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CASE REPORT

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# The *ASXL1* mutation p.Gly646Trpfs*12 found in a Turkish boy with Bohring-Opitz Syndrome

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**Key Clinical Message**

In line with a recent study showing that *ASXL1* mutations found in the common population cannot be ruled out as pathogenic, we have identified the *ASXL1* p.Gly646Trpfs*12 mutation—present in 132 individuals in ExAC—as a very probable cause of the disease in a Bohring-Opitz syndrome patient.

**KEYWORDS**

ASXL1, Bohring-Opitz syndrome, intellectual disability, mutation prioritization, variants of unknown significance

## 1 | INTRODUCTION

Bohring-Opitz syndrome (BOS, MIM #605039) is a rare and severe disease characterized mainly by intrauterine growth retardation, feeding difficulties, severe to profound developmental delay, nonspecific brain abnormalities, microcephaly, flexion at the elbows with ulnar deviation and flexion of the wrists and metacarpophalangeal joints (known as BOS posture) and distinctive facial features.[1] Heterozygous *ASXL1* truncating mutations have been identified as the main cause of BOS.[1, 2] A recent publication [3] called the attention to the fact that mutations associated with BOS are also present in the ExAC (Exome Aggregation Consortium) database.[4] As *ASXL1* is one of the genes most commonly mutated during hematopoietic clonal expansion of cells, the authors hypothesized that the presence of this mutation in public databases could be due to somatic mosaicism, and they could confirm the hypothesis by manual examination of the ExAC WES reads.

We have recently identified a new BOS case, in which Sanger sequencing of *ASXL1* revealed the p.Gly646Trpfs*12 mutation, also present in ExAC.

## 2 | CASE REPORT

The patient is a 4-year-old Turkish boy, only child of a healthy nonconsanguineous couple and born at term via normal vaginal delivery after an uneventful pregnancy. The birthweight was 2.3 kg (1st percentile, −2.4 SD), height was 45 cm (−2.3 SD), and head circumference was 32 cm (4th percentile, −1.7 SD). The patient was referred to the genetics department at the 9th day of life. Physical examination revealed trigonocephaly, microcephaly, nevus simplex (flammeus), dysmorphic features, intrauterine growth retardation, and BOS posture with fixed contractures. In the postnatal period, the patient was intubated due to respiratory problems. As he could not be extubated, at 4 months tracheostomy was performed. He was fed through G tube since the 4th month of life. At 2 months of age, the patient started suffering seizures. Abnormalities were detected by EEG, and antiepileptic treatment with levetiracetam and phenobarbital was initiated. Seizures have been under control thereafter with 3-4 attacks annually of a very short duration.

At 15 months, his weight was 10 kg (24th percentile, −0.69 SD), height was 62 cm (<3rd percentile, −5.4 SD), and head circumference was 41.5 cm (<1st percentile, −4.25 SD). At 3.5 years, he had microcephaly (45.5 cm; <3rd percentile, −2.43 SD), trigonocephaly, up-slanting palpebral fissures, narrow forehead, nevus simplex, hypertrichosis, low-set ears, low frontal hairline, microretrognathia, high palate and delayed teeth eruption (no eruption at 3 years of age; Figure 1A,B). He also had bilateral cryptorchidism and brachydactyly (Figure 1C), as well as hypotonia and spasticity on both his upper and lower extremities. In particular, the patient had contractures of the hands and fingers. Additionally, overlapping toes were detected in the feet (Figure 1D). Cranial magnetic resonance revealed corpus callosum agenesis and atrophy of the optic nerve, and he failed the hearing test bilaterally. The patient also underwent echocardiography, which revealed a small patent ductus arteriosus. Abdominal ultrasound and metabolic tests were normal. The patient's developmental milestones were severely delayed (he was not able to sit, crawl or walk independently and he was not able to speak). His karyotype and aCGH results were normal.

