INTRODUCTION

A number of inherited disorders are known to be caused by mutations in genes involved in the biosynthesis of heparan sulfate (HS), a linear polysaccharide that binds protein ligands, forming proteoglycans that are components of cell surfaces and extracellular matrices and are involved in many developmental processes. EXT2 (MIM: 608210) and NDST1 (MIM: 600853) are two such genes critical to the polymerization and processing of heparan sulfate. EXT2 belongs to the exostosin family of glycosyltransferases involved in the chain elongation step of heparan sulfate biosynthesis, while NDST1 encodes the bifunctional enzyme GlcNAc N-deacetylase/N-sulfotransferase that modifies the glycosaminoglycan component of heparan sulfate and plays a role in establishing the extent and pattern of HS sulfation. EXT2 is implicated in the autosomal dominant disorder multiple Exostoses type 2 (MIM: 133701). Biallelic EXT2 mutations, however, have also been reported in four siblings born to consanguineous parents, manifesting scoliosis, seizures, and macrocephaly (MIM: 616682) without exostosis, following an autosomal recessive pattern of inheritance. Recently, two more families have been reported with multiple siblings in each displaying intellectual disability, facial dysmorphisms, and seizures.
with autosomal recessive EXT2 variants and this disorder has been renamed with the acronym AREXT2 (autosomal recessive EXT2-related syndrome).\(^4,5\) Biallelic pathogenic variants within NDST1 are implicated in autosomal recessive intellectual disability type 46 (MIM: 616116).

This case report describes a patient with distinctive facial features, developmental delay, autism, and epilepsy, who was found to have two compound heterozygous likely pathogenic variants in EXT2 and a heterozygous variant of uncertain significance (VUS) within NDST1 through whole exome sequencing (WES) performed by the Genomics Laboratory at Mayo Clinic.

## 2 | CLINICAL REPORT

### 2.1 | Clinical description

The patient is a 14-year-old female evaluated through the Mayo Clinic Department of Clinical Genomics for developmental delay, autism, epilepsy, and distinctive craniofacial features including macrocephaly, prominent tall forehead, hypertelorism, long hypoplastic philtrum, bilateral preauricular pits, upturned and round broad nasal tip.

She was born to a fraternal twin pregnancy at 36-5/7 weeks’ gestation via C-section. Her birth weight was 7 pounds 2 ounces and birth length was 20.5 inches. Apgar scores are unknown, but she required treatment for respiratory difficulties with an oxygen tent and a CPAP for 4 days. She had slightly delayed motor milestones, sat unassisted at 7-8 months, and walked at 14 months of age. At the age of 2 years, language delay was also evident. Developmental regression of verbal and social skills along with cognitive decline was reported at 27 months. At age 5 years, she was found to be positive for antibodies to neuronal voltage gated potassium channels. She showed some improvement in language and socialization with immunotherapy but did not tolerate this treatment well so it was discontinued. She developed seizures at 10 years of age. She had aggressive behavior toward self and others, rocking stereotypies and sleep difficulty, which improved over time. She underwent surgery for strabismus bilaterally at the age of 5 years. Additional features include sensitive skin prone to acne and gastrointestinal problems such as constipation and gastroesophageal reflux.

### 2.2 | Laboratory evaluations

The patient had a normal karyotype, array CGH, and molecular analyses for Fragile X, Rett and Angelman/Prader Willi syndromes. Biochemical screening for mucopolysaccharidoses was performed in view of the coarse facial features and revealed low levels of heparan sulfate in dried blood spot (12 nmol/L), serum (4.46 ng/mL), and urine (0.5 mg/mmol creatinine) specimens (Reference ranges given in Table 1). Brain MRI was normal. EEG was abnormal and revealed occipital midline spike and waves consistent with focal seizures.

## 2.3 | Family history

Her fraternal twin sister has Asperger syndrome (diagnosed at 3 years of age), mild motor delays, and a history of cognitive and speech delays, which had improved over time. There was no other relevant family history, and brother and parents were unaffected (Figure 1).

## 3 | METHODS

### 3.1 | Editorial policies and ethical considerations

The patient provided written informed consent to research protocol IRB#12-009346 approved by the Mayo Clinic Institutional Review Board for this study and publication of this paper.

