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SHORT REPORT

GLI3 variants causing isolated polysyndactyly are not restricted to the protein's C-terminal third

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Abstract

Loss of function variants of *GLI3* are associated with a variety of forms of polysyndactyly: Pallister-Hall syndrome (PHS), Greig-Cephalopolysyndactyly syndrome (GCPS), and isolated polysyndactyly (IPD). Variants affecting the N-terminal and C-terminal thirds of the GLI3 protein have been associated with GCPS, those within the central third with PHS. Cases of IPD have been attributed to variants affecting the C-terminal third of the GLI3 protein. In this study, we further investigate these genotype–phenotype correlations. Sequencing of *GLI3* was performed in patients with clinical findings suggestive of a *GLI3*-associated syndrome. Additionally, we searched the literature for reported cases of either manifestation with mutations in the *GLI3* gene. Here, we report 48 novel cases from 16 families with polysyndactyly in whom we found causative variants in *GLI3* and a review on 314 previously reported *GLI3* variants. No differences in location of variants causing either GCPS or IPD were found. Review of published data confirmed the association of PHS and variants affecting the GLI3 protein's central third. We conclude that the observed manifestations of *GLI3* variants as GCPS or IPD display different phenotypic severities of the same disorder and propose a binary division of GLI3-associated disorders in either PHS or GCPS/polysyndactyly.

KEYWORDS
GCPS, genotype–phenotype correlations, GLI3, PHS, polydactyly, syndactyly

1 | INTRODUCTION

The Gli-Kruppel family member 3 (*GLI3*) gene encodes a zinc finger transcription factor that plays an important role in the sonic hedgehog signaling pathway and thus in various developmental processes including limb development (MIM: *165240).[1,2] Genetic variants in *GLI3* are well known causes of the allelic disorders Pallister-Hall syndrome (PHS; MIM: #146510), Greig-Cephalopolysyndactyly syndrome (GCPS; MIM: #175700), postaxial polydactyly type A and B (PAP-A and -B; MIM: #174200), and preaxial polydactyly type IV (PPD-IV;

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TABLE 1 Sixteen novel families with *GLI3*-associated disorders: Indices and their affected family members

Family	ID	Mutation	Inherited variant?	Polydactyly hands	Syndactyly hands	Polydactyly feet	Syndactyly feet	Macrocephaly	Hypertelorism	Broad forehead	Broad nasal bridge	Hamartoma	Headache	Seizures	Developmental delay	Others
1	1–1	c.2374C>T, p.(Arg792*)	+	PAP-A both	–	PPD-IV both	I/II both	–	–	–	–	–	–	–	–	
	1–2			PAP-A both	–	PPD-IV both	–	–	–	–	–	–	–	–	–	
	1–3			PAP-A both	–	PPD-IV both	–	–	–	–	–	–	–	–	–	
	1–4			PAP-A both	–	PPD-IV both	–	–	–	–	–	–	–	–	–	
2	2–1	c.4172delG, p.(Gly1391Alafs*28)	+	PAP-A both	–	PAP-A and PPD-IV R, PAP-A L	–	+	+	–	–	–	–	–	–	Speech delay
	2–2			–	–	+	–	+	+	+	+	–	–	–	–	Speech delay
	2–3			+	–	+	–	+	+	–	–	–	–	–	–	
3	3–1	c.2059delG, p.(Glu687Lysfs*6)	+	PAP-A both, duplicated IV R	R	Duplicated IV L, duplicated III R	both	–	–	–	–	–	–	–	–	
	3–2		n.t.	+	–	One foot	–	–	–	–	–	–	–	–	–	
	3–2		+	PAP-B both	–	+	–	–	–	–	–	–	–	–	–	
	3–4		n.t.	+	–	+	–	–	–	–	–	–	–	–	–	
	3–5		n.t.	+	–	–	–	–	–	–	–	–	–	–	–	
	3–6		n.t.	+	–	One foot	–	–	–	–	–	–	–	–	–	
	3–7		n.t.	+	–	–	–	–	–	–	–	–	–	–	–	
4	4–1	c.1999C>T, p.(Arg667*)	+	PAP-B both	II-V both	PPD-IV both	I-IV R, I-III L	–	+	–	+	–	–	–	–	
	4–2		n.t.	+	–	+	+	+	–	–	–	–	–	–	–	
	4–3		+	PAP-B both	–	–	I-II both	–	–	+	–	–	–	–	–	
5	5–1	c.1880_1881delAT, p.(His627Argfs*48)	De novo	PAP-B both	–	PPD-IV both	I-III both	–	–	–	–	–	–	–	–	
6	6–1	c.1793dupA; p.(Asn598Lysfs*7)	+	PPD-IV both, PAP-B both	–	PPD-IV both	II-IV both	+	+	+	–	–	–	–	–	
	6–2		+	PPD-IV both, PAP-B both	–	PPD-IV both	II-IV both	–	–	–	–	–	–	–	–	
7	7–1	c.1028 + 1G>A, p.?	+	–	–	PPD-IV both	I-III both	–	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
	7–2		n.t.	PAP-B both	–	PPD-IV both	I-II both	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	CAH (simple-virilizing)
	7–3		n.t.	–	IV-V both	PPD-IV both	I-II L, I-III R, IV-V both	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
	7–4		n.t.	–	IV-V both	–	I-III both	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
	7–5		n.t.	–	IV-V both	–	I-III both	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
8	8–1	c.366C>A, p.(Tyr122*)	+	PPD-IV both, PAP-B both	III-IV both	PPD-IV both	I-IV both	–	–	–	–	–	–	–	–	

