

## GLI3 MEDIATED POLYDACTYLY : A REVIEW

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### Abstract

*GLI3* is one of the major pathogenic genes associated with both syndromic and non-syndromic polydactyly. Mutations in different domains of *GLI3* exhibit variable type of clinical manifestations viz. Greig Cephalopolysyndactyly Syndrome (GCPS), Pallister-Hall Syndrome, Acrocallosal Syndrome, Pre-axial Polydactyly type IV, Post-axial Polydactyly type A, trigonocephaly with craniosynostosis and polydactyly and some types of oral-facial-digital syndrome. The present review demonstrates the clinical features of syndromic and non-syndromic polydactyly and recent progresses in underlying molecular genetics of *GLI3*. This review further demonstrates phenotypes with overlapping manifestations, genetic heterogeneity, and distinct phenotypes generated from mutations in *GLI3*. These updates improve our understanding of the *GLI3* mediated polydactyly and have implications on genetic counselling.

**Keywords :** Polydactyly, *GLI3*, GCPS, PHS, Mutation, Limb 3

Polydactyly refers to the presence of more than the usual number of fingers or toes (figure 1). Its incidence is 1:1,000 live births (Bellovits 2003, Phelps 1985) however frequency differs among racial groups (Stevenson *et al.* 1966). Often the extra digit is incompletely formed and lacks normal muscular development. Polydactyly can be classified into preaxial, central, and postaxial types depending on the location of the duplication. About 79 % of all duplications are postaxial, 15 % are preaxial and remaining 6 % are central (Phelps *et al.* 1985). If the hand is affected, the extra digit is most commonly medial or lateral rather than central. In the foot, the extra toe is usually on the lateral side. Polydactyly can occur as an isolated malformation or as part of a genetic syndrome (Mumoli *et al.* 2008). Currently, there are about 221 syndromes described with polydactyly and 120 syndromes with oligodactyly (Phadke and Sankar 2010). Despite this wide prevalence of limb abnormalities, to date only, 84 genes have been associated with syndromes that include limb defects, 15 of which have described polydactyly (Phadke and Sankar 2010).

*GLI3* is one of the major pathogenic genes, expressed early in development and is a downstream mediator of the sonic hedgehog pathway associated with both syndromic and non-syndromic polydactyly. *GLI3* is a large gene located on chromosome locus 7p14.1 with exons spanning 296 kb. The current reference sequence for the cDNA is an 8,228 nucleotide sequence: NM\_000168.5.

## **GLI3 protein**

*GLI3* gene encodes a protein of 1,580 amino acids. GLI3 has a dual function as a transcriptional activator and a repressor of the sonic hedgehog (SHH) pathway which plays an important role during limb development. The full-length GLI3 form (GLI3FL) after phosphorylation and nuclear translocation, acts as an activator (GLI3A) while GLI3R (C-4 terminally truncated) acts as a repressor. A proper balance between the GLI3 activator and the repressor GLI3R (the activator/repressor ratio gradient) specifies limb digit number and identity. GLI3 is composed of several functional domains, i.e. repressor domain (RD, aa106-aa263), DNA binding domain (DBD, aa480-aa632), cleavage site (CS, aa650-aa750), CREB binding protein domain (CBP, aa827-aa1131), transactivation domain 2 (TA2, aa1044-aa1322) and transactivation domain 1 (TA1, aa1376-aa1580) (Krauß et al., 2009).

