ORIGINAL ARTICLE

GENETIC MAPPING OF THREE LARGE CONSANGUINEOUS KASHMIRI FAMILIES WITH AUTOSOMAL RECESSIVE POLYDACTYLY SCREENED FOR FIVE PREVALENT GENES

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Background: Polydactyly, a congenital hand defect characterized by extra digits, is more complicated than simple duplication, delicately weaving aberrant anatomical components with hypoplasia, uneven joint shapes, and unusual tendon and ligament placements. The dominant theory attributes its genesis to a group of five genes: *GLI1* (chromosome 12q13.3), *ZNF141* (chromosome 4p16.3), *IQCE* (chromosome 7p22.2), *KIAA0825* (chromosome 5q15), and *FAM92A* (chromosome 8q22.1). The objective of this study is to identify the most prevalent genes responsible for polydactyly in the population of Azad Jammu and Kashmir. **Method:** The microsatellite markers used for PCR amplification and subsequently testing linkage to known genes are presented below. Represent electropherograms of ethidium bromide-stained 8% non-denaturing polyacrylamide gels (PAGEs) obtained by genotyping microsatellite markers linked on chromosome 8q22.1, 5q15, 12q13.3, 4p16.3, and 7p22.3 in family A, B, C. Genetic positions (in centiMorgan) for these marker loci were obtained from Rutgers combined linkage-physical map of the human genome. **Results:** Screening of most prevalent genes that include *GLI1* (chromosome 12q13.3), *ZNF141* (chromosome 4p16.3), *IQCE* (Chromosome 7p22.2), *KIAA0825* (chromosome 5q15) and *FAM92A* (8q22.1) showed heterozygosity on every locus. Already known disease loci were further narrowed down with highly polymorphic markers which failed to find any linkage. **Conclusion:** The previously reported genotypic-phenotypic relation was not revealed in these 3 families signifying the probable involvement of unexplored genetic segments in intricate pathogenesis of this condition, emphasizing the need for further exploration beyond the established genetic association.

Keywords: Polydactyly, *IQCE* gene, *GLI1* gene, heterozygosity, unexplored genetic segment

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INTRODUCTION

Congenital deformities of the hands, such as polydactyly, are extremely common. As a common limb-related birth defect.[1] Polydactyly, also known as hyperdactyly, has an incidence between 0.37 and 1.2 per 1,000 live births, depending on race.[2] Polydactyly or polydactylism refers to the appearance of 'supernumerary' fingers.[3] Polydactyly is the most common congenital hand anomaly, it is not simply a duplication, the anatomy is abnormal with contoured joints, hypoplastic structures, and anomalous ligament and tendon insertions. Genetic positions (in centiMorgan) for these marker loci were obtained from Rutgers combined linkage-physical map of the human genome (build 36.2).[4] According to estimation above 100 genetic syndromes are linked with polydactyly.[5] Percentage prevalence of polydactyly is reported to be 519/10,000 deliveries.[6]

Polydactyly is classified into 2 broad categories such as syndromic and non-syndromic polydactyly. Most common types of polydactyly are the pre-axial polydactyly (PPD) and the postaxial polydactyly (PAP) therefore rare types are: Mesoaxial or central polydactyly; mirror image polydactyly; palmer; and dorsal polydactyly, etc.[7,8] Temtamy–McKusick classified 4 pre-axial, 2 postaxial, and complicated polydactylous entities, all of which segregated autosomal dominant.[7] Polydactyly exhibits a classification encompassing four distinct types: type-I thumb/hallux polydactyly, type-II triphalangeal thumb polydactyly, type-III index finger polydactyly and type-IV polysyndactyly or crossed polydactyly. Goldstein *et al* suggested extending the Temtamy-McKusick method by including subtypes (10 pre-axial, 9 postaxial, 4 high-degrees, and 7 complicated kinds.[9] Castilla *et al*[2] proposed that hand PAP were distinct conditions. Sonic hedgehog (SHH) pathway disruptions cause diverse types of polydactyly.[10] Thumb and hallux polydactylies were diverse in genetics according to Orioli and Castilla.[11] The Temtamy-McKusick polydactyly category is the most often used among geneticists and dysmorphologists. In this view, the 3 primary polydactylies are pre, postaxial, and complicated, each of which has several subtypes.[7]