(Continues)

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TABLE 1 (Continued)

Family	ID	Mutation	Inherited variant?	Polydactyly hands	Syndactyly hands	Polydactyly feet	Syndactyly feet	Macrocephaly	Hypertelorism	Broad forehead	Broad nasal bridge	Hamartoma	Headache	Seizures	Developmental delay	Others
	8–2		n.t.	PPD-IV both	II-IV L, III-V R	PPD-IV both	II-III both	–	–	–	–	–	–	–	–	
	8–3		+	Synpolydactyly both hands and feet	–	Synpolydactyly both hands and feet	–	–	–	–	–	–	–	–	–	
	8–4		n.t.	Synpolydactyly both hands and feet	–	Synpolydactyly both hands and feet	–	–	–	–	–	–	–	–	–	
9	9–1	c.1033_1048del; p.(Ala345Thrfs*15)	+	–	I-II R	–	I-III both	–	–	–	–	–	–	–	–	
	9–2		n.t.	–	+	–	+	–	–	–	–	–	–	–	–	
	9–3		+	PAP L	III-IV both	PPD-IV both	–	–	–	–	–	–	–	–	–	
	9–4		n.t.	Hexadactyly both	–	–	–	–	–	–	–	–	–	–	–	
10	10–1	c.2103 + 2 T>A, p.?	De novo	PAP-A both	–	PAP-A L	–	–	–	–	–	–	–	–	–	
11	11–1	c.2595C>G, p.(Ser865*)	?	PAP both	Synostosis III-IV both	–	–	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
12	12–1	c.868C>T, p.(Arg290*)	+	PPD-IV both, PAP-B both	III-IV L	Central polydactyly both	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
	12–2		+	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
13	13–1	c.1133_1134insC, p.(Pro379Serfs*33)	?	PAP both	III-IV R	PPD-IV both	II-IV both	–	–	–	+	–	–	–	–	
	13–2		n.t.	Broad thumbs both, PAP-B both	III-IV R	PPD-IV both	II-IV both	–	–	–	+	–	–	–	–	Elongated head
	13–3		n.t.	Broad thumbs, polydactyly	+	PPD-IV both	II-IV both	–	–	–	+	–	–	–	–	Elongated head
14	14–1	c.1880A>C, p.(His627Pro)	+	PAP-B both	–	PPD-IV both	I-II R	–	–	–	–	–	–	–	–	
	14–2			PAP-B both	–	–	–	–	–	–	–	–	–	–	–	Malposition of the feet
15	15–1	c.2374C>T, p.(Arg792*)	de novo	PPD-IV both, PAP-B both	III-V both	PPD-IV both	I-III both	–	–	–	–	–	–	–	–	
16	16–1	c.3667_3670delinsATCAA, p.(Tyr1228Ilefs*24)	?	+	–	+	–	+	–	–	–	–	–	–	–	
	16–2		n.t.	PPD-IV both, PAP-B both	+	+	+	+	–	–	–	–	–	–	+	
	16–3		n.t.	–	+	+	+	+	–	–	–	–	–	–	+	
	16–4			+	–	+	–	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	
	16–5		n.t.	PPD-IV both	–	–	–	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	

