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Extended analysis of exome sequencing data reveals a novel homozygous deletion of exons 3 and 4 in *FUCA1* gene causing fucosidosis in an Indian family

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1. Background

Fucosidosis (MIM# 230000) is a neurodegenerative disorder characterised by coarse facial features, growth retardation, recurrent upper respiratory infections, seizures, dysostosis multiplex and angiokeratoma corporis diffusum as seen in other lysosomal storage disorders. It is caused by biallelic pathogenic variants in *FUCA1* (MIM*612280) which results in α-L-fucosidase deficiency. Due to deficiency of α-L-fucosidase enzyme, there is accumulation of fucose-containing glycolipids and glycoproteins in various tissues of the body and urine (Willems *et al.*, 1999). Till date, approximately 120 families have been described with fucosidosis. The highest incidence is seen in Italy and Hispanic-American population. Only 38 pathogenic variants are reported in the *FUCA1* gene that is associated with fucosidosis (Willems *et al.*, 1991). We discuss the importance of deep phenotyping using magnetic resonance imaging in the diagnostic process. Further, we discuss the significance of extended analysis of exome sequencing data using Integrative Genomics Viewer.

2. Case presentation

Proband, a 2-years-and-4-months female, second-born to fourth degree consanguineously married parents, presented with developmental delay and neurological deterioration (Figure 1A). She was born at term via vaginal delivery. Her birth weight was 3 Kg (−0.5 SD) and the perinatal period was uneventful.

At one and a half months of age, she was admitted with complaints of fever and respiratory distress and received supportive treatment for persistent pneumonia with lung abscess

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Conflict of interest statement

The authors declare no conflict of interest. All the authors have read and approved the manuscript.

aspergillosis and gastroesophageal reflux in the neonatal intensive care unit for a period of 45 days. She was started on antibiotics like cefalexin, sulfamethoxazole, trimethoprim and oxiconazole, an antifungal medication for the management of pneumonia and upper respiratory tract infection. Following this, she had recurrent episodes of lower respiratory tract infections requiring three hospital admissions within the first year of life. She was given nutritional support through nasogastric feeding until 10 months of age. There was a delay in her development which was followed by neuroregression after two years of age. She attained social smile at 2 months, head control at 8 months, rolling over at 12 months, sitting with support at 15 months, standing with support at 18 months and monosyllables at 18 months. At 2 years 4 months of age, she was admitted with complaints of seizures, manifesting as involuntary movements of all four limbs with upward rolling of eyes lasting for 5 to 10 seconds observed once in every 2 days. For management of seizures, phenobarbitone 30 mg twice a day was advised. Over a period of 2 months, the frequency of seizures increased to 4 to 5 times a day. At 2 years 6 months of age, she was hospitalised with complaints of fever, left focal seizures and impaired awareness. She was started on intravenous antibiotics such as piperacillin/tazobactum, amikacin and vancomycin as well as the antifungal medication, fluconazole. In addition, she was given multivitamin supplementation containing thiamine, riboflavin, carnitine and Coenzyme Q. At the age of 3 years, she had another episode of refractory seizures. She did not respond to supportive treatment and succumbed during this illness.

On examination, her occipitofrontal circumference was 47 cm (−0.46 SD) and height was 88 cm (−0.3 SD). She had coarse facial features, square face, flat facial profile, hypertelorism, broad nose, thick lips, high arched palate, mild pectus excavatum, everted umbilicus and Mongolian spots on the back (Figure 1B). She had hypotonia in all four limbs and had right ankle contractures.

3. Investigations

At one and a half month of age, her hemoglobin electrophoresis showed beta thalassemia trait, macrocytic normochromic anemia, low B cell count and NK cell count. At 2 years of age, her electroencephalography (EEG) showed moderate encephalopathy. Considering, the presence of seizures, autoimmune workup was done which came back negative. Her brain MRI was reviewed in detail and it was indicative of hypomyelination, with confluent and symmetric T2W hyperintensities in the periventricular and subcortical white matter and T2W hypointensities in the globi pallidi suggestive of fucosidosis (Figure 1C). Radiographs of this patient were unavailable.

4. Molecular testing

After the initial clinical evaluation, informed consents approved by institutional ethics committee were obtained from the family in accordance with Helsinki declaration. Peripheral blood samples were collected from the proband and her parents. DNA was extracted using QIAamp DNA Mini Kit. Exome sequencing (ES) was performed for the proband as described previously (Girisha *et al.*, 2019). The ES data was processed using ANNOVAR and customised scripts(Kausthubham *et al.*, 2021). Further, manual inspection

of exome sequencing data by a genome visualisation tool and copy number variation (CNV) analysis was performed using exome data by ExomeDepth algorithm. Validation of the causative variant identified was done by quantitative PCR (qPCR) by comparative quantification Ct (ΔΔCt) method (Livak and Schmittgen, 2001) on genomic DNA of the proband and clinically normal parents using Applied Biosystems StepOneTM Real-Time PCR System, PowerUp SYBR Green PCR Master Mix and StepOne Software v2.3 for data analysis. The relative exon copy number was calculated by the expression 2 × 2-ΔΔCt and is approximately two for a diploid sample, one for heterozygous deletion and zero for homozygous deletion.

