Case report

# Phenotype-genotype correlation in a case of infantile hypotonia and epilepsy: study of the clinical significance of two variants of uncertain significance in HIVEP2 and LINGO1

ABSTRACT

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| Genetic causes of neurodevelopmental disorders are frequent and complex. We Report The case of a 10-month-old infant followed for psychomotor delay, severe hypotonia, spastic movements of the lower limbs, and complex focal epilepsy. Whole-exome sequencing revealed rare variants in the HIVEP2 (NM\_006734.3: c.6528\_6539del; p. (Gly2177\_Gln2180del)) and LINGO1 (NM\_032808.5: c.829G>A; P. (Ala277Thr)) genes, classified as two variants of uncertain significance. Our observation finds a correlation between the patient's clinical presentation and genetic findings and proposes to expand the phenotypic spectrum associated with mutations in HIVEP2 and LINGO1 genes. It also illustrates their possible convergence in the genesis of severe neurodevelopmental disorders. We emphasize the importance of multidisciplinary care in managing severe neurodevelopmental disorders, the potential synergistic effect of mutations in distinct genes involved in neurodevelopmental pathways, and a reflection on the challenges related to vus interpretation, especially when multiple genes are associated. |

***Keywords:*** *neurodevelopmental disorder, hypotonia, epilepsy, HIVEP2, LINGO1, VUS, genetics*

1. INTRODUCTION

Hypotonia and infantile epilepsy of genetic origin are common neurodevelopmental disorders in neuropediatric, posing diagnostic and therapeutic challenges. Variants in HIVEP2 and LINGO1 genes are described as responsible for neurodevelopmental disorders. HIVEP2 is responsible for hypotonia, movement disorders, cerebral palsy, language delay, epilepsy, microcephaly, autism spectrum disorder, and intellectual disability 1. LINGO1 is associated with motor delay, severe intellectual disability, spastic hypertonia, language delay, microcephaly, epilepsy, and global developmental delay2. Whole-exome sequencing of our patient revealed variants of uncertain significance in these two genes. Many mutations are classified as variants of uncertain significance (VUS) because the evidence available at the time of their discovery is insufficient to establish their involvement in the disease3. Result interpretation is difficult when the patient's phenotype corresponds to described mutations, complicating genetic counseling. The objective of our work is to highlight a potential correlation between very rare mutations affecting the HIVEP2 and LINGO1 genes classified as VUS and the phenotype of our infant. We also suggest the synergistic effect of these mutations in the severity of our patient's clinical presentation. For this purpose, we performed a clinical evaluation, including a complete neurological examination, magnetic resonance brain imaging, waking and sleeping EEG, and metabolic assessment.

**2. CASE PRESENTATION**

A 10-month-old male infant, born on 07/15/2022, is being followed for psychomotor delay, hypotonia, epilepsy, and spastic limb movements. He was born to non-consanguineous parents, with a 32-year-old mother who had a history of three pregnancies, including one stillborn female. He has a healthy 5-year-old brother.

**2.1 Clinical History:**

Birth occurred at 34 weeks of gestation via cesarean section following premature rupture of membranes, with a birth weight of 1900g (intrauterine growth restriction). At 2 months of age, the infant was hospitalized for cyanotic episodes, revealing an interatrial communication. Chronic postprandial vomiting appeared at 4 months. At 5 months, psychomotor regression was recorded with loss of head control and grasping ability.

The clinical course was marked by initially pharmacosensitive epilepsy. Treatment began with sodium valproate, and the subsequent addition of carbamazepine led to significant improvement, notably reducing seizure frequency from 1-2 per week to 2 per 3 months, and their duration from 5 minutes to less than 1 minute. Persistent axial hypotonia was noted, with several admissions to intensive care for post-infectious febrile respiratory distress, though mechanical ventilation was not required.

Psychomotor development has remained stagnant since 2 months of age: absence of head control, unachieved sitting position, absence of grasping, and language delay (monosyllabic).

