# Novel Bi-Allelic *LGI3* Variants and Intellectual Developmental Disorder: Clinical and Genetic Insights from Four New Families

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## INTRODUCTION

Bi-allelic loss-of-function variants in *LGI3* (MIM: #608302) have been implicated in intellectual developmental disorder with muscle tone abnormalities and distal skeletal defects (IDDMDS). Current data are limited to a small number of families (16 patients from eight unrelated families), and the phenotypic variability observed suggests the presence of additional, as-yet-unidentified variants with distinct clinical manifestations[1]. Furthermore, the mechanisms through which *LGI3* dysfunction leads to the observed phenotypes remain poorly elucidated, highlighting the need for further research to dissect its role in neuronal and neuromuscular development[2]. In this study, we expand the genetic and phenotypic spectrum of IDDMDS by identifying four novel homozygous *LGI3* variants from four unrelated consanguineous families, comprising seven clinically affected patients.

## RESULTS

**Clinical Findings:** All families reported consanguinity (first-cousin). Clinical features varied widely (Table 1), but the common symptoms include global developmental delays (7/7 patients), microcephaly (6/7 patients), distinctive skeletal deformities (6/7 patients), failure to thrive (6/7 patients), intellectual disabilities of varying severity (5/7 patients), and facial dysmorphism (5/7 patients). Interestingly, we observed short stature, failure to thrive, and microcephaly in 6 patients, while this combination was observed in only 1 out of 16 patients in the literature[1] (Figure 1).

**Table 1: Developmental and neurological features.**

| Individuals | F1 - II.5 | F1 - II.6 | F2 - II.2 | F2 - II.4 | F2 - II.5 | F3 - II.2 | F4 - II.1 |
|---|---|---|---|---|---|---|---|
| Ethnic origin | Pakistan | Pakistan | Pakistan | Pakistan | Pakistan | Iran | Iran |
| Age at last exam | 5 years 5 months | 3 years 7 months | 8 years 11 months | 4 years | 2 years | 4 years | 3 years 5 months |
| Sex | Female | Male | Male | Female | Female | Female | Female |
| Global developmental delay | + | + | moderate | + (mild) | + (mild) | + | + |
| Intellectual disability | - | - | + (mild) | + (mild) | + (mild) | + | + |
| Areflexia/hyporeflexia | N/A | N/A | Uncertain | Uncertain | Uncertain | DTR 2+ | DTR 3+ Brisk |
| Distal deformities | + | - | + | + | + | + | N/A |
| Facial myokymia | + | + | + | + | + | - | - |
| Small mouth with restricted opening | - | - | - | - | - | - | - |
| Tongue fasciculations | - | + | + | + | + | - | N/A |
| Abnormal NCS | - | - | - | - | - | N/A | N/A |
| Myokymia or fasciculations | + | + | + | + | + | N/A | N/A |
| Short stature | + | + | + | + | + | - | + |
| Failure to thrive FTT | + | + | + | + | + | - | + |
| Microcephaly | + | + | + | + | + | - | + |

Abbreviations: F, Family; N/A, not available; NCS, nerve conduction study; DTR, Deep Tendon Reflex

