A Novel Loss-of-Function Variant in *SETBP1* Causing Autosomal Dominant Mental Retardation 29 in an Asian Indian Male Child

Asodu Sandeep Sarma[1], Shivangi Wagh[1], Ashwin Dalal[1], Prajnya Ranganath[1,2]

[1]*Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India*

[2]*Department of Medical Genetics, Nizam's Institute of Medical Sciences (NIMS), Hyderabad, India*

Correspondence to: Dr Prajnya Ranganath *Email:* prajnyaranganath@gmail.com

Abstract

Heterozygous gain-of-function variants in the *SETBP1* gene are known to cause Schinzel-Giedion syndrome, a multiple malformation condition with typical facial dysmorphism. Heterozygous, loss-of-function variants in the same gene have been recently identified to cause a very rare genetic disorder with a less severe phenotype characterized by mild dysmorphic features, mild to moderate intellectual disability with speech delay and epilepsy, referred to as autosomal dominant mental retardation 29. Here, we describe a novel loss-of-function variant c.2622del; p.(Asp874GlufsTer87) in the *SETBP1* gene identified through exome sequencing, in an Asian Indian male patient with facial dysmorphism, global developmental delay, and seizures.

Keywords: *SETBP1*, Loss-of-function variant, Autosomal dominant mental retardation 29

Introduction

SETBP1 (OMIM * 611060), located on chromosome 18q21.1, encodes an oncogene-binding protein and binds to SET domains that are involved in methylation of lysine residues on histone tails and ubiquitously expressed in all tissues (Hoischen et al., 2010). Germline, de novo heterozygous gain-of-function (GoF) variants in *SETBP1* cause a rare neurodevelopmental disorder characterized by typical craniofacial dysmorphism including severe midface retraction, congenital heart defect, hydronephrosis, clubfeet, hypertrichosis, epilepsy, and profound neurodevelopmental delay, which is referred to as Schinzel-Giedion midface retraction syndrome (SGS; OMIM #269150) (Hoischen et al., 2010; Schinzel and Giedion, 1978). Heterozygous loss-of-function (LoF) variants in the same gene, have been recently identified to cause a phenotype that is distinct from SGS, with milder intellectual disability and expressive speech impairment, which is referred to as autosomal dominant mental retardation-29 (MRD29; OMIM # 616078), SETBP1 LoF syndrome, or SETBP1 haploinsufficiency disorder (Filges et al., 2011). There are only few reports on LoF variants in the *SETBP1* gene causing MRD29, and none from the Asian Indian population.

Clinical Details

Here, we describe this 2-years-3-months-old male child, the only offspring of normal nonconsanguineous parents, who was referred for evaluation of global developmental delay and seizures. He was born at term gestation with a birth-weight of 2.5 kilograms, through an emergency Caesarean section, done for non-progress of labour. There were no adverse events in the antenatal or neonatal periods. He was noted to have delay in attainment of milestones from early infancy. Stable head control was attained at 6 months, rolling over from supine to prone position at 7 months, sitting without support by around 16 months, standing without support by 20 months, and walking without support by 24 months. At the time of presentation at 27 months of age, he had not attained the ability to run or climb stairs. He had attained cooing by around 6 months, monosyllabic speech by around 15 months, and bisyllabic speech by 24 months; meaningful words had not been attained at the time of evaluation at 27 months. He was able to comprehend simple commands such as when asked to move his hands, and imitate simple actions such as waving

Figure 1 Clinical photographs of the proband (**A.** frontal view & **B.** lateral view) showing facial dysmorphic features in the form of mild dolichocephaly, prominent forehead, frontal bossing, thick arched eyebrows with medial flare, periorbital fullness, down-slanting palpebral fissures, mild ocular hypertelorism, prominent infraorbital creases, depressed and wide nasal bridge, wide and bulbous tip of nose, prominent nasolabial folds, low-set ears, thin upper lip, and prominent chin.

goodbye. Bowel and bladder control had not been attained. Developmental quotient (DQ) assessment revealed the motor development to be corresponding to around 12 months, speech & language to around 10 months, and social development to around 18 months.

