Biallelic variants in *CSTB* cause a developmental and epileptic encephalopathy with dyskinesia

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Introduction

Biallelic *CSTB* (cystatin B) variants primarily cause Unverricht-Lundborg disease, a type of progressive myoclonic epilepsy. The vast majority of cases (90-99%) have two dodecamer repeat expansions in the promoter region, with the remainder caused by a single dodacamer repeat plus a single nucleotide variant (SNV) or indel. Compound heterozygotes have a more severe phenotype with earlier onset.[1,2] More recently, four patients in two families with homozygous *CSTB* loss of function variants were described with severe developmental delay, microcephaly, movement disorders, seizures, hypomyelination and cerebral atrophy.[3,4]

Methods

Individuals with severe neurodevelopmental phenotypes and biallelic CSTB indels or SNVs were identified either via collaborators or by searching for cases in large clinical exome databases. Clinical information was collected via a questionnaire. All research subjects provided written consent to participate. Probands were diagnosed via exome-based platforms with confirmation by Sanger sequencing at either CLIA-certified Baylor Genetics Laboratory or GeneDX. Family member analysis was completed as part of trio-based exome sequencing or via targeted sequencing.

Results

Eight individuals spread across six families were identified with biallelic *CSTB* indels or SNVs. Clinical features are summarized in Table 1. Their ages range from two to eight years of age. Three are deceased. All had severe developmental delay, with only one patient achieving independent sitting and feeding. Dystonia and epilepsy were seen in five individuals. Three individuals had medically refractory epilepsy. Chorea and myoclonus occurred in two individuals. Regression was observed in five individuals. MRI demonstrated delayed myelination and reduced brain volume (Table 1, Fig. 2). Progressive atrophy was seen when serial exams were performed (Fig. 2).

The majority of *CSTB* variants found in this cohort have been described previously. Two novel variants were identified, c.[10G>A] and c.[66+2>C]. These are detailed in Table 2 and Figure 1.

Table 1: Clinical Characteristics

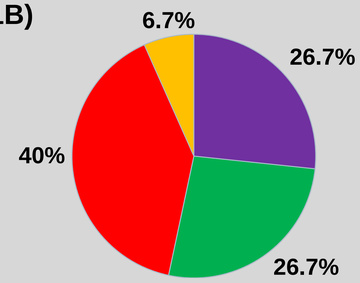
Individual	Age	DD	Microcephaly	Regression	Axial hypotonia	Appendicular hypertonia	Epilepsy	Dystonia	Chorea	Myoclonus	Delayed myelination	Reduced brain volume
Family 1, II-1	3 yo	+	+	+	+	+	+*	-	+	-	+	+
Family 2, II-1	2 yo	+	+	+	+	+	-	+	-	-	+	+
Family 3, II-1	Died at 23 mo	+	+	+	+	+	+	-	+	-	+	+
Family 3, II-3	Died at 5 yo	+	+	-	+	-	-	+	-	-	+	+
Family 3, II-4	7 yo	+	+	-	+	+	+	+	-	+	+	+
Family 4, II-1	Died at 8 yo	+	+	+	+	+	+*	+	-	+	+	+
Family 5, II-2	8 yo	+	+	+	+	+	+*	+	-	-	+	+
Family 6, II-1	2 yo	+	+	-	+	-	-	-	-	-	+	+

* Indicates medically refractory epilepsy.

Table 2 and Figure 1: Molecular Findings

Individual(s)	Nucleotide (Protein)	Variant Types
1:II-1	c.[67-1G>C];[202C>T] (p.R68*)	Splice, nonsense
2:II-2	c.[10G>A];[67-1G>C] (p.G4R)	Missense, splice
3:II-1,3,4	c.[66+2T>C];[202C>T] (p.R68*)	Splice, nonsense
4:II-1	c.[1_2insAT];[67_1G>C] (p.M1fs)	Indel, splice
5:II-2	Homozygous c.[67-1G>C]	Splice site
6:II-1	c.[1_2insAT];[202C>T] (p.M1fs, R68*)	Indel, nonsense

1B)



1A)