After reviewing the clinical presentation of the patient, Bohring-Opitz syndrome was suspected, and the *ASXL1* gene was manually sequenced. Written informed consent from the patient's family was obtained, and all protocols were approved by the Ethics Committee of the Universitat de Barcelona (IRB00003099). A *de novo* heterozygous

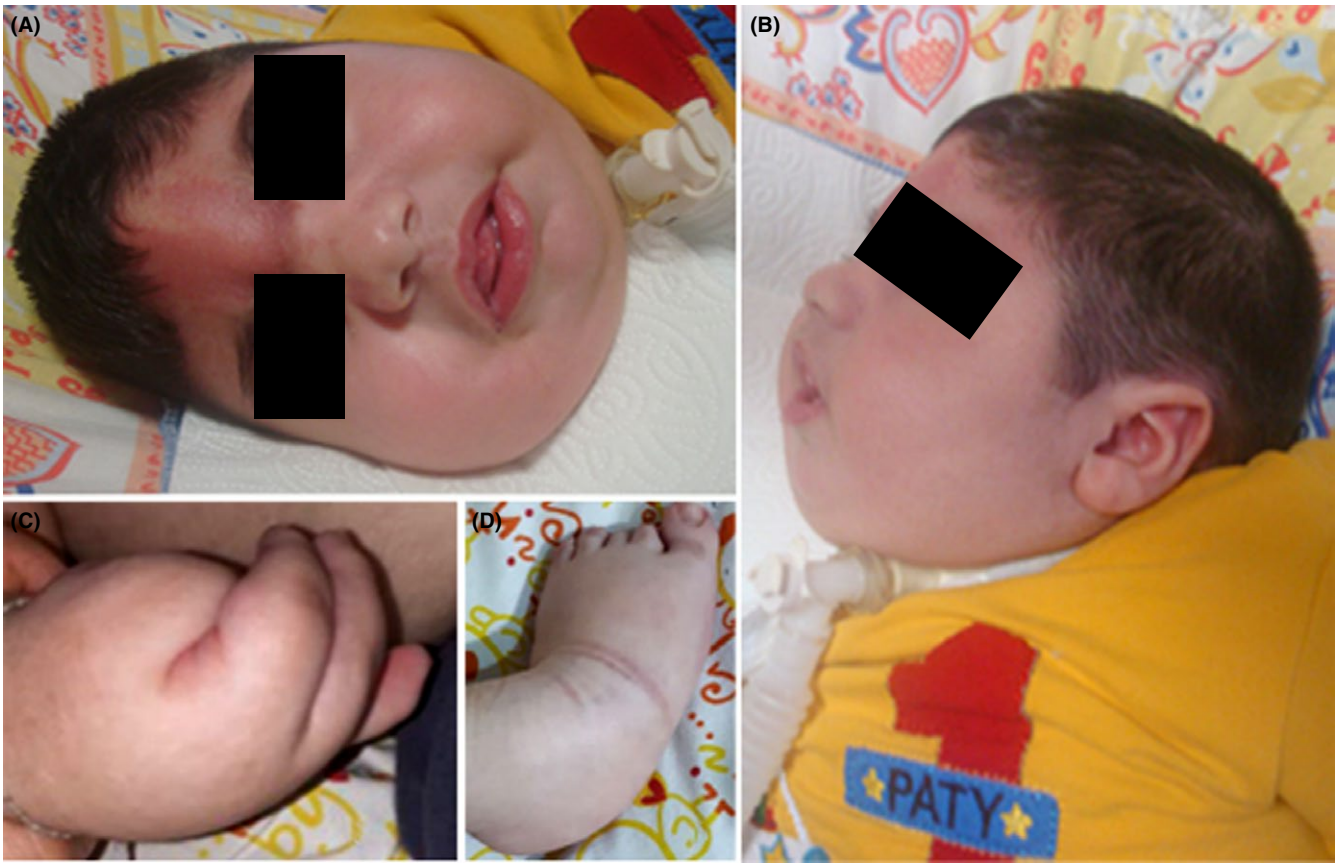


**FIGURE 1** Facial, hand and foot phenotypes of the patient at 3 y of age. A and B, Facial dysmorphisms, especially trigonocephaly and nevus simplex (flammeus) is clearly appreciated. C, brachydactyly is appreciable in the patient's hand. D, Foot phenotype with overlapping toes

mutation, c.1934dupG (p.Gly646Trpfs*12), was identified. The PCR amplification and Sanger sequencing were repeated twice, independently and the PCR fragment was cloned and sequenced (see Figure S1) to unequivocally confirm the mutation.