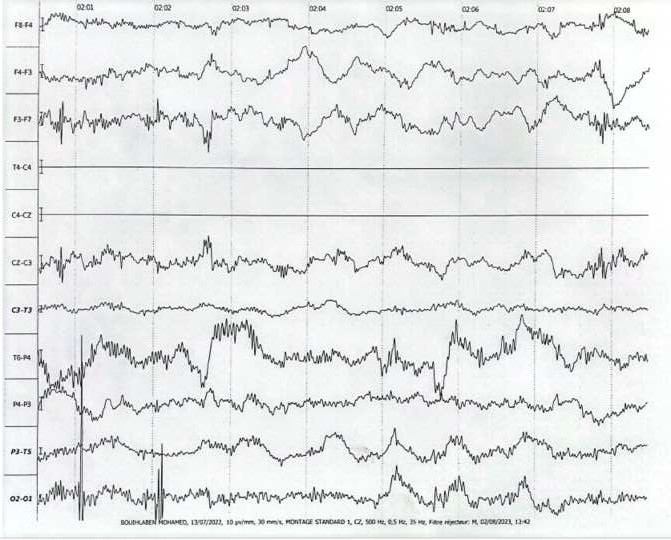
**2.2 Clinical Examination**:

****At 10 Months examination revealed axial hypotonia (**Figure 1**), spastic movements of the upper limbs, good visual contact, absence of ptosis, macroglossia or lingual fasciculations, present osteotendinous reflexes, systolic murmur, weight < -3 SD, height at-1SD, head circumference at -1 SD, and absence of hepatosplenomegaly and achromic patches.

***Figure 1.*** *Severe Axial Hypotonia in a 10-Month-Old Infant with HIVEP2 and LINGO1 Variants Clinical photograph demonstrating severe axial hypotonia (anterior view). Note: (A) marked head lag with inability to maintain head control (white arrow); (B) significant truncal weakness evidenced by poor postural control (black arrow); (C) characteristic limb positioning. Scale bar = 10 cm. Parental written consent was obtained for publication of this image.*

**2.3 Paraclinical Examinations:**

Cerebral MRI was normal. EEG showed paroxysmal abnormalities suggestive of complex focal epilepsy (**Figure 2**). Metabolic workup (lactate, ammonia, amino acid profile, and CPK) was normal Ophthalmological examination was normal Echocardiography revealed a left-to-right shunt interatrial communication of 14 mm.s.



***Figure 2.*** *Paroxysmal EEG Abnormalities Demonstrating Complex Focal Epilepsy Representative EEG recording showing epileptiform abnormalities. Recording parameters: sensitivity 7μV/mm, time constant 0.3s, high-frequency filter 70 Hz, sampling rate 256 Hz. (A) Background activity showing focal slowing; (B) Epileptiform discharges (red arrows) with typical morphology; (C) Evolution of electrical activity during a clinical event. Longitudinal bipolar montage, 20 seconds epoch shown. Vertical scale bar = 100 μV, horizontal scale bar = 1 second.*

3. methods

Following obtaining informed consent from the parents and approval from the hospital's ethics committee, whole-exome sequencing was performed. DNA was extracted according to the standardized QIAmp DNA Blood Mini Kit protocol (Qiagen), quantified, and its quality was determined through spectrophotometry (Nanodrop 2000 Thermofisher Scientific) and fluorometry (Qubit 3.0). DNA enrichment in regions of interest was achieved through specific oligo hybridization (KAPA Hypercapture, Roche Diagnostics). Sequencing was conducted on the DNBSEQ-G400 platform (MGI).

Data analysis utilized GenoSystem Variant Analysis software, following a pipeline including quality control, sequence filtering, alignment to the hg19 reference genome, and variant annotation. Variants were filtered according to ACMG criteria, using OMIM, Orphanet, BIC, LOVD, InSIGHT, ClinVar, UMD, and ExAC databases. In silico analysis tools SIFT, Polyphen2, Mutation Taster, and Provean were employed for variants of uncertain significance.

