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Supplemental Data

Mutations in *NALCN* Cause an Autosomal-Recessive Syndrome with Severe Hypotonia, Speech Impairment, and Cognitive Delay

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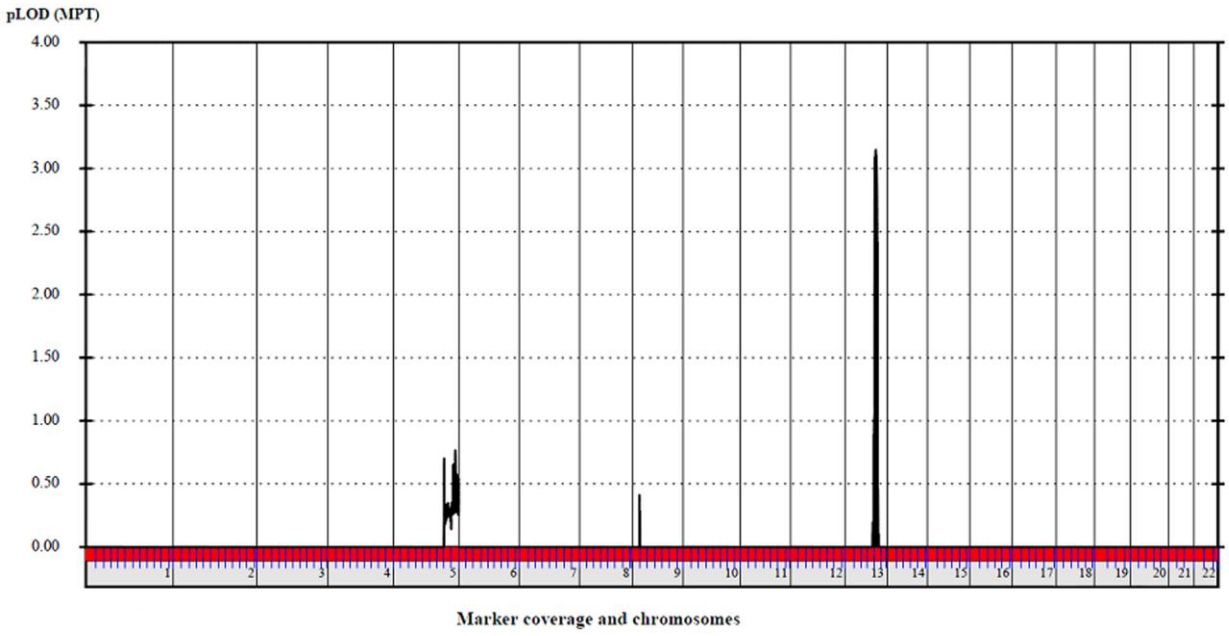

Figure S1. Linkage results for family 1.

Linkage analysis in the consanguineous Saudi family results in a peak of LOD score 3.1485 on chromosome 13. The analysis results were generated by GeneHunter/Easy Linkage software.

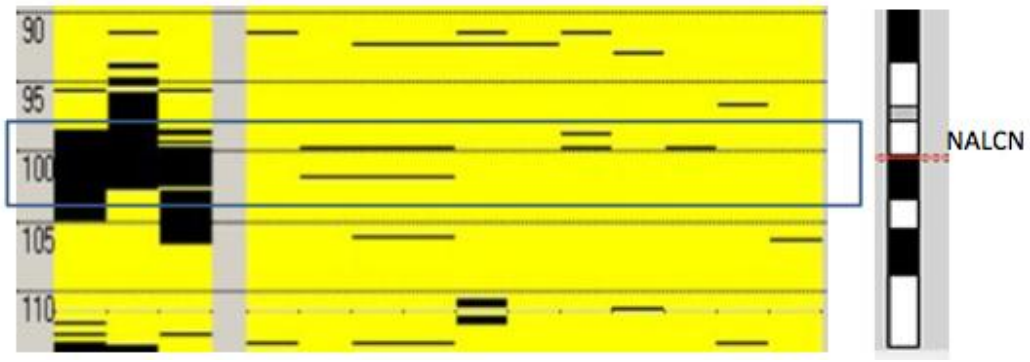

Figure S2. AutoSNPa results for family 1

DNA samples from the consanguineous Saudi family were extracted by standard procedures and genotyped on Affymetrix GeneChip Human Custom Mapping Axiom Arrays. Runs of homozygosity blocks from whole-genome DNA profiles were assembled using AutoSNPa software that disclosed a single shared homozygosity block on chromosome 13 (Left panel; affected individuals, IV-1, V-1, and V-2).