## **GLI3 morphopathies**

The clinical phenotypes associated with *GLI3* mutations was classified under “GLI3 morphopathies,” the term given by Radhakrishna U et al., 1999. Mutations in *GLI3* lead to three different clinical entities with polydactyly i.e. an isolated polydactyly (non-syndromic polydactyly), polydactyly along with other limb abnormalities or polydactyly in combination with other abnormalities other than limb (syndromic polydactyly). Syndromic polydactyly includes Greig cephalopolysyndactyly syndrome (GCPS; MIM# 175700) and Pallister–Hall syndrome (PHS; MIM# 146510), acrocallosal syndrome (MIM# 200990 (Elson et al., 2002), oral–facial–digital syndrome and trigonocephaly with craniosynostosis and polydactyly. The non-syndromic polydactyly includes “Postaxial polydactyly A/B”, “Preaxial polydactyly IV”, “Postaxial polydactyly A”, “Postaxial polydactyly B”, “Preaxial polydactyly”. A few cases were also reported with the presence of variable degree of syndactyly with polydactyly.

A strong genotype-phenotype correlation exists for truncating mutations in GLI3. Mutations within “amino terminal-encoding nucleotide 1–1997 or carboxy terminal encoding thirds after nucleotide 3481” are associated with GCPS, whereas in the “middle third of the gene” with PHS (Biesecker, 2008; Jamsheer et al. 2012). The degree of GLI3 involvement in the pathogenesis of non-syndromic postaxial polydactyly has not been reported.

### **I. Syndromic Polydactyly**

#### **1. Greig cephalopolysyndactyly syndrome (GCPS; MIM# 175700):**

Greig cephalopolysyndactyly (GCPS) syndrome is named after David Middleton Greig after reporting a patient with this disorder. The Greig cephalopolysyndactyly syndrome (GCPS) is clinically defined as a rare, pleiotropic, multiple congenital anomaly syndrome characterized by the primary clinical triad of polysyndactyly, macrocephaly, and hypertelorism. The estimated prevalence of GCPS is 1–9/1,000,000. In GCPS postaxial polydactyly is more common than preaxial and generally postaxial

polydactyly of the hands and preaxial polydactyly of the feet were observed in patients. The degree of severity of the polydactyly varies among affected family members and even among limbs of the same individual. The craniofacial manifestations include hypertelorism, telecanthus and macrocephaly which are also highly variable from individual to individual. One of our previous study revealed finger like thumb, dry skin and a tiny outgrowth from great toe which was visible only by X-ray analysis in a patient diagnosed with GCPS (Patel et al., 2014). Some less common clinical manifestation in GCPS include craniosynostosis, mental retardation, agenesis of the corpus callosum, and umbilical and diaphragmatic hernias. Some cases were also reported in GCPS patients along with tumors, such as leukemia and gliomas (Mendoza-Londono et al., 2005).

### 1.1 GCPS and GLI3

A study by NIH revealed that more than 75% of GCPS patients who have been evaluated in the study, have mutations in *GLI3* but some GCPS patients did not have mutations in *GLI3*. GCPS mutations include nonsense, missense, splicing mutations, translocations, deletions and insertions. Large deletions or truncating mutations in amino terminal-encoding or carboxy terminal-encoding thirds of the gene cause GCPS due to haploinsufficiency of *GLI3*. A total of 91 *GLI3* mutations and 6 mutations in sub-GCPS, 1 deletion of approximately 12 Mb in the 7p13-15 region in a case of GCPS with MODY2 (Zung 2011) and 1 deletion affecting multiple genes in the 7p14-13 locus in a case of GCPS with cerebral cavernous malformation (Bilguvar et al., 2007) have been reported on human gene mutation database (HGMD) database. Recently three studies from Indian population have shown milder forms of GCPS presenting polydactyly or polysyndactyly without craniofacial defects (Sethi et al. 2013; Patel et al. 2014; Patel et al. 2016).

## 2. Pallister–Hall syndrome (PHS; MIM# 146510)

PHS first described in 1980 by Hall as a lethal condition in neonatal period associates mainly hypothalamic hamartoma, postaxial polydactyly, bifid epiglottis and imperforate anus at the mild end to laryngotracheal cleft with neonatal lethality at the severe end. PHS is rare showing autosomal dominant pattern of inheritance with unknown prevalence. More than 100 PHS cases were reported by Biesecker et al. and a number of additional cases have been reported (Démurger et al., 2015). It is suspected that many individuals with postaxial polydactyly and asymptomatic hypothalamic hamartoma or bifid epiglottis may be misdiagnosed as having non-syndromic PAP-A.