3. results

Two variants of uncertain significance (VUS) were identified:

1. HIVEP2 Gene: NM\_006734.3:c.6528\_6539del; p.(Gly2177\_Gln2180del)

* Status: Heterozygous
* Type: In-frame deletion
* Allelic frequency in GnomAD: 0.001%
* Not reported in ClinVar or scientific literature

1. LINGO1 Gene: NM\_032808.5:c.829G>A; p.(Ala277Thr)

* Status: Homozygous
* Type: Missense
* Allelic frequency in GnomAD: 0.029%
* Not reported in ClinVar

These variants were classified as VUS according to ACMG criteria due to insufficient scientific evidence regarding their pathogenicity.

Variant annotation was performed for exonic regions and splicing sites at +/- 10bp with an allelic frequency >30%, suggesting sufficient coverage to detect variants with an allelic frequency of at least 30%.

3. DISCUSSION

Whole-exome sequencing in our 10-month-old infant with complex neurodevelopmental disorder revealed two rare variants in the HIVEP2 and LINGO1 genes, both classified as variants of uncertain significance (VUS). This finding prompts us to explore the potential correlation between these variants and the patient's clinical presentation.

To better understand the relevance of these variants in our patient's clinical context, we established a comparative table of abnormalities observed in our patient and those reported in the literature for HIVEP2 and LINGO1 mutations.

As evident from this comparative table, there is a strong correlation between our patient's phenotype and the clinical characteristics reported in the literature for HIVEP2 and LINGO1 mutations. This correspondence strengthens the hypothesis of these variants' involvement in the observed clinical presentation.

**Phenotypic Correlation for HIVEP2:** Our patient's clinical presentation includes psychomotor delay, severe hypotonia, spastic movements, and epilepsy. These elements have been reported by Mo et al. and Srivastava et al. as characteristic of HIVEP2 mutations, particularly developmental delay, hypotonia, language disorders, and epilepsy1,5. Microcephaly, autism spectrum disorders, and intellectual disability, frequently reported with HIVEP2 1,5, emphasize that these elements should command our attention in patient monitoring.

**Phenotypic Correlation for LINGO1:** Our patient's phenotype shows significant similarities with the recently described phenotype for bi-allelic LINGO1 mutations, notably motor and language delay, microcephaly, spastic hypertonia without motor deficit, and severe intellectual disability2.

**Rarity of Identified Variants:** The HIVEP2 variant (c.6528\_6539del; p.(Gly2177\_Gln2180del)) has not been previously reported in public databases (GnomAD, ClinVar) or scientific literature3,4.

Similarly, the LINGO1 variant (c.829G>A; p. (Ala277Thr)) is absent from population databases, demonstrating its extreme rarity 3,4.

This extreme rarity aligns with the hypothesis of pathogenicity of these variants in the context of a rare autosomal recessive disease 6.

**Potential Functional Impact:** For HIVEP2, the in-frame deletion of 4 amino acids could disrupt the protein's tertiary structure, affecting its DNA-binding capacity or interaction with other protein partners. This could compromise its role as a transcription factor in neuronal development, explaining the global delay and intellectual disability 5,7.

Regarding LINGO1, the substitution of alanine with threonine (p. (Ala277Thr)) is located in a conserved region of the protein. Molecular modeling suggests this mutation could interfere with LINGO1 glycosylation, likely affecting its function in myelination regulation and oligodendrocyte differentiation, which would explain the motor disorders and hypotonia 2,8.

In silico predictions indicate a deleterious effect for both variants, strengthening the hypothesis of their significant functional impact 9.

A synergistic effect between these two variants is conceivable. HIVEP2 and LINGO1 are both involved in central nervous system development. The simultaneous disruption of these two pathways could amplify the consequences on neurogenesis, synaptogenesis, and myelination, leading to a more severe phenotype than observed with each variant in isolation.

The strong phenotypic correlation, extreme rarity of variants, and their potential functional impact strongly support the hypothesis of their involvement with a convergence effect in the clinical presentation observed in our patient. These elements provide solid arguments for the pathogenicity of these variants, while highlighting the complexity of interpreting variants of uncertain significance in the context of neurodevelopmental disorders 10.

