the other finger originated from the fifth metacarpal. Later, the extra digits were removed surgically.

Molecular analysis

Using WES, we identified a previously reported splice site variant in the *IQCE* gene associated with PAPA-AR. The identified variant is a bi-allelic splice acceptor site variant (c.395-1G>A) in the intron 5 of the *IQCE* (NM_152558.5) located on chromosome 7p22.3-7p22.3 (Figure 1C). The variant was Sanger sequenced and segregated using standard protocols (Figure 1C). The variant was not observed in a homozygous state in an internal database, ExAC, gnomAD, and was predicted deleterious by several tools.

3D structure prediction

Using homology modeling, three-dimensional models of wild type and mutated IQCE protein (p.Gly132Valfs*22) were predicted and assessed using online structure analysis tools (Figure 2A and B). 3D protein modeling showed substantial changes and reduction of key

Table 1. *Genes associated with non-syndromic PAP.*

Genes	Disease	Inheritance	Locus	OMIM
GLI3	PAPA1	AD	7p14.1	174200
Unknown	PAPA2	AD	13q21-q32	602085
Unknown	PAPA3	AD	19p13.2-p13.1	607324
Unknown	PAPA4	AD	7q22	608562
Unknown	PAPA5	AR	13q13.3-q21.2	263450
ZNF141	PAPA6	AR	4p16.3	615226
IQCE	PAPA7	AR	7p22.3	617642
GLI1	PAPA8	AR	12q13.3	618123
FAM92A	PAPA9	AR	8q22.1	618219
KIAA0825	PAPA10	AR	5q15	618498
DACH1	PAPA11	AR	13q2133	603803