5. Result

No clinically relevant single nucleotide variations, small deletions/duplications or indels were observed in the proband on exome sequencing. Analysis of exome sequencing data, specifically the *FUCA1* gene was done using Integrative Genomics Viewer which revealed a homozygous deletion, spanning exons 3 and 4 of *FUCA1* (NM_000147.5) (Figure 2A). This deletion (chr1:23859648-23865720) was further confirmed by CNV analysis in the proband. Segregation and validation were done by qPCR. Values of amplicons in the proband was zero and one copy number in her parents in comparison to that of diploid unrelated control sample (Figure 2B). These findings indicate homozygous deletion in the proband and heterozygous deletion in the parents.

6. Discussion

We describe a 2-years-and-4-months child with fucosidosis caused due to biallelic deletion of exons 3 and 4 in *FUCA1.* Fucosidosis (OMIM# 230000) is a lysosomal storage disorder caused by biallelic pathogenic variants in *FUCA1* (MIM*612280) with the highest incidence described in Italy, in the Hispanic-American population (Willems *et al.*, 1999). Fucosidosis is a progressive neurodegenerative disorder with features like coarse facial features, growth retardation, recurrent respiratory infections, dysostosis multiplex and angiokeratoma corporis diffusum (Willems *et al.*, 1991). Till date, approximately 120 families and 38 pathogenic variants in FUCA1 have been associated with fucosidosis. As there is substantial phenotypic variability in clinical manifestation of fucosidosis, it was previously classified as type 1 and type 2. Type 1 follows a rapidly progressing neurodegenerative course with symptoms noticeable at the age of 1 to 2 years and death before the age of 10 years depending on the clinical severity. Whereas type 2 was a milder form of the condition and symptoms appear progressively and possibly survive into adulthood. As more cases have been reported, it is now considered as a continuous clinical spectrum with variable severity of phenotype (Willems *et al.*, 1991). Phenotypic variability and the overlapping symptoms of fucosidosis with metabolic and autoimmune diseases can cause a delay in diagnosis (Wynne *et al.*, 2018). Magnetic resonance imaging pattern recognition with signs of hypomyelination and signal abnormalities in globus pallidus can be suggestive of fucosidosis (Ediz *et al.*, 2016).

The *FUCA1* gene produces a lysosomal enzyme, α-L-fucosidase which helps in the degradation of fucose containing glycoproteins and glycolipids. Deficiency of α-L-

fucosidase enzyme results in the accumulation of fucosylated glycoconjugates in tissues such as liver and abnormal excretion in urine (Michalski and Klein, 1999). Most pathogenic variants seen in the *FUCA1* gene are intragenic small deletions/insertions (INDELs) and missense, nonsense, splice sites variants and can be identified through sequence analysis. Previously, two exonic deletions were reported in the *FUCA1* gene known to be associated with fucosidosis. We report a novel deletion of exons 3 and 4 in *FUCA1* identified by manual inspection of exome sequencing data using a genome visualizer tool and further confirmed by copy number variation analysis from exome sequencing data and qPCR. In cases where the clinician suspects fucosidosis to be the most probable diagnosis and no sequence variant is identified in the *FUCA1* gene, deletion/duplication analysis is recommended (Stepien, Ciara and Jezela-Stanek, 2020).

To conclude, deep phenotyping using neuroimaging is significant in the diagnostic process of fucosidosis. We report an additional individual with a novel homozygous deletion of exons 3 and 4 in the *FUCA1* gene associated with fucosidosis.