Pre-axial polydactyly (PPD) (radial, duplication of the thumb, PPD, MIM) is caused by disturbing the normal procedure of the anterior-posterior axis of the developing limb, with diverse aetiology and

variable inter- and intra-familial clinical features is the most common form of polydactyly with an incidence as high as 8.0 to 140 per 1,000 births. PAP (ulnar polydactyly) is the most prevalent and is characterized by the presence of extra fingers on the ulnar side of the hand.[12,13]

We screened three consanguineous families for autosomal recessive polydactyly manifestations, including both preaxial and postaxial variants. These families were genotyped across a panel that included the five most important genes linked to this condition: *FAM92A* on chromosome 8q22.1, *KIAA0825* on chromosome 5q15, *GLI1* on chromosome 12q13.3, *ZNF141* on chromosome 4p16.3, and *IQCE* on chromosome 7p22.3. Our focus on these pivotal genes is intended to elucidate their potential roles in the inheritance and manifestation of autosomal recessive polydactyly within consanguineous populations and to identify any correlations or associations that may contribute to the observed phenotypic presentations across these families.

MATERIAL AND METHODS

Before the start of this trial, the ethical review board gave its approval. Families with polydactyly were chosen based on Mendelian inheritance patterns. Pedigrees were constructed using data acquired from elder family members (Figure-1, 2 and 3).

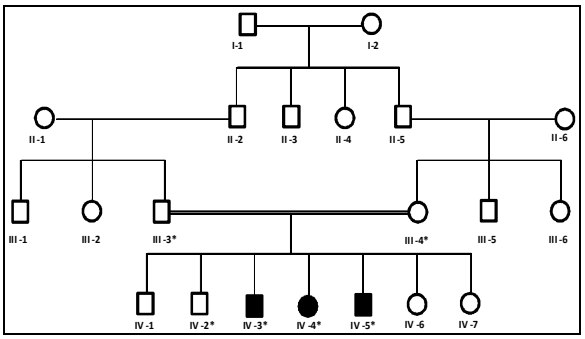

Figure-1: Pedigree of a consanguineous four-generational Kashmiri family A.

Symbols with stars represent samples that were available for the study.

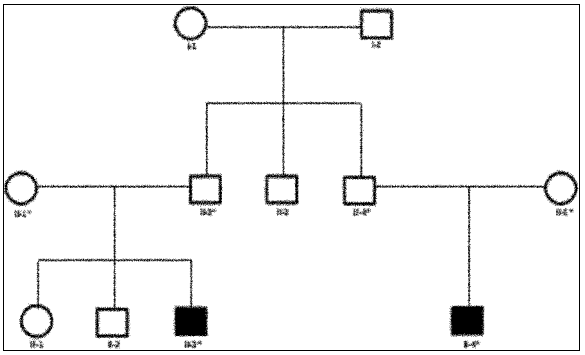

Figure-2: Pedigree of Kashmiri family B.

Symbols with stars represent samples that were available for the study.

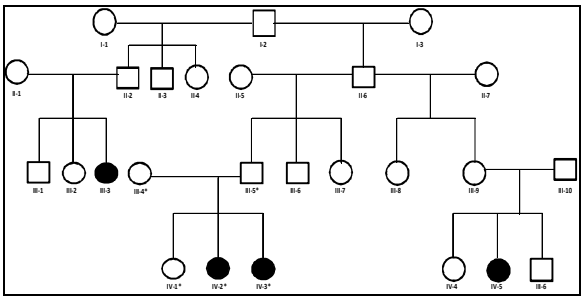

Figure-3: Pedigree drawing of multilobe Kashmiri family C.

