De novo frameshift mutation in ASXL3 in a patient with global developmental delay, microcephaly, and craniofacial anomalies

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Abstract

Background: Currently, diagnosis of affected individuals with rare genetic disorders can be lengthy and costly, resulting in a diagnostic odyssey and in many patients a definitive molecular diagnosis is never achieved despite extensive clinical investigation. The recent advent and use of genomic medicine has resulted in a paradigm shift in the clinical molecular genetics of rare diseases and has provided insight into the causes of numerous rare genetic conditions. In particular, whole exome and genome sequencing of families has been particularly useful in discovering de novo germline mutations as the cause of both rare diseases and complex disorders.

Case presentation: We present a six year old, nonverbal African American female with microcephaly, autism, global developmental delay, and metopic craniosynostosis. Exome sequencing of the patient and her two parents revealed a heterozygous two base pair de novo deletion, c.1897_1898delCA, p.Gln633ValfsX13 in ASXL3, predicted to result in a frameshift at codon 633 with substitution of a valine for a glutamine and introduction of a premature stop codon.

Conclusions: We provide additional evidence that, truncating and frameshifting mutations in the ASXL3 gene are the cause of a newly recognized disorder characterized by severe global developmental delay, short stature, microcephaly, and craniofacial anomalies. Furthermore, we expand the knowledge about disease causing mutations and the genotype-phenotype relationships in ASXL3 and provide evidence that rare, nonsynonymous, damaging mutations are not associated with developmental delay or microcephaly.

Keywords: ASXL3, Bohring-Opitz syndrome, Global developmental delay, Microcephaly, Craniofacial anomalies, de novo frameshift, Exome sequencing

Background

Obtaining a molecular diagnosis for many rare diseases can be an arduous task [1]. The process is often hindered by the rarity of conditions, which is further exacerbated by the clinical heterogeneity, genetic heterogeneity (genocopies) and phenocopies that rare diseases tend to exhibit [2]. Frequently, this results in a process that has been termed the diagnostic odyssey [3]. The NIH Office of Rare Diseases Research reported that it took 1 to 5 years to reach a proper diagnosis for 33% of patients with rare disorders and more than 5 years for 15% of these patients [4]. The recent advent and use of genomic medicine has resulted in a paradigm shift in the clinical molecular genetics of rare diseases – from phenotype-driven diagnosis to genotype-driven diagnosis – and has provided insight into the causes of numerous rare genetic conditions [1]. Briefly genomic medicine, defined as the structured approach to disease discovery, diagnosis, and management that prominently features next-generation sequencing and analysis at a genome scale [5], has provided the impetus for a paradigm shift in the clinical evaluation of rare diseases to identify underlying molecular genetic causes.
Whole exome and genome sequencing of families has been particularly useful in discovering de novo germline mutations as the cause of both rare diseases and complex disorders. For example, de novo mutations have recently been associated with rare growth disorders characterized by megalencephaly due to mutations in three genes, AKT3, PIK3R2, PIK3CA, [6] and Baraitser-Winter syndrome, characterized by brain malformations due to de novo mutations in the actin genes ACTB and ACTG1 [7]. We recently described a de novo mutation in MTOR as a cause of megalencephaly and intractable seizures (Smith et al., submitted). In addition, new research has implicated de novo mutations in the complex disorders of autism [8-10] and schizophrenia [11,12].

First described in 1999, Bohring-Optiz syndrome (BOS) [OMIM, 605039] has recently been shown through the use of next-generation exome sequencing, to be due to de novo heterozygous mutations in the additional sex combs-like 1 gene (ASXL1) [13]. Two further cases of de novo mutations in ASXL1 in patients with BOS supported the disease-gene association and gave limited insight into genotype-phenotype relationships [14]. Prior to this, the presence of seven of ten features were needed for a clinical diagnosis: trigonocephaly, microcephaly, flammeus nevus, prominent eyes, micro- or retrognathia, abnormal palate, typical BOS posture, feeding difficulties, intrauterine growth restriction (IUGR), and severe/profound learning difficulties [15].