### 2.1 PHS and GLI3

*GLI3* is the only gene in which pathogenic variants are known to cause Pallister-Hall syndrome. PHS *GLI3* mutations include nonsense, frameshift, and single splice mutations in the middle third of *GLI3*, which includes exons 13, 14, and part of 15 (Johnston et al., 2005). In one study, 40% (8/20) of persons affected with sub-PHS had pathogenic variants in *GLI3* that were similar to the pathogenic PHS variants (Johnston

et. al., 2010). All mutations identified to date predict a truncated protein and majority of truncating mutations occurred in the middle third of the protein. These mutations retain the C2H2 zinc finger but are missing the last third of the protein result in the production of a constitutive repressor protein leading to PHS. A total of 31 PHS mutations and 6 mutations for Sub-Pallister-Hall syndrome, 2 mutations in case of PHS with genital abnormalities which include a female with hydrometrocolpos secondary to vaginal atresia and a male with micropenis, hypoplastic scrotum and bilateral cryptorchidism (Narumi et al., 2010) have been reported in *GLI3* on HGMD database.

### **3. Acrocallosal syndrome (MIM# 200990):**

Acrocallosal syndrome (ACS) is a rare disorder, first described in 1979 by Schinzel. More than 50 patients have been described and a wide clinical spectrum exists. Clinical features of ACS include postaxial polydactyly, hallux duplication, macrocephaly and absence of the corpus callosum. Severe developmental delay was also noticed in the patients. Clinical variations and severity exist among affected family members. ACS showed autosomal recessive pattern of inheritance in family (Elson et al., 2002).

#### **3.1 ACS and *GLI3*:**

Only two mutations of *GLI3* have been reported for ACS. Elson et al. (2002) reported first heterozygous c.2800G>C mutation in the *GLI3* gene, which predicts p.Ala934Pro in a single patient with ACS (Elson et al., 2002). Second mutation in *GLI3* was c.2786T>C which predicts p.Leu929Pro reported by Speksnijder (2012). Apart from *GLI3*, some autosomal recessive mutations in the *KIF7* gene have been reported in a few ACS patients. These mutations hampered *GLI3* processing and dysregulation of *GLI3* transcription factors lead to ACS in patient (Putoux et al., 2011).

### **4. Oral-facial-digital syndrome**

In 1941, Mohr reported an OFD family with clinical entity oral (high-arched palate, lobate tongue with papilliform outgrowths), facial (broad nasal root, hypertelorism), and digital (syndactyly, brachydactyly, polydactyly of the hands and feet) anomaly. Oral-facial-digital-syndromes (OFDS) are a heterogeneous group of rare developmental disorders comprised of 13 groups of syndromes with affects mouth, face and digits. Involvement of central nervous system (CNS) and visceral organs such as kidney were also frequently observed in OFDS. OFD types I and VIII are X-linked whereas the majority of OFDS is transmitted as an autosomal recessive syndrome.

#### **4.1 OFD and *GLI3***

11 genes responsible for OFDS (OFDI, III, IV, VI, IX, XIV and two unclassified OFD subtypes) have been identified. *GLI3* were also studied as a candidate for OFDS and 6 mutations were reported in *GLI3* which leads to OFDS (Johnston JJ et al., 2010).

## 5. Trigenocephaly with craniosynostosis and polydactyly

Trigenocephaly is a premature fusion of the metopic suture results in abnormal head shape because of triangular appearance of the forehead when examined from above. Trigenocephaly can occur as an isolated malformation or as part of a multiple anomaly syndrome (Lajeunie et al., 1998). Craniosynostosis is a premature fusion of one or more cranial sutures resulting in an abnormal shape of head. It may result from a primary defect of ossification (primary craniosynostosis) or, more commonly, from a failure of brain growth (secondary craniosynostosis).