Symbols with stars represent samples that were available for the study.

Blood samples were taken from three unaffected individuals (III-3, III-4, and IV-4) and two affected with polydactyly (IV-2 and IV-3) in Family A. Extensive clinical assessments and testing was performed to rule out other disorders.

Family B, which spanned numerous generations, had two members (III-3, III-4) who had polydactyly. Clinically, trait carriers resembled unaffected family members phenotypically. At birth, the affected individuals had an extra radial finger but no other skeletal abnormalities. Two afflicted (III-2, III-3) and five healthy (II-1, II-4, II-5, II-7, III-6, III-2, III-3) members had blood drawn.

Four members of Family C (III-3, IV-2, IV-3, and IV-5) had polydactyly. Carriers of the trait resembled unaffected family members phenotypically. Since birth, the affected people have had six digits on both hands and feet, with no other bone abnormalities. Due to logistical challenges in obtaining the other two family members in remote places, blood samples were taken from two unaffected (III-1, III-3) and two affected (IV-2, IV-5) people (Figure-6).

To determine segments in all three families' microsatellite markers with increased heterozygosity within known loci were used to get clarity of linkage with disease.

Figures 1, 2, and 3 show the pedigree design of family B with hereditary Post-axial polydactyly. Consanguineous marriages are indicated by double lines. Circles represent females while squares represent males. Affected individuals are represented by filled symbols while unaffected symbols are represented by unfilled symbols.

To determine linkage, microsatellite markers linked with known genes were used. Electropherograms for genotyping these markers (linked on chromosomes 8q22.1, 5q15, 12q13.3, 4p16.3, and 7p22.3) were obtained using 8% non-denaturing polyacrylamide gels (PAGEs) stained with ethidium bromide.

RESULTS

For this investigation, three families with autosomal recessive inheritance patterns were selected from the

AJK area. One of these families had pre-axial polydactyly, while the other two had post-axial polydactyly, constituting the foundation for the linkage analysis (Figure-4, 5 and 6).

Homozygosity mapping using highly polymorphic microsatellite markers indicated that the condition appeared at birth, with no detectable effect from environmental circumstances, indicating an autosomal recessive mode of inheritance. This discovery was supported by pedigree research and family history.

Families A, B, and C were initially tested for genetic linkage to five known autosomal recessive postaxial polydactyly loci: *GLI1* (12q13.3), *ZNF141* (4p16.3), *IQCE* (7p22.2), *KIAA0825* (5q15), and *FAM92A* (8q22.1). Each locus in the families showed heterozygosity.

In current study focused on screening potential genes within these regions to uncover the disease's underlying processes by assessing their prevalence from the literature. However, genotyping of these appropriate sites failed to show linkage with any chromosome segment, indicating that other chromosomal regions may be involved in disease development.

Preaxial polydactyly (PPD) is less prevalent than PAP, with a frequency of 0.08% to 1.4% per 1,000 live births.[14] Affected members in Family B had preaxial polydactyly, which manifested as an additional radial finger from birth. Aside from the polydactyly, no other abnormalities were discovered during the clinical evaluation. Despite screening known loci with highly polymorphic microsatellites and potential genes within those loci, genotyping-based linkage analysis failed to demonstrate a link with any chromosomal segment. This finding shows that other chromosomal regions may play a role in illness pathogenesis.

Family C displayed clinical symptoms of autosomal recessive postaxial polydactyly. Since birth, affected relatives have six digits on both hands and feet, with no additional abnormalities observed. Similarly to Families A and B, genotyping failed to reveal a linked chromosomal segment despite screening for linkage to known loci using appropriate microsatellites and candidate genes.

Despite the refinement of disease loci, no substantial association with the indicated loci was found. The extra material accompanying this study contains detailed results and further information on this topic.

Figures: Clinical presentation of Family A (Figure-4), family B (Figure-5), and Family C (Figure-6). Family A and C show post-axial polydactyly, and Family B shows Pre-axial polydactyly.