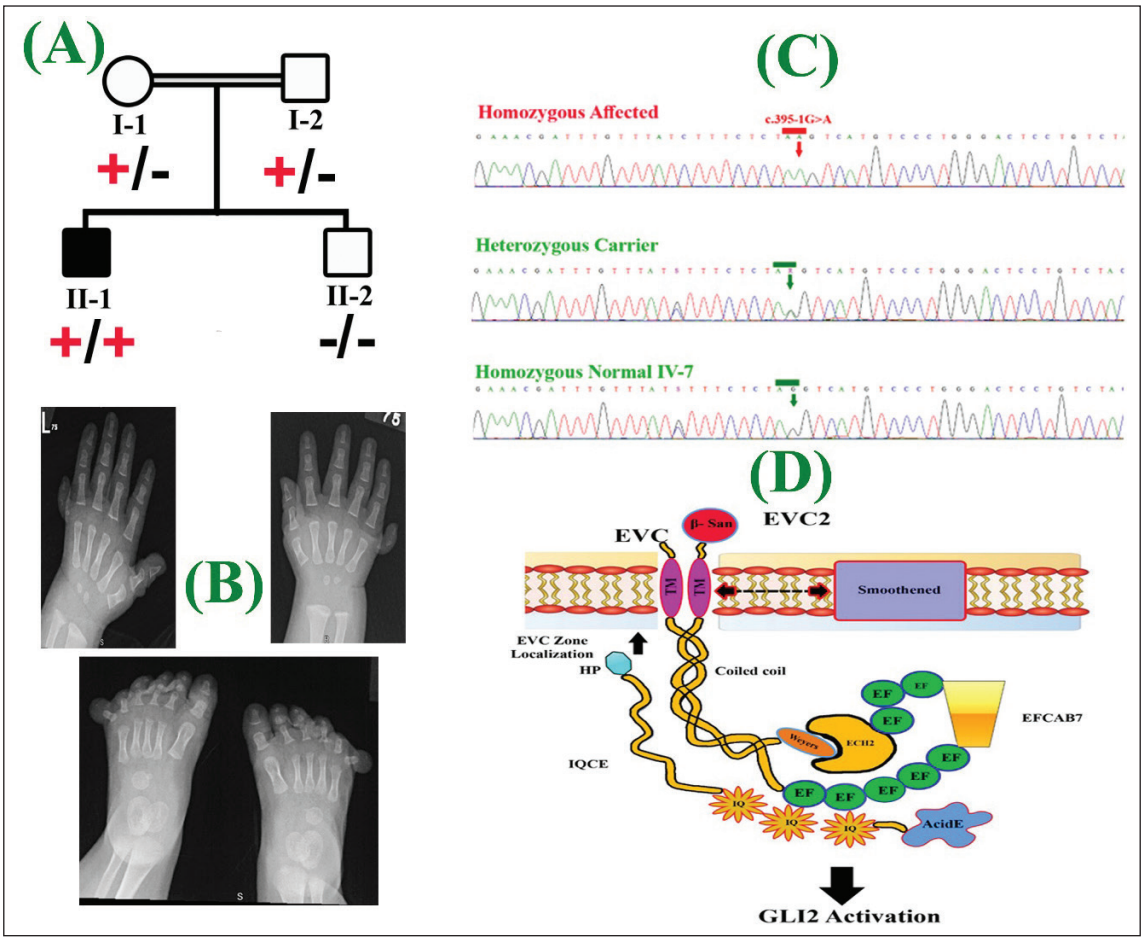

Figure 1. *(A) Pedigree of the family showing AR pattern of inheritance. (B) Xrays of the proband (II-1). (C) Sanger electrograms of the affected, carrier and wildtype. (D) Schematic representation of IQCE with EFCAB7 that intern interacts with EVC/EVC2 proteins that ultimately regulate the HH signalling pathway responsible for limb patterning and development.*

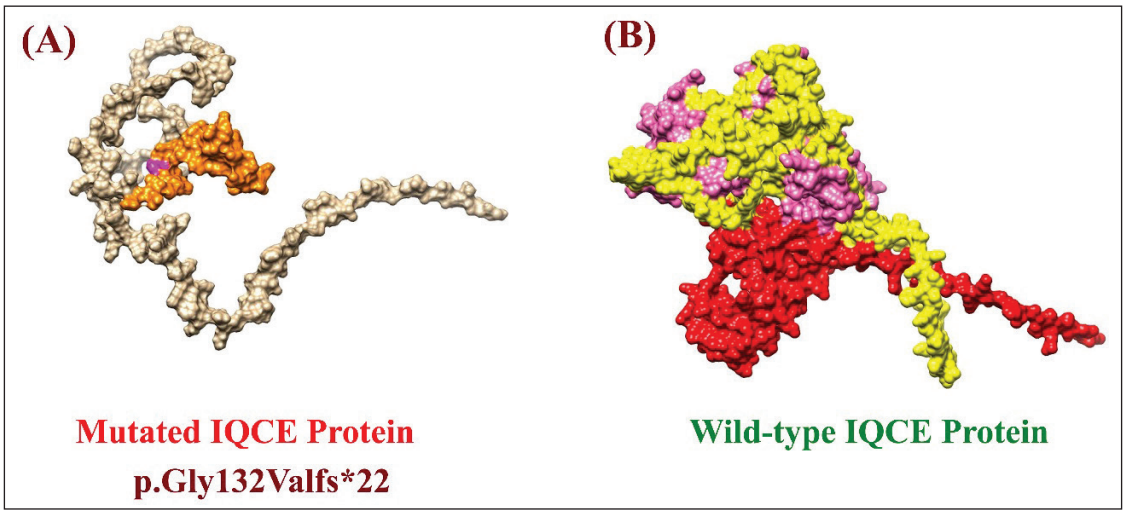

Figure 2. *IQCE protein modelling. (A) IQCE^{Mutated} structure showing the complete reduction. (B) IQCE$^{Wild\text{-}type}$ structure.*

domains in the mutated IQCE protein secondary structure compared to the wild-type IQCE.

Discussion

Herein, we used clinical and molecular methods to characterize a proband having bilateral non-syndromic PAPA without syndactyly. However, previously the same variant has been associated with PAPA-restricted lower limbs only. Later, it was confirmed that variants in *IQCE* cause PAPA in both upper and lower limbs (29). WES data analysis revealed an already reported bi-allelic variant (c.395-1G>A) in the *IQCE* gene. Variants in

Table 2. *PAPA types and associated clinical phenotypes.*

Title	PAPA1	PAPA6	PAPA7	PAPA8	PAPA9	PAPA10	PAPA11
Gene	*GLI3* - 165240	*ZNF141* - 194648	*IQCE* - 617631	*GLI1* - 165220	*CIBAR1* - 617273	*KIAA0825* - 617266	*DACH1- 603803*
Inheritance	Autosomal dominant	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Skeleton	***Hands*** - PAP(bilateral, sometimes extra digits are well formed and articulated) - Preaxial polydactyly (bilateral or unilateral) - Triphalangeal thumb - Syndactyly - Broad thumbs	***Hands*** - Fifth finger duplication, well-formed - Broad fifth finger, unilateral or bilateral - Deviation of fifth finger, radial or ulnar, to variable degree - Duplicated distal phalanx of fifth finger - Small central phalanx of fifth finger (in some patients)	***Hands*** -PAP, bilateral - Brachydactyly	***Hands*** - PAP - No duplication of metacarpals	***Hands*** - PAP	***Hands*** -PAPtype A -PAP, type B (rare)	***Hands*** -PAP, type A
	Feet - PAP - Preaxial polydactyly - Syndactyly	***Feet*** - Fifth toe duplication, well-formed	***Feet*** - PAP, unilateral or bilateral - Thick, broad, 2-headed fifth metatarsal - Cutaneous 2-3 toe syndactyly - Brachymetatarsia of fifth toe	***Feet*** - PAP	***Feet*** - PAP	***Feet*** -PAP, type A	***Feet*** -PAP, type A

IQCE have been previously associated with PAPA7, and the identified variant has been validated using minigene splice assay (8), showing deletion of G nucleotide from exon 6. This deletion results in frameshift and premature stop codon (p.Gly132Valfs*22) that might lead to small IQCE protein or mRNA nonsense-mediated decay.