Note: *GLI3* variants refer to the transcript NM_000168.6.

Abbreviations: I-V, respective digits; L, left; R, right; n.p., not phenotyped; n.t., not tested; PAP-A/B, postaxial polydactyly type A/B; PPD-IV, preaxial polydactyly type IV.

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MIM: #174700).[3,4] They all feature poly- and syndactylies of varying severity. While PAP-A, PAP-B, and PPD-IV are defined by non-syndromic polysyndactyly, PHS and GCPS depict more complex syndromic phenotypes. GCPS is characterized by polysyndactyly, macrocephaly, and facial dysmorphisms (especially hypertelorism, broad nasal bridge, high forehead, and frontal bossing). Mild mental

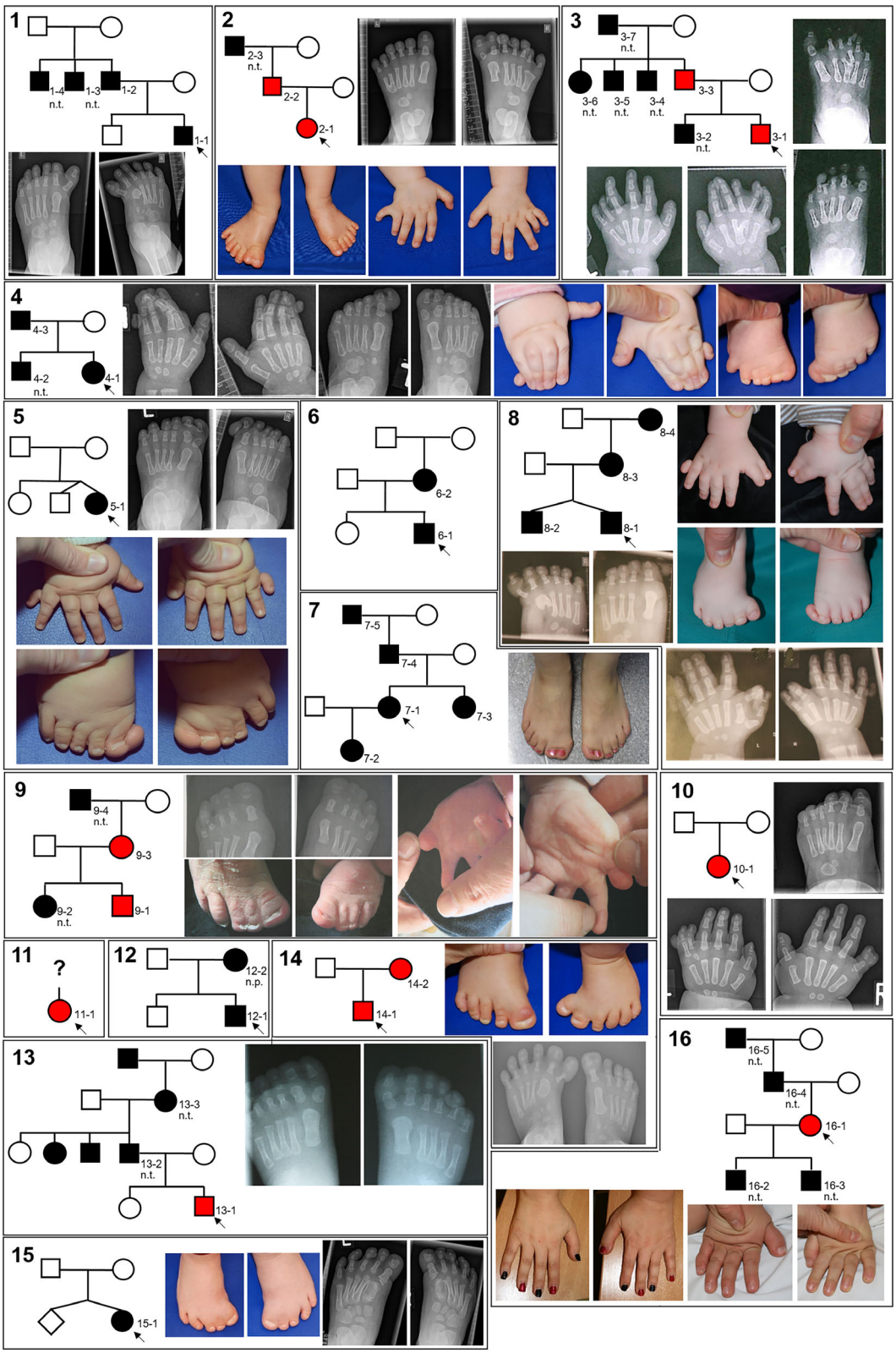

FIGURE 1 Family pedigrees and images of index patients. Pedigrees: red: affected individuals carrying novel variants; solid black: affected individuals; n.t.: not tested; n.p.: not phenotyped; arrow: index patient [Colour figure can be viewed at wileyonlinelibrary.com]