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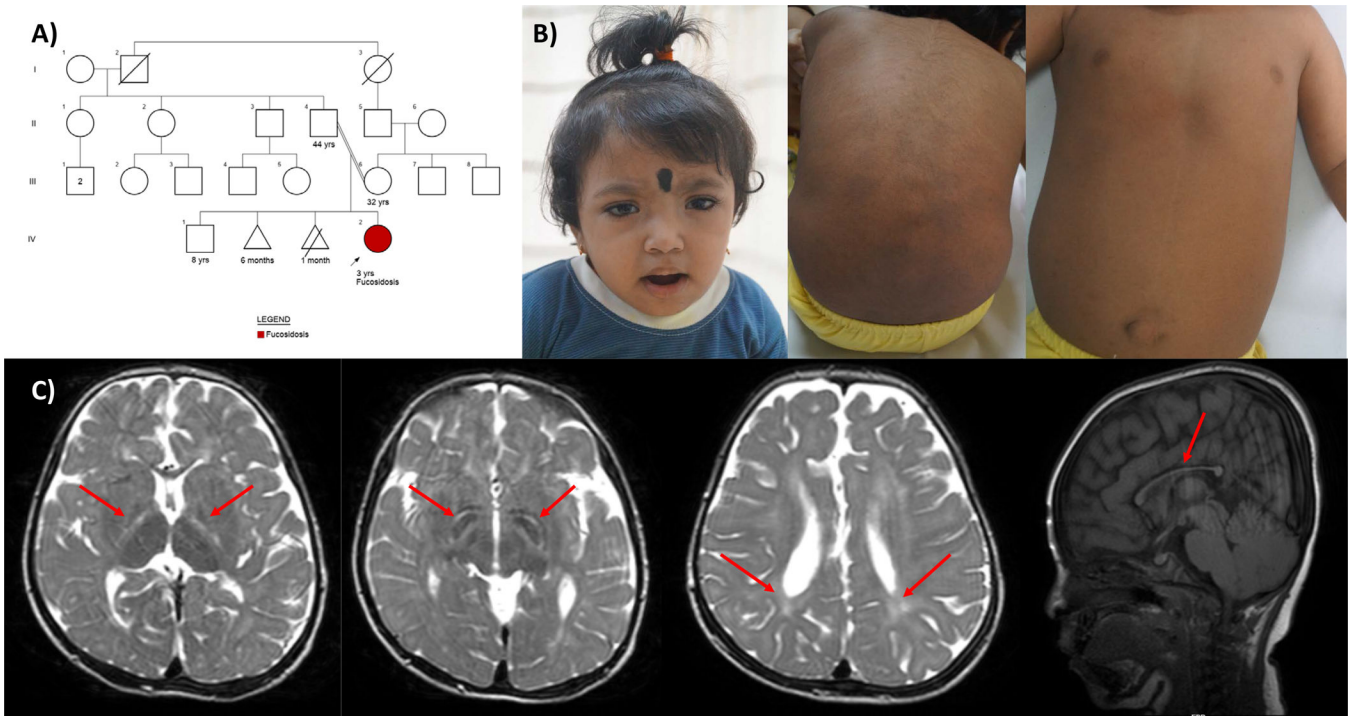

Figure 1:

A) Pedigree of the family. B) Clinical photograph of the proband showing coarse facial features, mild pectus excavatum, and Mongolian spots over back. C) MRI showing confluent and symmetric T2W hyperintensities in the periventricular and subcortical white matter suggesting hypomyelination, T2W signal hyperintensity in the posterior limb of the internal capsule, significant T2W hypointensities in the globi pallidi, and T1W images showing thin corpus callosum.

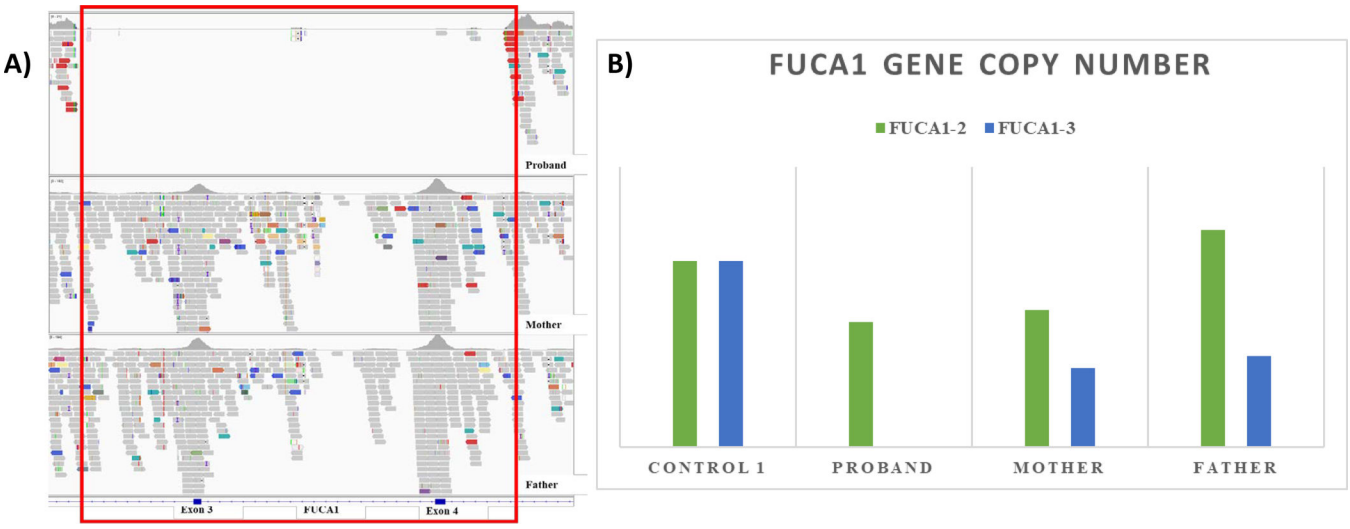

Figure 2:

A) Integrative genomic viewer showing absence of reads of exons 3 and 4 in *FUCA1* B) qPCR analysis depicting homozygous deletion in proband and heterozygous deletion in the parents in *FUCA1*