### 3.2 | Whole exome sequencing

Whole exome sequencing through the Mayo Clinic Genomics Laboratory was performed on DNA extracted from blood

### Table 1 Reference range for Heparan Sulfate

| Heparan sulfate | DBS N = 173 (nmol/L) | Serum N = 268 (ng/mL) | Urine N = 525 (mg/mmol CT) |
|-----------------|----------------------|-----------------------|---------------------------|
| 1%ile           | 12.0                 | 7.06                  | 0.014                     |
| 10%ile          | 16.2                 | 9.30                  | 0.031                     |
| 50%ile          | 32.1                 | 15.06                 | 0.067                     |
| 90%ile          | 48.9                 | 28.24                 | 0.188                     |
| 99%ile          | 96.1                 | 53.89                 | 0.292                     |

**FIGURE 1** Pedigree of our family showing EXT2 variants
samples from the proband and her unaffected parents. The exome was captured utilizing a custom reagent developed by Mayo Clinic and Agilent Technologies. Sequencing was performed on an Illumina HiSeq 2500 next-generation sequencing instrument, using HapMap sample NA12878 as an internal control. Paired-end 101 base-pair reads were aligned to a modified human reference genome (GRCh37/hg19) using Novoalign (Novocraft Technologies, Malaysia). Sequencing quality was evaluated using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/). All germline variants were jointly called through GATK Haplotype Caller and GenotypeGVCF.6 Each variant was annotated using the BioR Toolkit.7 Approximately, 97% of the exome is sequenced to a depth of at least 20X. Variants of interest in the patient were confirmed by automated fluorescence dideoxy sequencing and subsequently submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000782709, SCV000782708, and SCV000782711.

3.3 | In silico analysis tools used

Putative effects of the variants on protein structure and function were predicted using SIFT,8 PolyPhen-2,9 Mutation Taster,10 M-CAP,11 Predict SNP2,12 and CADD.13

4 | RESULTS

Trio WES testing revealed three possibly relevant variants. Two compound heterozygous VUSs (one paternally inherited—c.1118T>A p.(Val373Asp) and a maternally inherited—c.2015C>T p.(Thr672Met)) were identified within the EXT2 gene. This gene encodes a ubiquitously expressed enzyme involved in heparan sulfate biosynthesis, localized in the Golgi apparatus. Heterozygous pathogenic variants within EXT2 are causative of an autosomal recessive disorder known as multiple Exostoses type 2 in which patients develop multiple benign bone tumors called osteochondromas, not present in our patient. Recently, biallelic mutations in EXT2 have been described as causative for a new disorder named AREXT2 (autosomal recessive EXT2-related syndrome). This syndrome includes phenotypes of developmental delay, intellectual disability, seizures, scoliosis, and micro/macrocephaly. Patients with this syndrome also have been reported to have coarse facial features, GI-related issues, and poor speech.13

