ERCC6L2-associated inherited bone marrow failure syndrome

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Abstract
Background: ERCC6L2-associated disorder has recently been described and only five patients were reported so far. The described phenotype included bone marrow, cerebral, and craniofacial abnormalities. The aim of this study was to further define the genetic and phenotypic spectrum of the disorder by summarizing the five published cases and an additional case that we identified through whole-exome sequencing performed at the University of Toronto.

Methods: Clinical data was extracted from the Canadian Inherited Marrow Failure Registry. Whole exome sequencing was performed to identify causative mutations.

Results: All six cases had homozygous truncating mutations either at or upstream of the helicase domain of ERCC6L2. All patients displayed bone marrow failure, learning or developmental delay and microcephaly. Our patient was unique in displaying features of cerebellar disease, including ataxia and dysmetria as well as an interval deterioration of the corpus callosum and generalized volume loss on MRI. Another unique feature of our patient was retinal dystrophy with macular involvement. Along with one other patient, our patient displayed craniofacial abnormalities by presenting with low-set prominent ears, a pointed prominent chin, and deep-set eyes. Leukemia is common among patients with inherited bone marrow failure, but thus far, none of the patients have developed this complication.

Conclusions: ERCC6L2-associated disorder is a multisystem disorder. The phenotype spectrum includes bone marrow failure, cerebral, and craniofacial abnormalities, as well as cerebellar and retinal abnormalities.

KEYWORDS
bone marrow failure, developmental delay, ERCC6L2, microcephaly, thrombocytopenia

1 | INTRODUCTION

ERCC6L2-associated disorder has recently been described. The prevalence of the disorder is unknown; however, the disorder is probably very rare, and thus far, only five cases have been reported (Tummala et al., 2014; Zhang et al., 2016; Jarviaho et al., 2017). The spectrum of clinical manifestations and mutations is yet to be defined, but all the reported patients had bone marrow failure, and three of the given patients had both...
| Features               | Case 1 (Family 1) (Tummala et al., 2014) | Case 2 (Family 2) (Tummala et al., 2014) | Case 3 (Family 3) (Zhang et al., 2016) | Case 4 (Family 4) (Jarviahno et al., 2017) | Case 5 (Family 5) (Jarviahno et al., 2017) | Case 6 (Family 6) (Present study) |
|-----------------------|------------------------------------------|------------------------------------------|----------------------------------------|--------------------------------------------|------------------------------------------|----------------------------------|
| Gender                | Male                                     | Female                                   | Male                                   | Male                                       | Female                                   | Female                           |
| Age at presentation (years) | 12                                       | 19                                       | 13                                     | 8                                          | 8                                        | 7                                |
| Ethnic origin         | French                                   | Pakistani                                | UnK                                    | Finnish                                    | Finnish                                  | Pakistani                        |
| First-cousin parents  | Yes                                      | Yes                                      | Yes                                    | No                                         | No                                       | Yes                              |
| Platelet counts (×10^9/l) | 9                                        | 33                                       | Initially 4; then improved to moderate level of thrombocytopenia | 5                                          | 23                                      | 58                               |
| Hemoglobin (g/l)      | 99                                       | 93                                       | Initial: 90 Resolution: 120             | 81                                         | 106                                      | 133                              |
| Reticulocytes (×10^9/l) | UnK                                      | UnK                                      | 75 (At time of improved counts)         | UnK                                        | UnK                                      | 64.9                             |
| MCV (fl)              | UnK                                      | UnK                                      | 104 (initially) 104 (at the time of improved counts) | UnK                                        | UnK                                      | 88.9                             |
| WBC (×10^9/l)         | 2.4                                      | 5.3                                      | 1.47 (at the time of improved counts)   | 2.3                                        | 2.0                                      | 3.0                              |
| Neutrophils (×10^9/l) | UnK                                      | UnK                                      | 0.7 (initially) Then improved to moderate neutropenia | UnK                                        | UnK                                      | 1.1                              |
| HbF                   | UnK                                      | UnK                                      | 7%                                      | UnK                                        | UnK                                      | 3.7%                             |
| Bone marrow cellularity | Hypocellular                        | Hypocellular                             | Hypocellular                           | Hypocellular Reduced cellularity           | Hypocellular                             |                                  |
| Course of bone marrow failure | UnK                                  | UnK                                      | Spontaneous recovery                    | UnK                                        | UnK                                      | Stable over 3 years               |
| Developmental delay and/or learning difficulties | Yes                                   | Yes                                      | Yes                                    | No                                         | No                                       | Yes                              |
| Microcephaly          | Yes                                      | Yes                                      | Yes                                    | No                                         | No                                       | Yes                              |
| Signs of upper motor neuron palsy | UnK                                  | UnK                                      | UnK                                    | UnK                                        | UnK                                      | Hypertonia                      |
| Cerebellar features   | UnK                                      | UnK                                      | UnK                                    | UnK                                        | UnK                                      | Ataxia, dysmetria, nystagmus      |
| Hypotonia             | UnK                                      | During infancy                           | UnK                                    | UnK                                        | UnK                                      | UnK                              |
| Eyes (internal compartments) | UnK                                  | UnK                                      | UnK                                    | UnK                                        | UnK                                      | Rod and cone dystrophy.          |
| Craniofacial abnormalities | Abnormal facies and ear abnormalities | UnK                                      | UnK                                    | UnK                                        | UnK                                      | Low set prominent ears, pointed prominent chin, deep-set eyes |

(Continues)
microcephaly and developmental delay. Herein, we describe in detail a female patient with a novel pathogenic homozygous mutation in ERCC6L2 (OMIM 615667) with several new clinical features that have not been previously reported. In addition, we summarize the findings of all six identified cases and update the spectrum of phenotypes and genotypes of this rare syndrome.