## 3 | DISCUSSION

While the clinical outcome of the patient clearly pointed to a Bohring-Opitz Syndrome, the fact that he was carrying a mutation (p.Gly646Trpfs*12) present in 132 individuals from the general population hindered taking a final decision on the genetic diagnosis. In this sense, other mutations present in ExAC (among them the p.Arg404Ter described by Carlston et al[3]) were previously found to be BOS -causing (Table 1). The p.Gly646Trpfs*12 mutation described in our BOS case is one of the two *ASXL1* loss of function (LoF) changes especially frequent in ExAC, the other being p.Gly645Valfs*58 (found in 118 individuals). Both changes are located in an eight-nucleotide G-homopolymer tract and could be overrepresented due to sequencing errors. While these changes were not included in Carlston et al[3] analyses, the authors discussed the fact that the p.Gly646Trpfs*12 mutation had been identified in a large series of myeloid malignancies by deep sequencing (confirmed by manual methods in some cases) and had been described as the most common cancer-associated *ASXL1* mutation.[5] Besides, 66% of the ExAC carriers of the p.Gly646Trpfs*12 mutation belong to the Cancer Genome Atlas (TCGA) population.

This mutation is a truncating mutation affecting the last exons of the *ASXL1* gene, similarly to all the previously described BOS mutations (Figure 2). More recently, the p.Gly646Trpfs*12 mutation has been filtered from the GnomAD

**TABLE 1** BOS-causing ASXL1 mutations present in ExAC

| cDNA mutation | Prot. mutation | Reported phenotype | ExAc | GenomAD | Origen |
|---|---|---|---|---|---|
| c.1117C>T | p.Q373* | Focal Epilepsy[a] | no | 2/246256 | 6 |
| c.1129C>T | p.Q377* | ID[a] | no | no | 7 |
| c.1210C>T | p.R404* | Bohring-Opitz syndrome | 7/121378 | 4/246248 | 2, 3, 8, 9 |
| c.1269dupT | p.L424fs | Bohring-Opitz syndrome | no | no | 10 |
| c.1272_1273delGT | p.T425Qfs*12 | Bohring-Opitz syndrome | no | 1/246262 | 1 |
| c.1544_1545delTG | p.V515Gfs*13 | Focal Epilepsy[a] | no | no | 6 |
| c.1924 G>T | p.G642* | Bohring-Opitz syndrome | no | no | 1 |
| c.1934insG | **p.G646Wfs*12** | Bohring-Opitz syndrome | 132/80804 | no[b] | **Present study** |
| c.2013_2014 del | p.C672Tfs*4 | Bohring-Opitz syndrome | no | no | 1 |
| c.2036_2037insG | p.G680Rfs*38 | Bohring-Opitz syndrome | no | no | 11 |
| c.2100dupT | p.P701Sfs*16 | Bohring-Opitz syndrome | no | no | 12 |
| c.2197C>T | p.Q733* | Bohring-Opitz syndrome | 1/121070[c] | idem ExAC | 2 |
| c.2324 T>G, | p.L775* | Bohring-Opitz syndrome | 1/121162 | no[b] | 1 |
| c.2332C>T | p.Q778* | Bohring-Opitz syndrome | no | no | 2 |
| c.2407_2411del5 | p.Q803Tfs*17 | Bohring-Opitz syndrome | 3/120748[c] | no[b],[c] | 2 |
| c.2468T>G | p.L823* | Bohring-Opitz syndrome | 2/120758[c] | 2/244338[c] | 2 |
| c.2535dup | p.S846Qfs*5 | Bohring-Opitz syndrome | no | no | 1 |
| c.2759_2762dup | p.V922Ifs*3 | Bohring-Opitz syndrome | no | no | 1 |
| c.2773C>T | p.Q925* | Bohring-Opitz syndrome | no | no | 2 |
| c.2893C>T | p.R965* | Bohring-Opitz syndrome | 1/121306 | 3/246200 | 13 |
| c.3077del | p.G1026Dfs*21 | Bohring-Opitz syndrome | no | no | 1 |
| c.3083C>A | p.S1028* | Bohring-Opitz syndrome | no | no | 2 |
| c.4060 G>T | p.E1354* | Bohring-Opitz syndrome | no | no | 14 |
| c.4116_4117del2 | p.F1373fs | Bohring-Opitz syndrome[d] | no | no | 15 |