As illustrated in Table 2, our patient has overlapping features with this disorder, including macrocephaly, coarse facies, long philtrum, hypotonia, strabismus, gastroesophageal reflux disease, constipation, sensitive skin, development/language delay, seizures, and delayed psychomotor development. However, skeletal issues such as scoliosis and decreased bone density and muscular issues such as hypotonia, observed with this disorder, were not observed in our patient. There is clinical heterogeneity in this disorder, with two of four patients with biallelic EXT2 alterations noted to have a ventricular septal
| Features                  | Farhan et al<sup>1</sup> | El-Bazzal et al<sup>4</sup> | Gentle et al<sup>5</sup> | This study                  |
|--------------------------|---------------------------|-----------------------------|--------------------------|-----------------------------|
|                          | Patient 1 II-1            | Patient 2 II-3              | Patient 3 II-4            | Patient 1 II-2              |
|                          | Patient 4 II-5 (deceased) | Patient 2 II-3              |                          | Patient 2 II-3              |
|                          |                           | Patient 1                   | Patient 2                | Proband                     |
|                          |                           | Patient                      |                          | Sister                      |
| Sex                      | M                         | M                           | M                        | M                           |
| Age at examination (y)   | 19                        | 14                          | 11                       | 10                          |
| Genotype                 | EXT2 variants             | G                           | G                        | G                           |
|                          |                          | c.260T>G, p.Met87Arg (hom)  | c.260T>G, p.Met87Arg (hom)| c.260T>G, p.Met87Arg (hom)  |
|                          |                          | c.283C>T, p.Arg95Cys (hom)  | c.283C>T, p.Arg95Cys (hom)| c.11C>T, p.Ser4Leu (hom)    |
|                          |                          |                             |                          |                             |
| Neurological issues      |                           |                             |                          |                             |
| Developmental Delay      | +                         | +                           | +                        | +                           |
| Intellectual disability  | +                         | +                           | +                        | +                           |
| Speech delay             | +                         | +                           | +                        | +                           |
| Autism                   | –                         | –                           | –                        | +                           |
| Seizures                 | +                         | +                           | +                        | +                           |
| Facial dysmorphisms      |                           |                             |                          |                             |
| Macrocephaly             | +                         | +                           | +                        | –                           |
| Microcephaly             | –                         | –                           | –                        | –                           |
| Forehead                 | Flat                      | Flat                        | High                     | High                        |
| Hypertelorism            | +                         | +                           | +                        | –                           |
| Long philtrum            | +                         | +                           | +                        | +                           |
| Bulbous nose             | –                         | –                           | –                        | +                           |
| Ear anomalies            | –                         | –                           | +                        | –                           |
| Other issues             |                           |                             |                          |                             |
| Exostoses                | –                         | –                           | –                        | +                           |
| Cardiovascular issues    | +                         | +                           | –                        | +                           |
| Renal issues             | +                         | + (Kidney failure)          | –                        | –                           |
| Hypotonia                | +                         | +                           | +                        | –                           |

(Continues)
The onset of this disorder is reported in infancy, with seizures starting between 2 and 5 years. Our patient's developmental delay was noted during the first 2 years of life and seizures were noted around 10 years of age. Despite some differences, there was significant overlap seen between this clinically variable condition and the patient's phenotype. The two rare EXT2 variants found in our patient have not been reported clinically in the literature or clinical variant databases (HGMD, OMIM, or ClinVar). However, in silico tools predict they are putatively pathogenic. Notably, the patient's fraternal twin sister who is mildly affected as compared to the proband, with some learning delays and speech concerns, was also found to have the two compound heterozygous EXT2 variants while the patient's unaffected brother was found to carry only the heterozygous paternally inherited EXT2 variant (Figure 1).

NDST1 encodes a ubiquitously expressed transmembrane protein residing in the Golgi apparatus, involved in the pathway for formation of heparan sulfate. The gene is implicated with autosomal recessive intellectual disability type 46. This condition has an onset in infancy or early childhood and is characterized by delayed psychomotor development, delayed or absent speech, intellectual disability and hypotonia. Patients in some cases have also been shown to display behavioral abnormalities such as agitation, aggression and sleep disturbances, seizures, and postnatal growth deficiency.3,15 The patient had aggressive behavior, intellectual disability, seizures, and sleep disturbance but no evidence of growth retardation. Notably, the mildly affected sister was also found to carry this variant. The discordance in the phenotype of the two sisters might be due to the presence of potassium channel antibodies seen in proband but not her sister. However, as this condition is autosomal recessive and no variant was found in the patient's second allele, this remains a VUS. It is interesting to note that both EXT2 and NDST1 are genes that encode enzymes involved in heparan sulfate synthesis. This raises the possibility that the EXT2 gene variants are possibly causative and the NDST1 variant has a modifier effect on the patient's clinical characteristics. At this time, there is no sufficient evidence to prove this hypothesis and this remains an open question for future investigation.

| Features | Farhan et al | El-Bazzal et al | Gentile et al | This study |
|----------|-------------|----------------|-------------|-------------|
| Scoliosis | + | + | + | − |
| Skin issues | Sensitive skin | Sensitive skin | Sensitive skin | − |
| Gastrointestinal issues | Gastro-esophageal reflux & associated ulceration, constipation and diarrhea | Gastro-esophageal reflux, constipation | − | + |

6 | CONCLUSION

In summary, the genetic variants found in EXT2 are likely pathogenic according to ACMG/AMP guidelines,16 based on the collective evidence of familial cosegregation, biochemical analysis, and emerging gene-phenotype reports in the literature. At this point, we do not have enough evidence to rule in or out a potential contribution of the NDST1 variant despite the fact both EXT2 and NDST1 proteins are located in the ER-Golgi, are components of the heparan sulfate biosynthesis pathway, and interact with each other affecting the
structure and levels of heparan sulfate,¹⁷ and this remains a finding of uncertain significance. The findings described above could allow for a better characterization of distinct features of such clinically variable and nonspecific phenotypes and may also provide potential strategies for therapeutic intervention by enzyme replacement therapy or administration of heparan sulfate to the patients.