2 | CASE DESCRIPTION

The patient in this report was enrolled on the Canadian Inherited Marrow Failure Registry and informed written consent was obtained. The study was approved by the Hospital for Sick Children’s Research Ethics Board. The patient was a female, who presented at the age of two
months with poor weight gain, requiring G-tube feeding and a height below the 3rd percentile. After 6 months of age, her head circumferences were noticed to be below the 3rd percentile. Previous evaluations of the child did not report microcephaly, but specific measurements were not available. Since about 6 months of age, the child was also noticed to have developmental delay. Other features noted on physical examination included low set prominent ears, a pointed prominent chin, deep-set eyes and one café au lait spot on the abdomen, which was about 1.5 × 1.5 cm. Neurological examination showed hypertonia, brisk deep tendon reflexes, bilateral clonus, down-going plantar reflexes, strabismus, and features of cerebellar disease that included dysmetria, ataxia and nystagmus. At 3½ years old, triple-evoked potentials and somatosensory-evoked potentials of the median nerve showed evidence of central conduction delay.

Brain MRI at the age of 6 years showed diffuse hazy T2 signal of hyperintensity throughout the supratentorial white matter. Repeat MRI at 10 years old displayed an interval increase in supratentorial FLAIR hyperintensity, involving the posterior limbs of the internal capsule, cerebral peduncle, external capsule, peririgmal white matter, and optic radiation. There appeared to be reduced white volume with mild thinning of the corpus callosum and overall generalized supra and infratentorial volume loss.

On eye examination, the macula showed patches of atrophy, whereas the rest of the retina showed generalized pigment epithelial changes; electoretinography showed generalized rod-cone dystrophy with additional selective involvement of the retinal ON-pathway. Visual-evoked potentials showed evidence of central conduction delay.

The patient had persistent mild thrombocytopenia and intermittent mild neutropenia. At 8½ years of age, her complete blood counts showed platelets 58 × 10⁹/L, WBC 3.0 × 10⁹/L, neutrophils 1.1 × 10⁹/L, Hb 133 g/L, MCV 88.9 fl, absolute reticulocyte count of 64.9 × 10⁹/L, and hemoglobin F of 3.7%. The patient’s bone marrow testing showed severe hypocellularity of <10%–20% with reduced trilineage hematopoiesis.

The patient underwent multiple investigations to reveal the cause for the above manifestations which all showed normal results, including testing for: karyotype, fragile X by FMR1 (CGG)n repeat, a metabolic screen, congenital disorders of glycosylation, mitochondrial disorders, alpha fetoprotein, chromosome breakage studies (for Fanconi Anemia), targeted sequencing of FNX (Friedreich Ataxia), SBDS (Shwachman-Diamond syndrome), MECP2 (Rett syndrome), ERCC6 and ERCC8 (Cockayne syndrome), and copy number variant analysis by oligonucleotide microarray. A microcephaly panel, which sequenced 41 known genes in autosomal recessive microcephaly, was also tested and found to be negative. Testing for Ataxia Telangiectasia, Nijmegen Breakage syndrome, and Bloom syndrome by chromosome analysis were all negative. Telomere length (for dyskeratosis congenita) of total lymphocytes and memory T cells and B cells was at the 1st percentile; length of telomere in granulocytes and natural killer cells was below the 1st percentile. These findings were not typical of dyskeratosis congenita.

At the age of 9 years, the patient underwent whole exome sequencing and was discovered to have a homozygous stop mutation in ERCC6L2 (NCBI RefSeq NG_034107.1), c.1687C>T (p.Arg563*). No other causal mutations were identified.

3 | DISCUSSION

Together with the present case, there are six published cases of patients with an ERCC6L2-related disorder. The features of these six patients are summarized in Table 1. The addition of our case further supports the link between ERCC6L2 and this recently characterized syndrome. All six cases were caused by truncating mutations either at or upstream of the helicase domain leading to premature termination of translation (Figure 1). Since ERCC6L2 belongs to the Snf2 family of helicase-related proteins, it is possible that deletion of the helicase domain is sufficient to cause the disease due to the importance of the Snf2 family of helicase-related proteins in regulation of nuclear processes (Tummala et al., 2014; Flaus, Martin, Barton, & Owen-Hughes, 2006). Alternatively, a complete loss of the protein due to either nonsense-mediated mRNA decay or protein degradation by the ubiquitin-proteasome system is the cause of the phenotype. Protein degradation by the ubiquitin-proteasome system plays an important role in several fundamental cellular processes, including regulation of the cell cycle, modulation of cell surface receptors and ion channels, as well as antigen presentation and has been linked to several hereditary human diseases (Sarikas et al., 2005).