Here, we report on the use of exome sequencing of a proband with considerable overlap with the BOS phenotype and her parents to discover a heterozygous frame shift variant in the additional sex combs-like 3, (ASXL3) gene. ASXL3 is in the same gene family as ASXL1 and mutations in ASXL3 appear to be associated with a disorder that is paralogous to BOS.

Case presentation

The proband is a 6 year old African American female (CMH000079) with microcephaly, autism, global developmental delay, and metopic craniosynostosis (Figure 1). She was born at 34-weeks gestation following a pregnancy complicated by insulin-dependent diabetes, with maternal blood sugar lability throughout pregnancy. The patient's mother was a 30 year old gravida 3, para 2 female, with a history of 1 first trimester miscarriage. There was no exposure to alcohol, tobacco, or drugs. Delivery was via cesarean section for breech presentation. At birth the proband had a weight of 1.88 kg (25% for gestational age), length 40.5 cm (3% for gestational age), and occipitofrontal circumference 30 cm (25% for gestational age). Apgar scores were 8 and 9 at one and five minutes, respectively; however, on the first day of life she was transferred to the Neonatal Intensive Care Unit (NICU) for hyperinsulinemic hypoglycemia.

Family history was notable for a maternal half-sister with type 1 diabetes mellitus, who also had a history of hyperinsulinemia and hypoglycemic seizures in infancy, but who has normal cognitive functioning. The mother also had a history of hypoglycemia in childhood that evolved to insulin dependent diabetes mellitus. The mother is otherwise healthy and has no history of learning problems. The patient’s father has hypertension, but is otherwise healthy.

In the first 18 months of life the patient was hospitalized 10 times for vomiting, hypoglycemia, failure to thrive, and episodic irritability. Blood glucose was labile, ranging from 40–215 mg/dL (reference range 60–110 mg/dL). She had two hypoglycemic seizures. A sulfonylurea receptor defect was suspected because of the blood sugar lability in the proband, sister, and mother. Molecular testing of the proband and her mother identified a shared novel variant in the ABCC8 (ATP-Binding Cassette, Subfamily, Member 8)
gene (c. 2143G > A; p.Val115Met) that was considered to be consistent with a diagnosis of Familial Hyperinsulinemic Hypoglycemia Type 1 (HHF1 OMIM #256450) [16,17]. She was therefore placed on diazoxide; a gastrostomy tube and a peripherally inserted central catheter (PICC) line were placed for nutrition and glucose stabilization.

By 18 months, length and weight were between the 10-25th percentiles but head circumference was below the 3rd percentile. She was hypotonic and hyperreflexic. She had dysmorphic facial features including a sloping forehead with metopic ridging, a flat nasal bridge, wide-set eyes and slightly posteriorly rotated ears. The nose had a short columella and hypoplastic alae nasae. Nasolabial folds were smooth and the upper lip had a prominent Cupid’s bow. She had no other dysmorphic features. Developmental milestones were delayed. At 18 months, she could army crawl, had inconsistent visual attention, and was nonverbal. She had episodic irritability with fits of screaming lasting hours, self-injurious behavior, and very poor sleep. She developed repetitive movements, predominately lateral head shaking. Developmental testing demonstrated delay: on the Cognitive Adaptive Test [18], her visual-motor/problem-solving quotient was 33 (mean 100, SD 10); on the Clinical Linguistic and Auditory Milestone Scale [18], language quotient was 42 (mean 100, SD 10). Brain MRI demonstrated mild white matter loss with enlarged lateral ventricles and mild prominence of the sulci. Brain spectroscopy was normal. A head CT confirmed larged lateral ventricles and mild prominence of the sulci. Brain MRI was normal. A head CT confirmed.

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However, there are 29 single nucleotide variants of with a frequency of less than 1 percent in our database (Additional file 1: Table S1). Characterization of these 29 rare variants revealed 19 that were predicted to deleterious by SIFT (Sorts Intolerant From Tolerant substitutions) [24] and 11 predicted to be probably or possibly damaging by PolyPhen2 (Polymorphism Phenotyping 2) [25]. Phenotypic evaluation of the samples with these rare, non-synonymous variants revealed no patients with development delay, microcephaly, or other craniofacial anomalies, suggesting that these variants are unlikely to be pathogenic in a heterozygous state.