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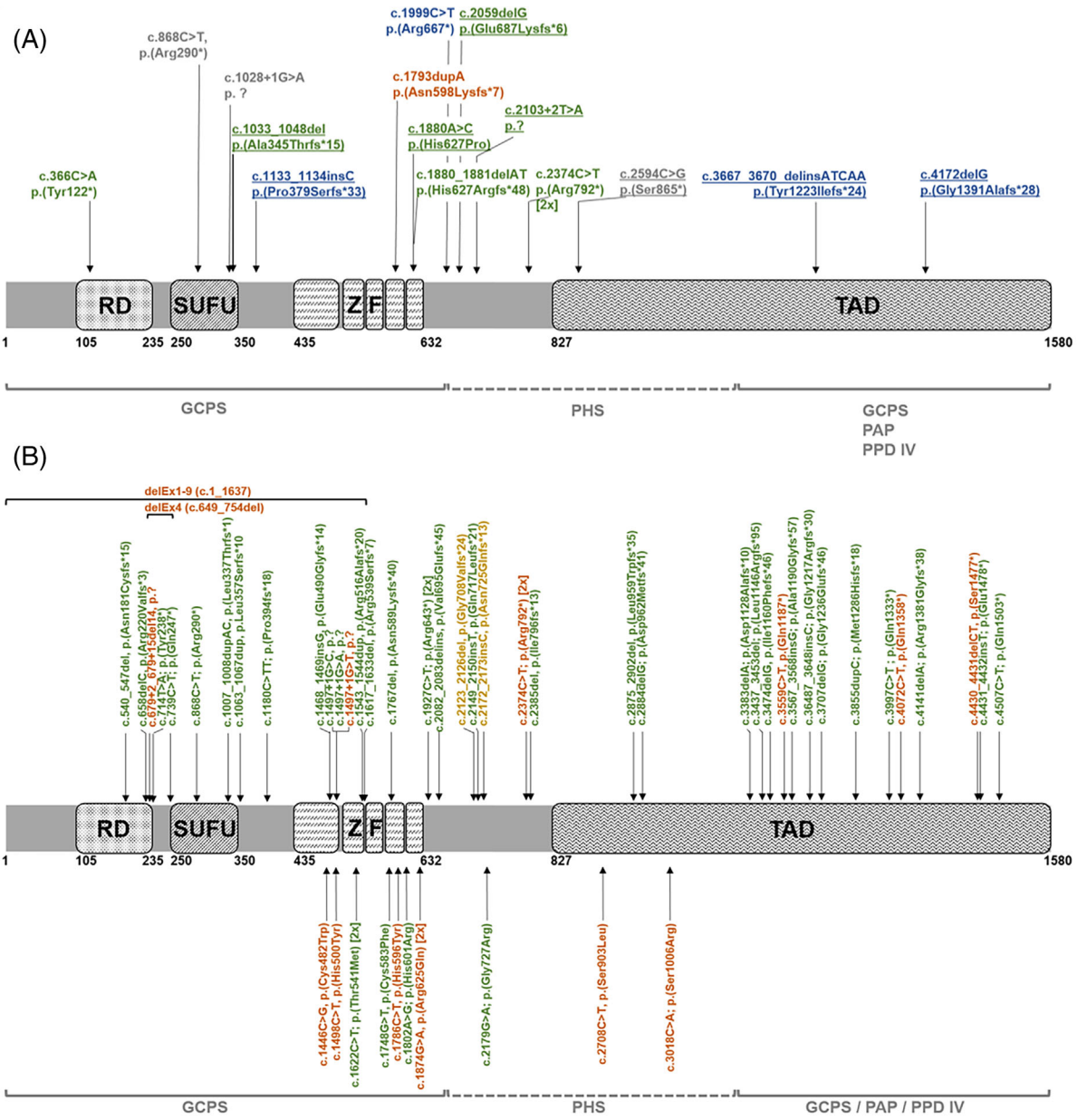

FIGURE 2 Legend on next page.

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retardation, trigonocephaly and craniosynostosis as well as further rare anomalies may occur. By contrast, abnormalities characteristic for PHS are a (mostly postaxial) polysyndactyly associated with hypothalamic hamartoma, hypopituitarism, bifid epiglottis, imperforate anus, headaches, seizures, and developmental delay. Mild, incidental forms to lethal courses have been described.

The GLI3 protein harbors two N-terminal transcriptional repressor domains (RD and SUFU), five DNA binding zinc finger domains (ZF), and a C-terminal transcriptional activator domain (TAD).[1,5] A certain genotype–phenotype correlation has been proposed: GCPS is predominantly attributed to variants affecting the N-terminal (amino acid position 1–660) and C-terminal third (amino acid position 1160–1580) of the GLI3 protein, whereas PHS is attributed to variants affecting the central third (amino acid position 661–1159) of GLI3.[6] Isolated polysyndactyly (IPD), however, is supposed to result from C-terminal variants of the GLI3 protein.[7,8]

2 | PATIENTS AND METHODS

Patients with either syndromic or nonsyndromic polysyndactyly were included in our study and phenotyped by clinical geneticists and/or primary physicians. Patients with clinical findings suggestive of a *GLI3*-associated polysyndactyly syndrome were selected for targeted Sanger sequencing of the *GLI3* gene performed on genomic DNA isolated from whole blood samples. Whenever possible trio sequencing of the affected index and both parents was applied. Raw Sanger sequencing data were analyzed using SeqPilot (JSI medical systems, USA) and variants were evaluated using ClinVar (NCBI, USA), HGMD (Qiagen Digital Insights, Denmark), and gnomAD[9] databases. For missense variants, an additional pathogenicity prediction was conducted using the bioinformatic prediction tools MutationTaster,[10] Polyphen2,[11] and SIFT.[12]

3 | RESULTS

We tested 94 individuals with polysyndactyly and detected 15 different causing, mostly amorphic, *GLI3* variants in 16 families (Table 1 and Table S1; Figures 1 and 2A). Eight were novel variants and seven had been reported previously. The variants spanned almost the entire coding region of *GLI3* (c.366—c.4172).