There was an episode of lower respiratory tract infection requiring hospitalization and administration of intravenous antibiotics for 1 week, at 6 months of age. Seizures started at the age of 12 months, and he had recurrent generalized tonic-clonic seizures, which could be controlled only after therapy with two antiepileptic drugs i.e., levetiracetam and phenytoin, was started. There were no similarly affected individuals in the family.

On examination, his anthropometric measurements were as follows: length was 86 cm (-1 SD), weight was 11 kgs (-1.6 SD), and head circumference was 48 cm (-1.3 SD) [standard deviations (SDs) mentioned with reference to the mean anthropometric measurements for age for normal Asian Indian male children]. He was noted to have craniofacial dysmorphism in the form of mild dolichocephaly, a prominent forehead, frontal bossing, thick arched eyebrows with medial flare, periorbital fullness, down-slanting palpebral fissures, mild ocular hypertelorism, prominent infraorbital creases, low-set ears, depressed and wide nasal bridge, wide and bulbous tip of nose, prominent nasolabial folds, thin upper lip, and a prominent broad chin (**Figures 1A & 1B**). Neurological examination was normal except for mild hypotonia of both lower limbs and a developmental quotient (DQ) of around 50% in all domains. Per abdominal, cardiovascular, respiratory, and musculoskeletal system examination was normal.

Complete hemogram, liver function tests, serum creatinine, serum electrolytes, serum creatine phosphokinase, and serum calcium and phosphorus, were normal. Radiographs of the chest and of the left hand and wrist were normal, with the bone age corresponding to around 2 years. Thyroid function tests which had been done initially at the age of 6 months, at the time of

hospitalization for the respiratory infection, had shown a borderline low level of thyroid stimulating hormone (TSH) of 0.5 mU/L (reference range 0.55 – 5.3 mU/L); however, repeat TSH and free thyroxine (T4) assays done at 7 months of age, and again at 2 years 3 months, were in the normal range. Magnetic resonance imaging (MRI) of the brain did not show any specific abnormality.

As no specific etiology could be ascertained clinically, whole-exome sequencing was done for the child. Genomic DNA was isolated from blood lymphocytes of the proband using the QIAamp DNA Blood Mini kit (Qiagen, Germany) and exome enrichment was done using the TruSeq exome capture kit (Illumina, USA). The captured library was sequenced to mean 100X coverage on Illumina HiSeq2000 sequencing platform (Illumina, USA). Exome sequence analysis revealed a total of 12,0231 variants. After stringent filtering and clinical correlation, a likely pathogenic novel variant was identified in the *SETBP1* gene, NM_015559.2:c.2622del:p.(Asp874GlufsTer87) (**Figure 2A**). The variant is absent in the population databases 1000 Genomes and gnomAD, and also in the in-house database of 1400 exomes. This variant is also not reported in ClinVar, even though a proximal missense variant c.2621A>G; pAsp874Gly, is listed as a pathogenic variant. It is predicted to be disease-causing by MutationTaster. Sanger sequencing confirmed the heterozygous status of the variant in the proband (**Figure 2B**). The variant was confirmed to be absent in both the normal parents through targeted Sanger sequencing (**Figures 2C & 2D**), indicating that it is most likely a de novo variant. The novel SETBP1 variant was interpreted using the American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) guidelines (Richards et al., 2015), and was classified as pathogenic based on the following ACMG-AMP criteria: PM2, PP3, PM5, PM4, PVS1, and PS2.

The child was thus diagnosed to have *SETBP1*-associated autosomal dominant mental retardation 29. The parents were advised to continue the antiseizure medications, as well as developmental therapy and speech therapy for the child, on a regular basis, and 3 monthly follow-ups for assessment of growth and development were suggested. Though this pathogenic variant is most likely of de novo origin in the proband, the likelihood of gonadal mosaicism for this variant in either parent cannot be ruled out; therefore, there may be an empiric risk of recurrence of around 1% in subsequent offspring of the parents. This, along with the option of prenatal genetic testing for their future pregnancies, was conveyed to the parents.