Discussion

In this study we provide the second report and fifth patient with pathogenic mutations in ASXL3. Our case provides additional evidence that, indeed, truncating frameshift mutations in the ASXL3 gene are the cause of a newly recognized distinct disorder characterized by global developmental delay and craniofacial anomalies that shares significant clinical features with Bohring-Opitz syndrome. Our patient, CMH000076, exhibited multiple overlapping clinical features of BOS, most notably trigonocephaly, microcephaly, feeding difficulties, and severe learning difficulties (Table 1). Unlike the previously reported cases with ASXL3 mutations, our patient had trigonocephaly, thus, the lack of trigoncephaly may not be a useful in differentiating BOS from this newly recognized condition [23]. To date, none of the five patients described with ASXL3 mutations has displayed the typical BOS-posture, whereas 100 percent of the 30 described patients with ASXL1 mutations did [14], suggesting that this feature might be useful in discriminating between the two related conditions.

The mutation in our patient was at amino coding position 633, which is closest to subject #3 reported by Bainbridge et al. [23] (659_660del). In contrast to subject #3, however, CMH000079 exhibited a severe phenotype with feeding difficulties, growth restriction and severe

Table 1 Comparison of clinical features of CMH000079, patients with ASXL3 mutations described by Bainbridge et al. [23], and those reported in patients with BOS

| CMH000079 | Reported in Bainbridge et al. | Reported in Bohring-Opitz |
|-----------|-----------------------------|---------------------------|
| Trigonocephaly | Y | N |
| Microcephaly | Y | Y |
| Flamméus nevus | N | N |
| Prominent eyes | Y | Y |
| Micro- or retrognathia | N | Y |
| Abnormal palate | N | Y |
| Typical BOS posture | N | N |
| Feeding difficulties | Y | Y |
| IUGR | Y | Y |
| Severe/profound learning difficulties | Y | Y |
| Upplanting palpebral fissures | Y | NA |
| Posteriorly rotated ears | Y | Y |
| High arched palate | N | Y |
| Deep palmar creases | N* | Y |
| Slight ulnar deviation of the hands | N | Y |
| Recurrent infections | Y | NA |
| Seizures | N | NA |
| Apneas | Y# | NA |
| Epicanthal folds | Y | NA |
| Broad alveolar ridges | N | NA |
| Cleft/notch lip | N | NA |
| Cleft palate | N | NA |
| Buccal frenulae | N | NA |
| Depressed nasal bridge | Y | Y |
| Anteverted nares | Y | Y |
| Strabismus | N | NA |
| Anterior chamber abnormalities | N | NA |
| Myopia | Y | NA |
| Retinal/optic nerve abnormalities | N | NA |
| Low hairline | N | NA |
| Hypertrichosis | Y | NA |
| Fixed contractures | N | NA |
| Congenital dislocations | N | NA |
| Hypotonia | Y (trunk) | Y |

Table adapted from [15], bolded symptoms are the 10 common/specific features of BOS.

NA- Feature not commented on.

*- Some redundancy of skin on hands and palms noted.

#- History of obstructive sleep apnea; no history of central sleep apnea.
global developmental delay. Both the mutation described here and that of the previously described subject #3 occur in an evolutionarily conserved serine-rich motif found between residues ~600-800 [23]. The considerable clinical variability between our patient and that of subject #3 emphasize the need for additional studies of the complex phenotype-genotype associations in this disorder and the paragocial disorder BOS. The diagnosis of HHF1 due to mutation in ABCC8 in this patient likely contributed to her recurring hospitalizations and growth difficulties, and may have impacted her early development. Importantly, the ABCC8 variant (c. 2143G > A; p.Val115Met) was also identified by the whole exome sequencing. Labile maternal blood glucose during pregnancy was also considered in the early differential of her developmental delay. However, both the mother and sister of the proband share HHF1 diagnosis and have histories of severe hypoglycemia and with resultant episodes of altered mental status and seizures in early childhood; both have normal cognitive functioning and achieved normal growth parameters.