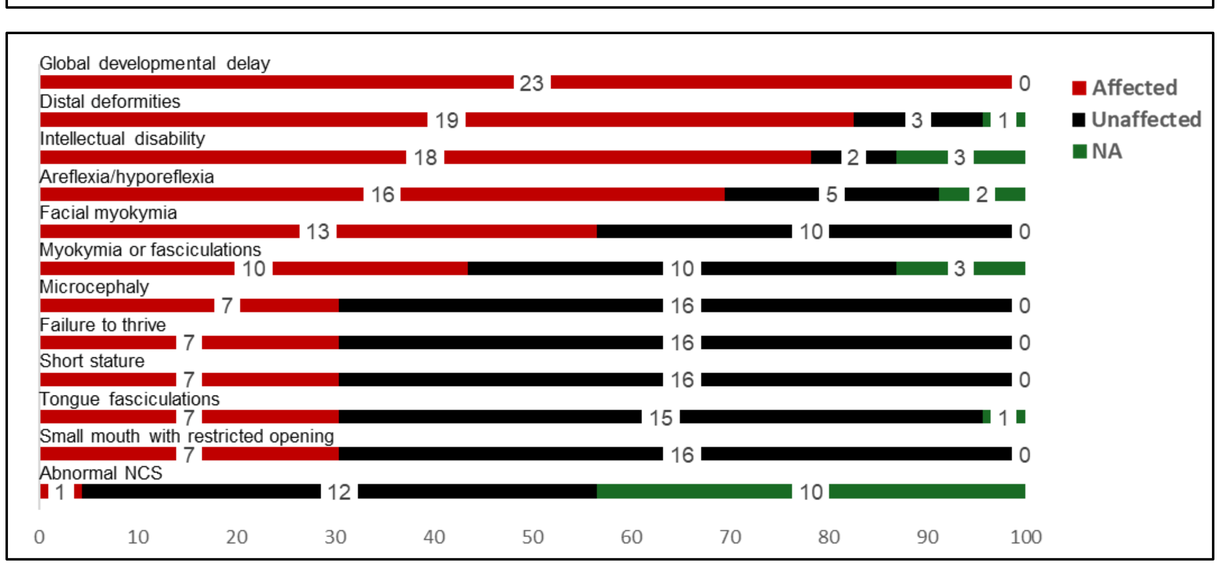


**Figure 1**: **Summary of clinical features in patients with biallelic *LGI3* variants**. The proportion of patients showing specific clinical features among the 7 newly reported individuals (this study) and 16 individuals from previously published reports[1]. The x-axis represents the percentage of patients, while the numbers inside the bars indicate the actual count of patients in each category. Clinical findings are categorized as affected (red), unaffected (black), and no available information (NA, green).

**Genetic Analysis:** We identified four novel biallelic *LGI3* variants in affected individuals, including a stop-gain variant [c.397C>T, p.(Arg133*)], two frameshift variants [c.841_850del; p.(Val281Serfs49)]; and [c.1358del; p.(Gly453Valfs171)], and one missense variant [c.415A>C; p.(Thr139Pro)] (Figure 2).