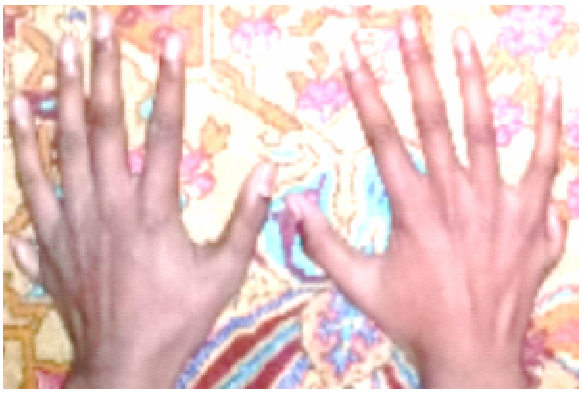

Figure-4: Clinical presentation of an individual (IV-2) of family A showing postaxial polydactyly.

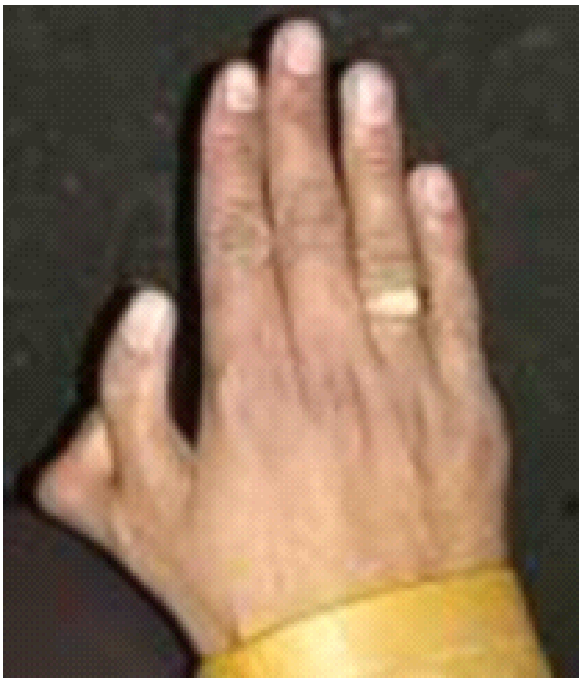

Figure-5: Clinical finding of the affected individual (III-3) with isolated preaxial polydactyly in family B.

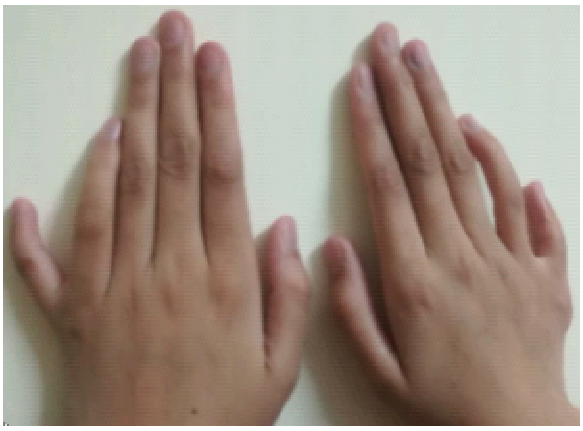

Figure-6: An affected individual (III-1) of Kashmiri family C, showing postaxial polydactyly clinical manifestations.

DISCUSSION

Our research into postaxial and preaxial polydactyly indicated adversities in finding causal genetic variables among the families analysed. The observed phenotypic differences within each family highlight the variety of these illnesses and the difficulties in identifying particular genetic loci. Polydactyly affects 519 out of every 10,000 births. Polydactyly results from defective patterning of the anterior-posterior axis of the developing limb bud. Some of these events lead to defects in signalling pathways, which are involved in regulating limb morphogenesis. Disruption of the gene expression results in abnormalities such as digit number and identity.[15]

Family A, which exhibits postaxial polydactyly, corresponds to the PAP-A classification, which is distinguished by well-formed additional digits on both hands and feet. The lack of linkage with previously known candidate genes such as *GLI1, ZNF141, IQCE, KIAA0825,* and *FAM92A* shows the presence of unknown genetic components. Upon genotyping of these suitable loci were remained failed to find linkage at any segment of chromosome.