Discussion

Schinzel and Giedion first described the phenotype of SGS in 1978 (Schinzel and Giedion, 1978), but the causative gene mutations were identified much later through exome sequencing of 4 affected individuals by Hoischen et al. in 2010 (Hoischen et al., 2015). All the SGS-causing variants that have been reported till date have been found to be missense gain-of-function (GoF) variants and confined to exon 4 of the gene. In 2011, Filges et al. reported a less severe disease phenotype when compared to SGS, caused by heterozygous loss-of-function (LoF) variants in *SETBP1*; this condition was designated autosomal dominant mental retardation-29 (MRD29) (Filges et al., 2011). The LoF mutations in *SETBP1* that have been reported to cause MRD29 include contiguous gene deletions (Marseglia et al., 2012; Coe et al., 2014), heterozygous deletion including *SETBP1* exclusively (Filges et al., 2011), small indels, and stop-gain variants (Leonardi et al., 2020), which are distributed throughout the gene, unlike the SGS-causing missense mutations which are localized to the hotspot region. In addition, while germline GoF and LoF variants in *SETBP1* are associated with SGS and MRD29 respectively, somatic mutations in *SETBP1* have been implicated in myeloid malignancies (Makishima et al., 2013).

Patients with *SETBP1*-associated autosomal dominant mental retardation (MRD29) have been reported to have delayed motor development, delayed speech, attention deficit hyperactivity disorder (ADHD), and mild to moderate intellectual disability; severe intellectual disability and seizures have also been reported in a few patients. These patients also have variable and subtle craniofacial dysmorphism including dolichocephaly, elongated facies, a high forehead, arched eyebrows, down-slanting palpebral fissures, hypertelorism, periorbital fullness, low-set ears, wide nasal bridge, upturned nasal tip, smooth philtrum, thin and tented upper lip, and mild micrognathia (Coe et al., 2014; Leonardi et al., 2020). The phenotype is usually less severe than that of Schinzel-Giedion syndrome, which is caused by GoF variants in the same *SETBP1* gene, and is characterized by severe intellectual disability, coarse facies with severe midface hypoplasia, multiple congenital