Upon identification of a causing variant in *GLI3* further and more detailed phenotypic information was collected. Eight families showed no further abnormalities beyond IPD (families 1, 3, 5, 8, 9, 10, 14, 15) whereas five families showed additional features in line with GCPS (families 2, 4, 6, 13, 16). Three families did not take part in further phenotypic characterization (families 7, 11, 12). We did not observe a distinct correlation between type of mutation (nonsense vs. missense) and phenotype. Notably, specific phenotypes and severity varied within families. However, in the majority of families diagnosis of either IPD or GCPS was uniform within families. Only in family 6 both GCPS and IPD occurred (Table 1; Figure 2A). Subgroup analysis of polydactyly subtypes revealed a co-occurrence of PAP-B and PPD-IV in hands. In feet PPD-IV was the leading manifestation of polydactyly, in one case also accompanied by PAP-A (Figure 1 and Figure S1).

Conducting a retrospective analysis of studies on *GLI3*-associated disorders, we analyzed 309 published cases of *GLI3* variants (Table S2). Of those, 65 cases showed IPD. In 32 out of these 65 cases (49.2%) of IPD, variants affected the N-terminal third of the GLI3 protein, while 20 cases (30.8%) harbored variants affecting the central and 12 cases (18.5%) the C-terminal third of the GLI3 protein. One case (1.5%) was caused by a large structural variant. Interestingly, none of the identified missense variants (12 out of 65 cases) affected the C-terminal third of the GLI3 protein (Figure 2B,C).

While GCPS cases were also associated with variants spread across the entire GLI3 protein (79/157 (50.3%) in the N-terminal third, 24/157 (15.3%) in the central third, 37/157 (23.6%) in the C-terminal third and 17/157 (10.8%) large SVs), PHS cases showed a distinct genotype–phenotype correlation with the majority of cases (68/74 (91.9%)) harboring variants affecting the central third of the GLI3 protein (Figure 2C).

4 | DISCUSSION AND CONCLUSION

In 16 families with identified *GLI3* variants, we observed GCPS as well as IPD. Notably, none of the individuals in our study presented with PHS. This can most likely be attributed to a prior selection bias since classic PHS presents with a severe phenotype.

Previous studies suggested a genotype–phenotype correlation of *GLI3*-associated disorders with GCPS being associated with variants affecting the protein's N-terminal or C-terminal third and IPD only occurring when variants affected the C-terminal third.[6–8] Yet, in this study, we could not observe such a distinct genotype–phenotype correlation. Strikingly, *GLI3* variants associated with IPD even occurred exclusively in the N-terminal and central third of the GLI3 protein. Interestingly, we observed only one variant affecting the protein's C-terminal third which was associated with the presence of GCPS. Three further variants causing GCPS affect the N-terminal third of the GLI3 protein, whereas only one variant associated with GCPS affects the protein's central third, so that locations of variants causing GCPS are

FIGURE 2 (A) *GLI3* variants identified in 16 families and their location within the GLI3 protein. Green: variants causing IPD. Blue: GCPS. Orange: IPD as well as GCPS. Gray: Patients could not be fully phenotyped. Underscored: novel variants. Brackets below indicate regions previously described to be associated with the respective *GLI3* disorders (B) and (C) Review of published *GLI3* variants and associated phenotypes (B) Location of *GLI3* variants causing IPD. Top: truncating variants, bottom: missense variants. Green: variants causing IPD. Orange: IPD as well as GCPS. Yellow: IPD as well as PHS. (C) Descriptive statistics of location of *GLI3* variants and the associated phenotypes. Top: affected regions of the GLI3 protein and the phenotypes associated with each affected region. Bottom: vice versa, different *GLI3*-associated phenotypes and regions of the GLI3 protein the respective causing variant is found in [Colour figure can be viewed at wileyonlinelibrary.com]