Family B had preaxial polydactyly, which was characterized by the presence of a radial additional finger on the hand. Despite extensive screens of putative genes related to polydactyly, the absence of confirmed connections highlights the complex genetic basis of these diseases. This intricacy is consistent with. This supports the findings of[16,17] who highlighted the importance of SHH pathway abnormalities caused by *LMBR1* and *ZRS* mutations in comparable polydactyly phenotypes.

The paucity of detected connections with known candidate genes in Family C, which has autosomal recessive postaxial polydactyly, underscores the mysterious nature of the underlying genetic causes. Despite screening putative genes such as *FAM92A, GLI1, ZNF141, IQCE, and KIAA0825*, the search for other genetic locations may be fruitful.

Polydactyly genes tend to influence particular physiological areas, such as the zone of polarizing activity, which regulates limb morphology and positional identity.[18,19] Apart from the zone of polarizing activity (which produces fibroblast growth factor 8 (FGF8) the apical ectodermal ridge also produces FGF8. HOX genes, hedgehog pathways (sonic Hedgehog (SHH) and Indian Hedgehog (IHH), FGFs, bone morphogenetic proteins, and cartilage-derived morphogenetic proteins they have significant role in limb development. Chondrocyte ossification and differentiation are two processes that the IHH signalling pathway may influence.[20] *GLI, IQCE* and *ZNF141* genes are known to play role in SHH pathway. Following genes are already reported to be implicated in formation of digits that include. *FAM92A, GLI1, ZNF141, IQCE* and *KIAA082*.

GLI1 gene linked to PAPA8 was described by Palencia-Campos *et al*[21]. The *GLI1* gene has undergone many different mutations that have been linked to PAPA8. It codes for a protein with 1,106 amino acids, is found on chromosome 12q13.3.[21] On chromosome 8q21.13-q24.12, the homozygous variations of the *FAM92A* gene are linked to this autosomal recessive disease.[22]

Bi-allelic missense mutations in the *KIAA0825I* gene, is found on chromosome 5q15. The protein that is encoded by *KIAA0825* (also known as C5orf36) has a frame- shift and nonsense variant and its function is uncertain.[23] PAPA6 has been linked to a homozygous missense mutation in the Zinc Finger Protein 141 gene on chromosome 4p16.3.[24]

Our study demonstrating direct genetic connections underscores the need for future investigation employing improved sequencing techniques such as whole-genome sequencing and functional investigations of regulatory elements. These techniques have the potential to uncover novel genetic variations, enhancers, or modifiers that contribute to the complexity of polydactyly.

CONCLUSION

Our findings highlight the presence of previously unknown genetic variables that contribute to various presentations of postaxial and preaxial polydactyly. Further work is required to uncover the entire genetic landscape controlling these congenital deformities.