All six cases manifested hematopoietic features. Thrombocytopenia was the most prominent phenotypic feature and was seen in all cases; it was moderate in two cases and severe in four cases. Anemia was seen in five cases and ranged from mild to moderate. In one case, reticulocyte counts were available when the patient was anemic and were low for the degree of anemia. Hemoglobin F was tested in two cases and was elevated at 7% and 3.7%. Neutrophils were reported in two cases and were moderately low in both of them. MCV was reported in two cases and was elevated in one patient. Bone marrow was hypocellular in all cases. Myelodysplastic syndrome and leukemia were not reported among the six patients; however, due to the small series and the predicted functions of ERCC6L2, it is
reasonable to manage these patients with a leukemia surveillance program until a leukemic risk is confidently excluded. Importantly, most syndromes with inherited bone marrow failure are leukemia prone (Dror, 2017). Therefore, establishing a diagnosis in such cases immediately changes the management of these patients and a leukemia surveillance program is initiated. Further, a significant proportion of patients with inherited bone marrow failure syndromes may not manifest prominent bone marrow failure before developing leukemia. Hence, inclusion of ERCC6L2 in the list of genes to be tested when the features described in the present manuscript are encountered may be the only tool that allows for early institution of a leukemia-surveillance program.

The neuronal presentation was noteworthy with four patients showing learning difficulties and developmental delay. Microcephaly was also present in four of the cases. In our case, nerve conduction velocity was measured which showed central delay. Our patient was unique in displaying features of cerebellar disease, including ataxia and dysmetria. Importantly, in our patient, we have noticed an interval deterioration of the corpus callosum and generalized volume loss on MRI. This suggests the possibility of a neurodegenerative process. Nevertheless, the patient has not shown clinical signs of deterioration.

Two of the patients presented with craniofacial abnormalities, while information on the other four patients was not provided. Case 1 displayed “abnormal facies” and ear abnormalities, while our patient had low-set prominent ears, a pointed prominent chin, and deep-set eyes.

The patient in the present report also had early onset retinal dystrophy with macular involvement. The patient had compound heterozygous variants in C2orf71, a gene associated with retinitis pigmentosa, of (c.3739G>A (p.Gly1247Ser) and c.1882G>A (p.Ala628Thr)). The reported minor allele frequencies of the variants in the GnomAD database were 3% (p.Gly1247Ser) and 1.1% (p.Ala628Thr) among South Asians, and both variants were also reported in a homozygous state in the general population. Further, bioinformatics analysis showed inconsistent results with regard to potential damaging effect. Thus, these variants were excluded as a cause of the retinal phenotype. Interestingly, retinitis pigmentosa was associated with several other hereditary bone marrow failure disorders, including DNAJC21-related Shwachman-Diamond syndrome (Tummala et al., 2014; Dhanraj et al., 2017) and Revesz syndrome (Savage et al., 2008). Therefore, it is possible that retinitis pigmentosa is part of the phenotypic spectrum of the ERCC6L2-related disorder or the disorder is caused by a variant(s) that was not detected by whole exome sequencing (Dhanraj et al., 2017). Reports of additional cases of ERCC6L2-related disorder are needed to determine a causal relationship.

The ERCC6L2 gene locus is on chromosome 9 (9q22.32) and spans 14 exons. It has two distinct domains: an N-terminal DEAH ATP-helicate domain, and a catalytic helicase C-terminal domain. ERCC6L2 belongs to the Snf2 family of helicase-like proteins, which are involved in chromatin unwinding, transcription regulation, and DNA recombination, translocation, and repair (Flaus et al., 2006). Most importantly, it was proposed to play a role in the DNA-damage response. Since a DNA-damage response is required during cell proliferation and tissue maintenance, ERCC6L2 deficiency would result in a slow accumulation of DNA damage. In response to genotoxic stress, ERCC6L2 genes translocate from the cytosol to both the mitochondria and nucleus (Tummala et al., 2014). Knockdown of ERCC6L2 genes in lung cancer cells showed an increase in DNA damage and intracellular ROS. Zhang and colleagues have identified a new protein, HEBO, which is formed from alternative splicing of ERCC6L2. HEBO is a DNA repair factor localized mainly in the nucleus that was recruited to sites of DNA lesions in an NBS1-dependent manner (Zhang et al., 2016). The authors identified a patient with an ERCC6L2 homozygous nonsense mutations (Case 3 in Table 1) that results in truncation of about half of the HEBO.

In summary, the data collected from cases of patients with biallelic mutations in ERCC6L2 results in a unique syndrome that includes bone marrow failure, cerebral and cerebellar abnormalities, as well as craniofacial abnormalities. The addition of new cases allows for opportunities to further study this bone marrow failure syndrome and continue defining its pathogenesis and phenotypic spectrum.

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CONFLICT OF INTERESTS

None declared.

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