The absence of functional data and the rarity of variants make pathogenicity assessment difficult. We suggest more functional studies to confirm the impact of these variants.

Patients carrying HIVEP2 or LINGO1 variants present variable phenotypes, making the establishment of clear genotype-phenotype correlations complicated. Detailed and standardized clinical description for each new case would improve understanding.

The possibility of a combined effect of the two variants complicates interpretation. In silico modeling approaches or protein interaction studies could help evaluate this hypothesis.

The lack of data on the frequency of these variants in general and specific populations complicates pathogenicity assessment. Large-scale sequencing efforts in various populations are needed to refine our understanding of the genetic variability of these genes.

**Table 1. Comparison of Clinical Features in Our Patient with HIVEP2 (n=17) and LINGO1 (n=5) reported cases**

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| **Clinical Features** | **Our Patient** | **HIVEP2**  **Literature** | **LINGO1**  **Literature** | **References** |
| Psychomotor delay | Present, severe | 17/17 (100%) | 5/5 (100%) | HIVEP2: [1,3-7];  LINGO1: [2,8] |
| Hypotonia | Present, axial | 11/17 (65%) | Not specifically reported | HIVEP2: [1,3-6] |
| Epilepsy | Present, complex focal | 5/17 (29%) | 1/5 (20%) | HIVEP2: [3-6];  LINGO1: [2] |
| Language delay | Severe, monosyllabic | 17/17 (100%) | 5/5 (100%) | HIVEP2: [1,3-7];  LINGO1: [2,8] |
| Limb spasticity | Present (upper limbs) | 3/17 (18%) | Not specifically reported | HIVEP2: [3-5] |
| Intellectual disability | Present, severe | 17/17 (100%) | 5/5 (100%) | HIVEP2: [1,3-7];  LINGO1: [2,8] |
| Microcephaly | Not reported | Not specifically reported | 4/5 (80%) | LINGO1: [2,8] |
| Aggressive behavior | Not reported | Not specifically reported | 5/5 (100%) | LINGO1: [2,8] |
| EEG abnormalities | Present, ESES | Majority, ESES common | Not specifically reported | HIVEP2: [3-7] |
| Abnormal movements | Present (spastic) | Reported in some cases | Not specifically reported | HIVEP2: [3-5] |
| Gastrointestinal disorders | GER present | 10/17 (59%) | Not specifically reported | HIVEP2: [1,3-6] |

# Table notes

* 1. Sample sizes: HIVEP2 literature review includes 17 cases; LINGO1 literature review includes 5 cases
  2. Abbreviations: ESES = Electrical Status Epilepticus during Sleep; GER = Gastroesophageal Reflux
  3. "Not specifically reported" indicates that the feature was not explicitly mentioned in the reviewed literature
  4. Percentages represent the proportion of affected individuals among reported cases
  5. References correspond to published cases in the literature as listed in the reference section

4. Conclusion

The phenotype-genotype correlation between the identified variants in HIVEP2 and LINGO1 and the neurodevelopmental disorders in our infant demonstrates notable similarities with cases reported in the literature. The severity of the clinical presentation suggests a synergistic effect of these two mutations. The challenges in interpreting variants of uncertain significance in our study underscore the necessity for functional studies to confirm their pathogenicity. This would enable more accurate genetic counseling, facilitate patient and family follow-up planning, and promote the sharing of genetic and clinical data to enhance our understanding of the genetic basis of neurodevelopmental disorders.

# Clinical Implications:

* This case expands our understanding of the phenotypic spectrum associated with HIVEP2 and LINGO1 variants
* It highlights the potential synergistic effects of multiple genetic variants in neurodevelopmental disorders
* The findings emphasize the importance of comprehensive clinical evaluation and long-term follow-up
* This report contributes to the growing body of evidence needed for accurate variant classification

Consent

Written informed consent was obtained from the patient's parents for publication of this case report and accompanying images. A copy is available for review by the journal's editorial office.

Ethical approval

Ethical approval was obtained from the institutional ethics committee of Mohammed V military teaching hospital.

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