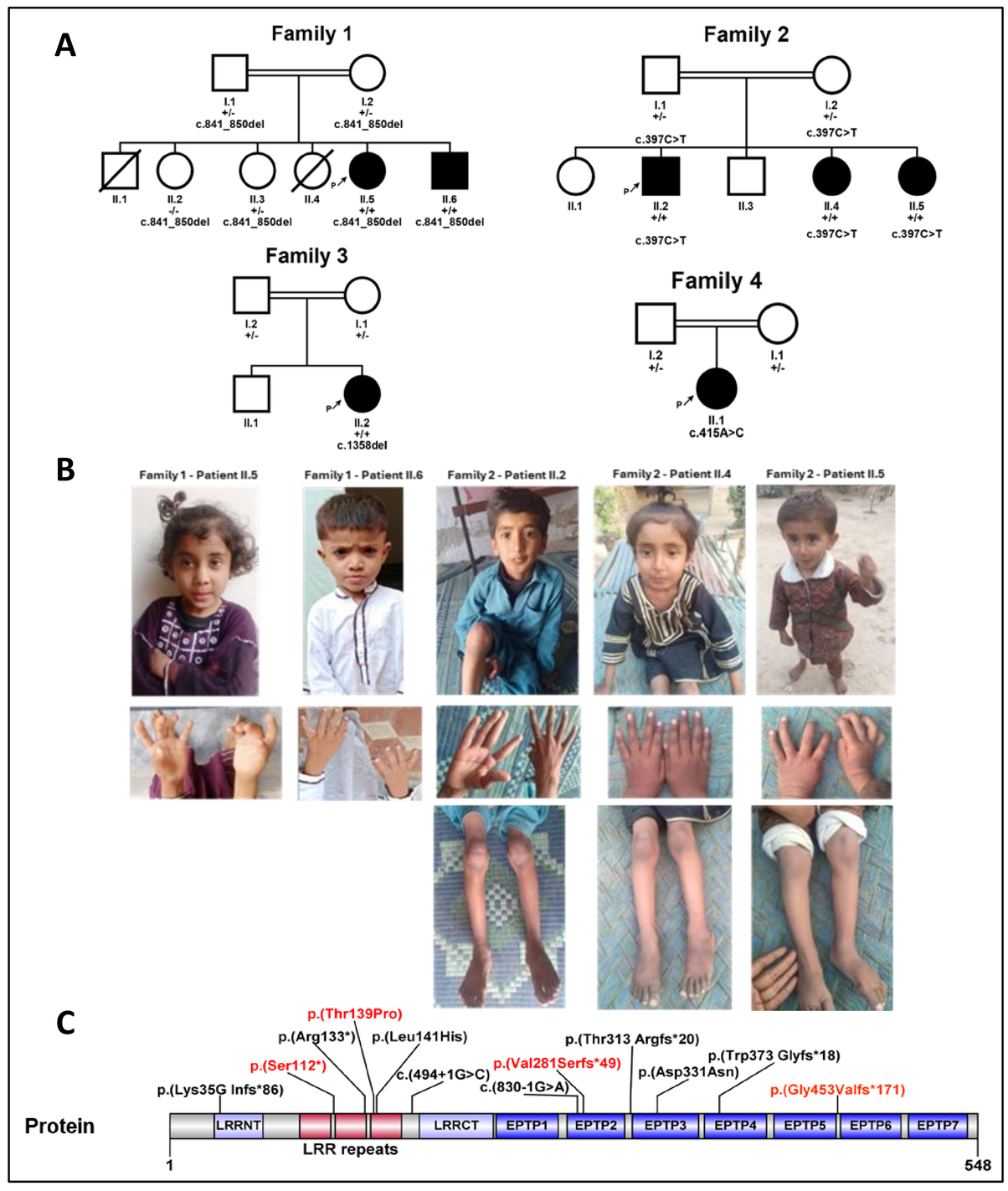


**Figure 2: Clinical features of patients with biallelic variants in *LGI3* gene**: **(A).** Pedigrees of four families in this study showing inheritance of pathogenic *LGI3* variants; **(B)** Facial features of five patients and illustrations of hand and leg anomalies from family 1 and 2. **(C)** Overview of known variants (black) and novel variants reported in this study (red) within the *LGI3* gene, shown at the protein domain level.

## METHODS

**Patient Recruitment:** Four unrelated families with varied clinical features were recruited via direct communication from different centers after obtaining informed consent from the patients or their parents. DNA was extracted from blood samples. **Whole Genome Sequencing - Family 2:** Libraries were prepared using the TruSeq Nano DNA HT kit and sequenced on NovaSeq 6000 (150-bp paired-end, ~30x nuclear, ≥1000x mitochondrial coverage). Reads were aligned to GRCh38 using DRAGEN v4.2.4. SNVs/indels were annotated with Geneyx; SVs with ANNOTSV3.1 and an in-house database. Variants were interpreted using ACMG guidelines and ClinGen recommendations. **Whole Exome Sequencing - Family 1**: Exome sequencing was conducted at CeGaT using the Twist Human Core Exome kit with mitochondrial enrichment. Sequencing achieved >98% coverage at 20x for autosomes and ≥300x for mtDNA. Analysis was performed with DRAGEN (v4.2.4) and Geneyx; CNVs were annotated using ANNOVARv3.1 and an in-house database. **Families 3 and 4:** Exome was performed by Macrogen using Agilent SureSelect V7 and sequenced on NovaSeqX Plus (150-bp paired-end, 100x). Reads were aligned to GRCh37 with BWA, variants called with GATK, annotated via ANNOVAR, and filtered using GATK best practices and in-house MAF <1%. **Sanger Validation**: Segregation of candidate variants was assessed in available family members using Sanger sequencing. The target region of the *LGI3* gene (NM_139278.4, GRCh38) was amplified via PCR and directly sequenced with flanking or internal primers. Trace data from Sanger sequencing were visualized and analyzed using FinchTV (V1.4.0).

## CONCLUSION

In this study, we expand the mutational and phenotypic landscape of *LGI3* by reporting four novel homozygous variants identified in four unrelated consanguineous families. Through comprehensive clinical evaluations and genetic analyses, we aim to better characterize the diverse manifestations of IDDMDS and to improve understanding of *LGI3*'s role in neurodevelopment disorders and its broader implications for rare human disease.

**CONFLICT OF INTEREST STATEMENT:** M. Ali, A. Rad and G. Oprea are current employees of Arcensus GmbH.

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**References:**

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