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overall compatible with previous reports (Figure 2A). Additionally, one individual showed GCPS whereas another individual from the same family presented with IPD. This phenomenon could also be observed in previously published cases (Figure 2B). Thus, we follow previous observations[13–15] and hypothesize that GCPS and IPD might be different extents of severity of the same disorder rather than distinct entities of disease. Also, we identified several variants affecting the central third of the GLI3 protein that were associated with IPD but not with PHS. These cases possibly represent mild forms of PHS similar to those reported previously.[13] Since brain imaging results of patients with IPD were not available, we cannot exclude the presence of such asymptomatic hamartomas.

A recent study focusing on the limb phenotypes associated with *GLI3* variants found a correlation of variants affecting the N-terminal half of GLI3 with an anterior polydactyly phenotype (leading manifestation of PPD-IV in feet) and variants affecting the C-terminal TAD region with a posterior polydactyly phenotype (leading manifestation of PAP in hands).[16] The majority of the variants identified in our study located to the N-terminal half of GLI3, whereas only three variants affected the C-terminal TAD. With our data, we can neither confirm nor exclude these observations by Baas et al. (Figure S2).

To further evaluate a genotype–phenotype correlation regarding GCPS versus IPD, we performed a retrospective analysis of published cases with *GLI3* variants. In line with observations from our study, we found no clear genotype–phenotype correlation for IPD in these cases — with 49.2% of *GLI3* variants associated with IPD affecting the protein's N-terminal, 30.8% the central and 18.5% the C-terminal third. The same holds true for GCPS cases, whereas we could confirm the genotype–phenotype correlation for PHS with 91.9% of causing variants affecting the central third of the GLI3 protein. Notably, nonsense variants causing IPD affected almost the entire coding region of *GLI3* (c.540–c.4507) whereas missense variants clustered in the central part of the GLI3 protein (c.1446–c.3018) and especially the zinc-finger domains (Figure 2B). We could also confirm the previously stated observation that both GCPS and IPD are caused by varying types of variants (missense, nonsense, splice site, larger deletions).[6,15]

These results from our broad retrospective analysis of 314 published cases with confirmed *GLI3* variants are in line with previous studies of larger cohorts: Kalff-Suske et al. also showed that nonsense variants in *GLI3* leading to GCPS are distributed over the entire protein while Johnston et al. as well as Démurger et al. found that variants in the central third of GLI3 cause PHS.[6,17,18] Also, previous studies reported variants affecting all thirds of the GLI3 protein associated with a spectrum from IPD to GCPS.[14,15] Neither in our cohort nor in previous studies patients with *GLI3* variants and an isolated non-limb phenotype only (e.g., macrocephaly, hypertelorism, broad forehead) could be identified. Interestingly however, Démurger et al. reported an even asymptomatic carrier of a familial *GLI3* variant (c.427G>T, p.(Glu143*)) highlighting the clinical variability of the phenotypes.[17]

Thus, IPD appears not to be restricted to cases with variants affecting the C-terminal third of the GLI3 protein but may manifest independently of variant location. PHS, however, is strongly correlated to variants affecting the protein's central third. Taken together, *GLI3* variants are associated with a phenotypically broad spectrum of only two distinct entities: GCPS on the one hand and PHS on the other, with IPD occurring as a mild manifestation of GCPS or in rarer cases of PHS.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14059.

DATA AVAILABILITY STATEMENT

Original sequencing data are available upon reasonable request.

ETHICS STATEMENT

Tested individuals provided written informed consent for participating in this study as approved by the ethical review board of the Charité — Universitätsmedizin Berlin.

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