Using patients' fibroblasts, the RNA expression analysis revealed that IQCE pathogenesis results in the dysregulation of several genes associated with the HH-signaling pathway. Furthermore, knock-out zebrafish trials revealed astonishing phenotypes associated with cilia dysregulation, such as left-right asymmetry, body curvature issues, misdirected cilia in the pronephric duct, kidney cysts, and retinal defects (29). Thus, suggesting the key role of IQCE in the ciliatory development and regulating genes associated with the HH pathway. As disease-causing variants in the Ellis-Van Creveld (EVC) and EVC2 are associated with EVC syndrome in humans. One of the phenotypes in EVC patients is PAP. The disorder is characterized by severe skeletal deformities, including PAP, cardiac anomalies, and facial dimorphism (3). EVC/EVC2 makes a complex with the smoothened, frizzled class receptor (SMO) and interacts with IQCE/EFCAB7 at the base of primary cilia associated with the activation of GLI2, which further causes HH signaling activation (Figure 1D). Thus, EVC is mostly caused by impaired HH signaling pathways. EVC/EVC2 inactivation does not affect the SMO phosphorylation or ciliary accumulation; however, it affects the GLI ciliary activation and localization. This suggests a key role of IQCE in the downstream HH signaling cascades (30). The discovery of cilia involvement has improved our in-depth knowledge regarding the HH signaling pathway. Still, we lack a precise understanding of how these newly identified players/genes, such as *FAM92A*, *KIA0825*, and *DACH1*, interact and how these key proteins are associated with cilia trafficking and how their dis-regulation leads to abnormal limb patterning.

Polydactyly in humans is a genetically and phenotypically heterogeneous disorder, as polydactyly is linked with syndromic and non-syndromic phenotypes. Syndromic types constitute 496 disorders searched in OMIM, including severe disorders such as Split-Hand/Foot Malformation, EVC, neurodevelopmental disorders, syndactyly, and many more (18,31-33). However, non-syndromic types are few, but they help us understand the prevalence of the variants in a population and help us understand the pathophysiology of the disorder in detail. Thus, identifying novel genes implicated in congenital limb abnormalities is important to understand limb development in humans and help manage associated syndromic disorders. In addition, proper genetic counseling of the family having severe skeletal disorders might help eradicate the disorder in future poignancies. In addition, introducing the newborn screening program in a developing country like Pakistan will be the first step in screening some severe genetic disorders. Parenteral diagnosis can play a major role in reducing the burden of such severe disorders (34,35). This can be accomplished by prenatal genetic testing for monogenetic disorders (PGT-M). PGT and in vitro fertilization are options for parents wishing to have future pregnancies (36,37). In conclusion, we have presented an association of a splice site variant in *IQCE* with an isolated PAP in humans. This information will help researchers understand the intricate signaling cascades needed for proper limb orientation and development and will also help them prevent the pathogenesis of limb deformities.

List of Abbreviations

GLI	Glioma-associated oncogene family zinc finger 1
HH	Hedgehog
IQCE	IQ domain-containing protein E
OMIM	Online Mendelian Inheritance in Man
PAPA	Postaxial polydactyly type A
SMO	Smoothened, frizzled class receptor

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Ethical approval
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Author details
Muhammad Bilal[1], Muhammad Raheel[2], Gul Hassan[3], Shah Zeb[4,5], Arif Mahmood[6], Zamrud Zehri[7], Hafiza Yasmin Manzoor[8], Muhammad Umair[9]

1. Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
2. Department of Microbiology, Shah Abdul Latif University, Khairpur, Pakistan
3. Department of Biochemistry, Shah Abdul Latif University, Khairpur, Pakistan
4. Institute for Advanced Study, Shenzhen University, Shenzhen, People's Republic of China
5. College of Physics and Optoelectronics Engineering, Shenzhen University, Shenzhen, People's Republic of China
6. Center for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, China
7. Shaheed Nawab Ghous Bakhsh Raisani Memorial Hospital, Mastung, Pakistan
8. Department of Laboratory, Carle Foundation Hospital, Urbana, IL, USA
9. Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

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