ACKNOWLEDGMENTS

The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. The authors would like to thank the Genome Aggregation Database (gnomAD) and the groups that provided exome and genome variant data to this resource. A full list of contributing groups can be found at http://gnomad.broadinstitute.org/about. The authors would also like to thank the patient and patient’s family for participation in this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest exist.

AUTHOR CONTRIBUTIONS

A.G., L.H., and E.W.K.: summarized the findings and characterized the patient’s genomic results. K.M.R.: measured heparan sulfate levels in the patient. R.H.G., D.L.R., and S.A.E.: evaluated the patient clinically, counseled the patient, and obtained consent from the patient. A.G., R.H.G., and E.W.K.: wrote the manuscript with input from all authors.

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REFERENCES

1. Farhan SM, Wang J, Robinson JF, et al. Old gene, new phenotype: mutations in heparan sulfate synthesis enzyme, EXT2 leads to seizure and developmental disorder, no exostoses. J Med Genet. 2015;52(10):666-675.
2. Busse M, Feta A, Presto J, et al. Contribution of EXT1, EXT2, and EXT3 to heparan sulfate chain elongation. J Biol Chem. 2007;282(45):32802-32810.
3. Armstrong L, Tarailo-Graovac M, Sinclair G, et al. A girl with developmental delay, ataxia, cranial nerve palsies, severe respiratory problems in infancy-Expanding NDST1 syndrome. Am J Med Genet A. 2017;173(3):712-715.
4. El-Bazzal L, Atkinson A, Gillart AC, Obeid M, Delague V, Megarbane A. A novel EXT2 mutation in a consanguineous family with severe developmental delay, microcephaly, seizures, feeding difficulties, and osteopenia extends the phenotypic spectrum of autosomal recessive EXT2-related syndrome (AREXT2). Eur J Med Genet. 2018. [Epub ahead of print] https://doi.org/10.1016/j.ejmg.2018.07.025.
5. Gentile M, Agolini E, Cocciaffèrro D, et al. Novel exostosin-2 missense variants in a family with autosomal recessive exostosin-2-related syndrome: further evidences on the phenotype. Clin Genet. 2018;95(1):165-171.
6. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297-1303.
7. Kocher JP, Quest DJ, Duffy P, et al. The Biological Reference Repository (BioR): a rapid and flexible system for genomics annotation. Bioinformatics. 2014;30(13):1920-1922.
8. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073-1081.
9. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248-249.
10. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):361-362.
11. Jagadeesh KA, Wenger AM, Berger MJ, et al. M-CAP eliminates a majority of variants of uncertain significance in clinical exomes at high sensitivity. Nat Genet. 2016;48(12):1581-1586.
12. Bendl J, Musil M, Stourac J, Zendulka J, Damborský J, Brezovský J. PredictSNP2: a unified platform for accurately evaluating SNP effects by exploiting the different characteristics of variants in distinct genomic regions. PLoS Comput Biol. 2016;12(5):e1004962.
13. Rentsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2018;47(1):D886-D894.
14. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291.
15. Reuter MS, Musante L, Hu H, et al. NDST1 missense mutations in autosomal recessive intellectual disability. Am J Med Genet A. 2014;164A(11):2753-2763.
16. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
17. Presto J, Thuveson M, Carlsson P, et al. Heparan sulfate biosynthesis enzymes EXT1 and EXT2 affect NDST1 expression and heparan sulfate sulfation. Proc Natl Acad Sci U S A. 2008;105(12):4751-4756.

How to cite this article: Gupta A, Ewing SA, Renaud DL, et al. Developmental delay, coarse facial features, and epilepsy in a patient with EXT2 gene variants. Clin Case Rep. 2019;7:632–637. https://doi.org/10.1002/ ccr3.2010