### 5.1 Trigenocephaly with craniosynostosis and polydactyly and GLI3

McDonald-McGinn et al., 2010 reported two frameshift mutations p.Ser340ValfsX7 and p.His1515ProfsX3 in GLI3 resulting in premature protein termination of protein. Hurst (2011) further extended 5 mutations of GLI3 for GCPS with metopic and sagittal synostosis.

## II. Non-syndromic Polydactyly

The non-syndromic polydactyly can be classified into preaxial polydactyly (PPD), axial (central) polydactyly and postaxial polydactyly (PAP) according to the position of the extra digit(s) in the hand or foot. PPD is defined as a supernumerary digit affecting the first digits, PAP involves the fifth digits, and axial (central) polydactyly involves duplication of three central digits. PAP can be further classified into types A and B; in PAP type A (type A, PAPA) extra digit(s) are well developed and in PAP type B (type B, PAPB) rudimentary digits were present. Non-syndromic polydactyly frequently exhibits an autosomal dominant inheritance pattern with variable penetrance.

### 1. Non-syndromic Polydactyly and GLI3

Two heterozygous p.L1216PfsX31 and p.R290X mutations in the GLI3 were reported in two families presented with PPD4 (Radhakrishna et al., 1999; Fujioka et al., 2005). A mutation p.A765PfsX14, in the GLI3 was identified in a family from Gujarat state of India affected with PAPA1 (Radhakrishna et al., 1999). A family affected with PAP of the hands with/without feet and variable syndactyly of digits was reported to carry a mutation p.R539TfsX12 in the GLI3 (Al-Qattan, 2012). Three mutations p.E147X, p.R643X and p. G727R in the GLI3 were also identified in three families reported from Gujarat, India, with dominant PAPA/B. Recently, a patient with both types of post-axial polydactyly type: PAP-A in feet and PAP-B in hands was reported to carry a mutation p.E1478X in GLI3 (Patel et al., 2016).

## III. Polydactyly with other limb abnormalities

Polydactyly can also occur with other limb anomalies like variable degrees of syndactyly of fingers and toes. Two different cases with polydactyly-syndactyly complex were reported to have mutations in GLI3. First report from China revealed a C-terminal frame-shift mutation p.Asp962MetfsX41 in GLI3 responsible for non-

syndromic and complex digital anomalies a family (Cheng et al., 2011). Another mutation p.His601Arg was reported in a Jewish Moroccan family with preaxial-postaxial polydactyly-syndactyly complex (Volodarsky et al. 2014).

### Conclusion

The phenotypes caused by mutations in *GLI3* are diverse, discrete, variable, and pleiotropic. As of December 2016, 221 different mutations in the *GLI3* gene were listed in the human gene mutation database (HGMD). The vast majority of mutations were detected in patients with syndromic polydactyly (GCPS and PHS) and only a few mutations were detected in non-syndromic polydactyly. The craniofacial manifestations of GCPS were highly variable and not all patients with GCPS have obvious macrocephaly or hypotelorism as observed in many cases. The milder end of the GCPS spectrum can include isolated polydactyly only. There is no clear delineation exists between GCPS and non-syndromic polydactyly. Furthermore *GLI3* mutations observed in Indian population is entirely different from other reported population and even patients manifest wide range of phenotypes. The precise molecular mechanism through which the specific mutation leads to variable disease phenotypes are yet to be elucidated.

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**FIGURE LEGENDS**

**Figure 1.** A, B) Clinical photographs of a patient showing bilateral polydactyly with syndactyly of lower limb and bilateral postaxial polydactyly of upper limb. C & D) Bilateral postaxial polydactyly of lower limb and unilateral postaxial polydactyly of left hand in second case. (Patel et al. 2016)