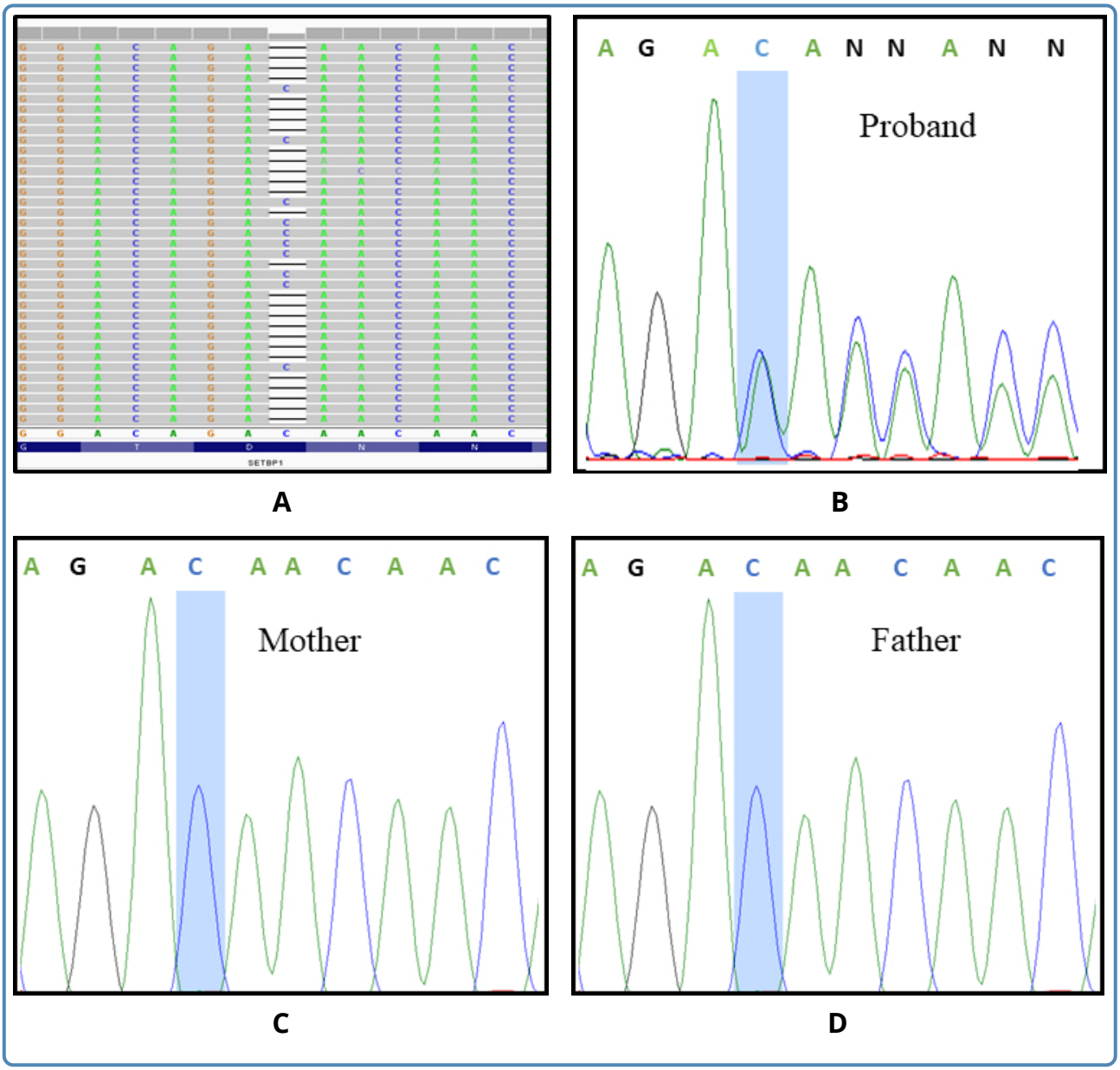

Figure 2 **A.** Integrative Genomics Viewer (IGV) screenshot showing the heterozygous variant c.2622del in *SETBP1* (NM_015559.2) in the proband; **B.** Sanger sequence chromatogram of the proband showing the heterozygous variant c.2622del in *SETBP1*; **C. & D.** Sanger sequence chromatograms of the parents of the proband showing absence of the variant c.2622del in *SETBP1*.

malformations including abnormalities of the skull, distal phalangeal hypoplasia, genitourinary and renal malformations, and congenital cardiac defects, and an increased risk of neuroepithelial neoplasia. Therefore, unlike for Schinzel-Giedion syndrome, a gestalt diagnosis is usually not possible for MRD29. However, detailed reverse phenotyping following identification of putative causative variants through NGS-based testing often helps in corroborating the diagnosis. Our patient, for example, on reverse phenotyping was found to have most of the dysmorphic features previously reported with *SETBP1*-associated MRD29, in addition to motor and speech delay,

and seizures requiring two antiepileptic drugs for adequate control.

Till date, only around 20 patients with MRD29 due to LoF mutation in *SETBP1* have been reported across the world (Filges et al., 2011; Coe et al., 2014; Hamdan et al., 2014; Eising et al., 2019; Leonardi et al., 2020). To the best of our knowledge, this is the first report of MRD29 in a patient of Asian-Indian origin.

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Web resources

- 1000 Genomes database - `http://phase3browser.1000genomes.org/index.html`
- ANNOVAR - `https://annovar.openbioinformatics.org/en/`
- Burrows-Wheeler Aligner (BWA) - `http://bio-bwa.sourceforge.net/`
- ClinVar - `https://www.ncbi.nlm.nih.gov/clinvar`
- Combined Annotation Dependent Depletion (CADD) software - `https://cadd.gs.washington.edu/`
- gnomAD (The Genome Aggregation Database) - `http://gnomad.broadinstitute.org/`
- Genome Analysis Toolkit (GATK) - `https://gatk.broadinstitute.org/hc/en-us`
- MutationTaster software - `http://www.mutationtaster.org/`
- Online Mendelian Inheritance in Man (OMIM) database - `https://www.omim.org/`
- Primer3 web version 4.1.0 - `https://primer3.ut.ee/`