[a]Clinical history is limited without description of presence or absence of other BOS features.
[b]Filtered in GenomAD (failed random forest filters).
[c]These numbers correspond to another mutation affecting the same residue.
[d]The patient is also carrying recessive mutations in the CFTR gene.
Bold type indicates the mutation in the case presented here

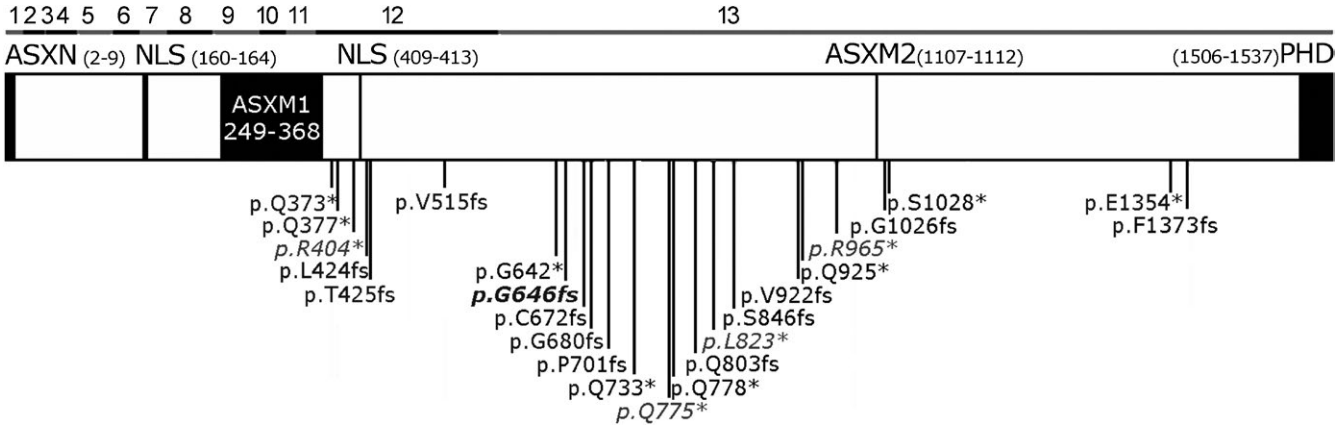


**FIGURE 2** ASXL1 protein structure and BOS-causing mutations. In italics, mutations also present in ExAC. ASXL1 coding exons are indicated above. ASXN, ASX N-terminal domain; NLS, Nuclear localization signal; ASXM, ASX middle domains; PHD, plant homeodomain

database as it failed random forest filters. In addition, very recently a new patient with this mutation was reported to ClinVar by GenDX, although the clinical outcome of the patient was not reported.

Taking all this into consideration, we conclude that the p.Gly646Trpfs*12 mutation is a disease-causing mutation responsible for the patient's BOS clinical presentation and that, as Carlston et al[3] stated, the assumption that pathogenic variants in genes associated with severe, pediatric-onset, highly penetrant, autosomal dominant conditions have to be absent or extremely rare in public databases has to be taken cautiously.

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## AUTHOR CONTRIBUTION

RU, SG, DG, and SB: contributed to the planning of this work. RU, SG, LCV, GC, RR, DE, DG, and SB: conducted this work. RU, LCV, and GC: performed the sequencing, cloning, and chromatogram analysis. RU and RR: performed the bibliographical, in silico and database analysis of the mutation. SG and DE: clinically evaluated the patient and have generated and written the clinical data. RU, SB, and DG: wrote the main manuscript text. RU and RR: elaborated Table 1. RU and SG: prepared Figure 1. RU, SB, and DG: prepared Figure 2. RU and GC: prepared Figure S1. All authors reviewed the manuscript.

## CONFLICT OF INTEREST

None declared.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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