REFERENCES

1. Dwivedi AP. Management of postaxial polydactyly by “Ksharsutra”: a minimally invasive Ayurvedic para surgical procedure. J Ayurveda Integr Med 2013;4(2):114–6.
2. Castilla E, Paz J, Mutchinick O, Muñoz E, Giorgiutti E, Gelman Z. Polydactyly: a genetic study in South America. Am J Hum Genet.1973;25(4):405–12.
3. Bell J. On syndactylies and its association with polydactyly. In: J Bell, (Ed). The Treasury of Human Inheritance. Cambridge: University Press, 1953: 30–50.
4. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 2001;19(3):180–92.
5. Matise TC, Chen F, ChenW, De La Vega FM, Hansen M, He C, *et al*. A second-generation combined linkage–physical map of the human genome. Genome Res.2007;17(12):1783–6.
6. Holmes LB, Nasri H, Hunt AT, Toufaily MH, Westgate MN. Polydactyly, postaxial, type B. Birth Defects Res 2018;110(2):134–41.
7. Schwabe GC, Mundlos S. Genetics of congenital hand anomalies. Handchir Mikrochir Plast Chir.2004;36(2–3):85–97.
8. Temtamy SA, McKusick VA. The genetics of hand malformations. Birth Defects Orig Artic Ser.1978;14(3):1–619. .
9. Goldstein DJ, Kambouris M, Ward RE. Familial crossed polysyndactyly. Am J Med Genet 1994;50(3):215–23.
10. Lettice LA, Horikoshi T, Heaney SJ, van Baren MJ, van der Linde HC, Breedveld GJ, *et al*. Disruption of a long-range cis-acting regulator for Shh causes preaxial polydactyly. Proc Natl Acad Sci USA 2002;99(11):7548–53.

11. Orioli IM, Castilla EE. Thumb/hallux duplication and preaxial polydactyly type I. Am J Med Genet 1999;82(3):219–24.
12. Singer G, Thein S, Kraus T, Petnehazy T, Eberl R, Schmidt B. Ulnar polydactyly: an analysis of appearance and postoperative outcome. J Pediatr Surg 2014;49(3):474–6.
13. Buck-Gramcko D, (Ed). Congenital malformations of the hand and forearm. London: Churchill Livingstone; 1998.
14. Mariani FV, Martin GR. Deciphering skeletal patterning: clues from the limb. Nature 2003;423(6937):319–25.
15. Todt WL, Fallon JF. Posterior apical ectodermal ridge removal in the chick wing bud triggers a series of events resulting in defective anterior pattern formation. Development 1987;101(3):501–15.
16. Manouvrier-Hanu S, Holder-Espinasse M, Lyonnet S. Genetics of limb anomalies in humans. Trends Genet 1999;15(10):409–17.
17. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 1996;273(5275):613–22.
18. Mori C, Nakamura N, Kimura S, Irie H, Takigawa T, Shiota K. Programmed cell death in the interdigital tissue of the fetal mouse limb is apoptosis with DNA fragmentation. Anat Rec 1995;242(1):103–10.
19. Volodarsky M, Langer Y, Birk OS. A novel *GLI3* mutation affecting the zinc finger domain leads to the preaxial-postaxial polydactyly-syndactyly complex. BMC Med Genet 2014;15:110.
20. Ibrahimi OA, Chiu ES, McCarthy JG, Mohammadi M. Understanding the molecular basis of Apert syndrome. Plast Reconstr Surg 2005;11:264–70.
21. Palencia-Campos A, Ullah A, Nevado J, Yildirim R, Unal E, Ciorraga M, *et al. GLI1* inactivation is associated with developmental phenotypes overlapping with Ellis-van Creveld syndrome. Hum Mol Genet 2017;26(23):4556–71.
22. Schrauwen I, Giese APJ, Aziz A, Lafont DT, Chakchouk I, Santos-Cortez RLP, *et al.* FAM92A underlies non-syndromic postaxial polydactyly in humans and an abnormal limb and digit skeletal phenotype in mice. J Bone Miner Res 2019;34:375–86.
23. Bilal M, Ahmad W. A frameshift variant in *KIAA0825* causes postaxial polydactyly. Mol Syndromol 2020;12(1):20–4.
24. Kalsoom UE, Klopocki E, Wasif N, Tariq M, Khan S, Hecht J, *et al.* Whole exome sequencing identified a novel zinc-finger gene ZNF141 associated with autosomal recessive postaxial polydactyly type A. J Med Genet 2013;50:47–53.

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IH: Experimental research and write-up
ZA: Write-up and supervision
RM: Write-up and sampling
MA: Write-up and review
FA: Experimentation
MA: Experimentation
RAWK: Write-up and supervision
AR: Experimentation
IA: Experimentation

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