To date, all reported potential pathogenic mutations in ASXL3 have been either frameshifting or truncating [23]. To further examine the variant burden in ASXL3 and the genotype-phenotype relationship, we utilized our clinical grade variant database with more than 1,300 exomes to evaluate rare variation (defined as present in less than 1% of samples) in the ASXL3 gene. Unlike what has been reported in other large-scale databases, our internal warehouse does not contain samples with truncating, nonsense mutations [23]; however we did uncover 29 rare, single nucleotide variants (SNVs) (Additional file 1: Table S1). ASXL3 is 6,747 nucleotides long and composed of 12 exons with the 3’ exons 11 and 12 being disproportionately large; comprising 1,957 and 3,708 nucleotides, respectively. The vast majority of the variants from our database were in exon 11, which included our de novo deletion or exon 12 (Additional file 1: Table S1). Interestingly, although our database contains 107 samples with neurodevelopmental disorders, none of the discovered rare variants were in any of these patients, suggesting that these variants are unlikely to be pathogenic in a heterozygous state and providing additional support that only frameshifting or truncating mutations in ASXL3 are pathogenic. However, additional functional studies to investigate the pathogenicity of ASXL3 variants are needed to completely conclude that SNVs are expected to be nonpathogenic.

**Conclusions**

In summary, we describe the use of familial exome sequencing to discover a de novo frameshifting mutation in the ASXL3 gene in a patient with feeding difficulties, microcephaly, severe global developmental delay, and craniofacial anomalies. Furthermore, we provide additional evidence that heterozygous, frameshifting, truncating mutations in ASXL3 are the cause of a newly recognized disorder [23]. In addition, we expand the knowledge about disease causing mutations and the genotype-phenotype relationships in ASXL3 and provide evidence that rare, nonsynonymous, and predicted damaging mutations are not associated with developmental delay or microcephaly, rather that pathogenic are likely to be either frameshifting or truncating.

**Consent**

The study was approved by the Institutional Review Board of Children’s Mercy Hospital (CMH). Written informed consent was obtained from the parents for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**Additional file**

**Additional file 1: Methods. Figure S1.** Next-generation sequencing results viewed in the Integrative Genome Viewer [12] of the de novo frameshifting mutation 1897_1898delCA, p.Gln633ValfsX13 in CMH000079 and not present in mother (CMH000080) or father (CMH000081) **Figure S2.** Capillary sequencing results of the de novo frameshifting mutation 1897_1898delCA, p.Gln633ValfsX13 in patient with forward and reverse PCR primers and control sample. **Table S1.** Variants at a frequency of less than 1% in ASXL3 more than 1,300 exomes sequenced at the Center for Pediatric Genomic Medicine at Children’s Mercy Hospital.

**Abbreviations**

ASXL1: Additional sex combs-like 1; ASXL3: Additional sex combs-like 3; BOS: Bohring-Optiz syndrome; CPGM: Center for pediatric genomic medicine; CMH: Children’s mercy hospital; HHF1: Hyperinsulinemic hypoglycemia, familial, type 1; IUGR: Intrauterine growth restriction; MR: Magnetic resonance imaging; PICC: Peripherally inserted central catheter; PolyPhen2: Polymorphism phenotyping 2; SIFT: Sorts intolerant from tolerant substitutions; SNVs: Single nucleotide variants.

**Competing interests**

The authors declare that that they have no competing interests.

**Authors’ contributions**

Conceptualized and designed the study SFK. Drafted the initial manuscript: NAM, SES. Coordinated and supervised sample and clinical data collection, obtained consent: SES, LDS. Undertook the exome sequencing: DLD, EGF. Designed the bioinformatics tools, and coordinated bioinformatics efforts: NAM. Carried out the initial analyses: DLD. Reviewed data analysis and overviewed clinical reporting of genetic findings: CJS. Developed clinical-pathological components of bioinformatic tools, and coordinated protocol implementation: NAM, SES. Reviewed and revised the manuscript: SFK, LDS. All authors read and approved the final manuscript.

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