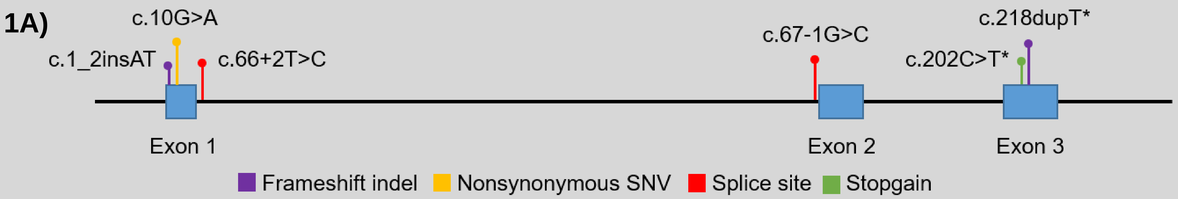


Fig. 1A depicts all *CSTB* variants linked to severe infantile onset *CSTB* disease to date. * indicates variants previously reported. Fig. 1B shows the relative proportion of variant types (frameshift indel - purple, nonsynonymous SNV - orange, splice site - red, stopgain - green) seen in the eight reported families with severe infantile onset *CSTB* disease.

References

1. Canafoglia, L., Gennaro, E., Capovilla, G. et al. (2012). Electroclinical presentation and genotype-phenotype relationships in patients with Unverricht-Lundborg disease carrying compound heterozygous CSTB point and indel mutations. *Epilepsia,53*(12), 2120-2127. doi:10.1111/j.1528-1167.2012.03718.x
2. Koskenkorva, P., Hyppönen, J., Äikiä, M., et al. (2011). Severer Phenotype in Unverricht-Lundborg Disease (EPM1) Patients Compound Heterozygous for the Dodecamer Repeat Expansion and the c.202CT Mutation in the *CSTB*Gene. *Neurodegenerative Diseases,8*(6), 515-522. doi:10.1159/000323470
3. Mancini, G. M., Schot, R., Wit, M. C., et al. (2016). CSTBnull mutation associated with microcephaly, early developmental delay, and severe dyskinesia. *Neurology,86*(9), 877-878. doi:10.1212/wnl.0000000000002422
4. O'brien, A., Marshall, C. R., Blaser, S., et al. (2017). Severe neurodegeneration, progressive cerebral volume loss and diffuse hypomyelination associated with a homozygous frameshift mutation in CSTB. *European Journal of Human Genetics,25*(6), 775-778. doi:10.1038/ejhg.2017.39

Figure 2: Typical imaging

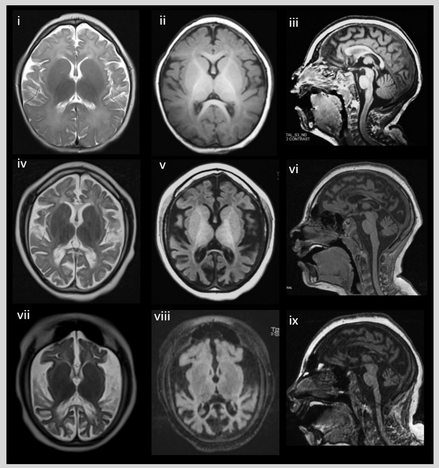


Figure 3 – Progressive atrophy in a patient (4:II-1) with severe infantile onset *CSTB* disease. Column 1, axial T2 imaging; column 2, axial T1 imaging; column 3, sagittal T1 imaging.

Table 3: Genotype-phenotype correlations

	Progressive myoclonic epilepsy		Developmental and epileptic encephalopathy with dyskinesia
Genotype	Homozygous dodecamer repeat	Compound heterozygous for dodecamer repeat and indel or SNV	Biallelic indel or SNV
Age of onset	6-16 years	5-10 years	Infantile
Seizure types	Tonic-clonic, myoclonic; induced by action and photic stimulation.	Tonic-clonic, myoclonic, myoclonic-atonic, absence	Tonic-clonic, tonic, myoclonic
Movement disorder	Non-epileptic myoclonus, tremor, ataxia	Non-epileptic myoclonus, ataxia	Dystonia, non-epileptic myoclonus, chorea, spasticity
Development /cognition	Normal to mild cognitive impairment	Mild to severe cognitive impairment	Severe developmental delay, regression
Other features	Mood disorders	Mood disorders, autistic traits	Microcephaly, dysphagia

Conclusions

Biallelic deleterious variants (frameshift, splice-site or missense variants affecting critical domains) in *CSTB* can cause a severe developmental and epileptic encephalopathy with dyskinesia distinct from Unverricht-Lundborg disease. This condition is characterized by severe developmental delay, microcephaly, dyskinetic movement disorder, epilepsy, hypotonia, hypomyelination and progressive cerebral atrophy.