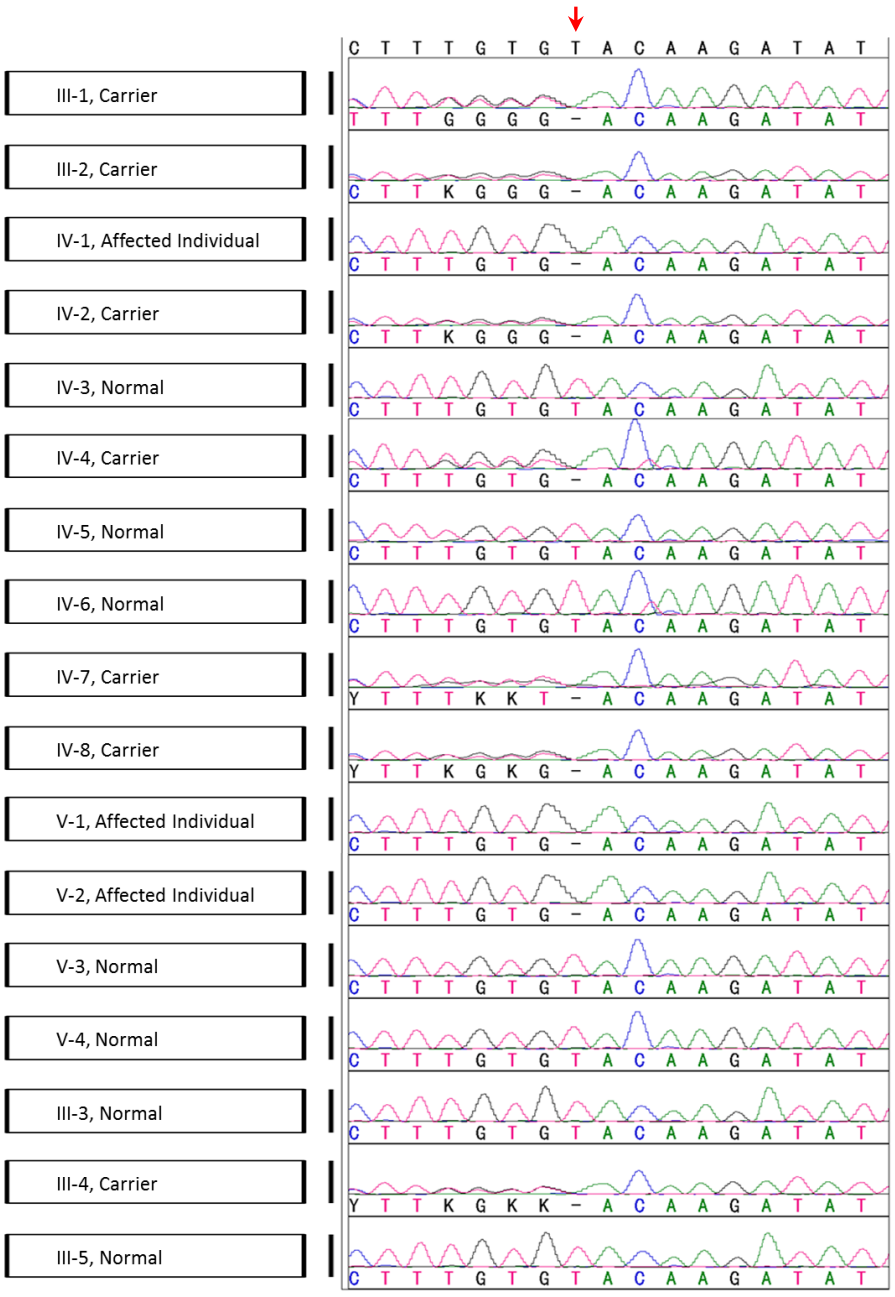

Figure S3. Chromatogram showing c1489delT; pTyr497Thr*fs21 in family 1**

Segregation analysis of c1489delT in the consanguineous Saudi family shows presence of the carriers, and affected and normal individuals in the family 1.

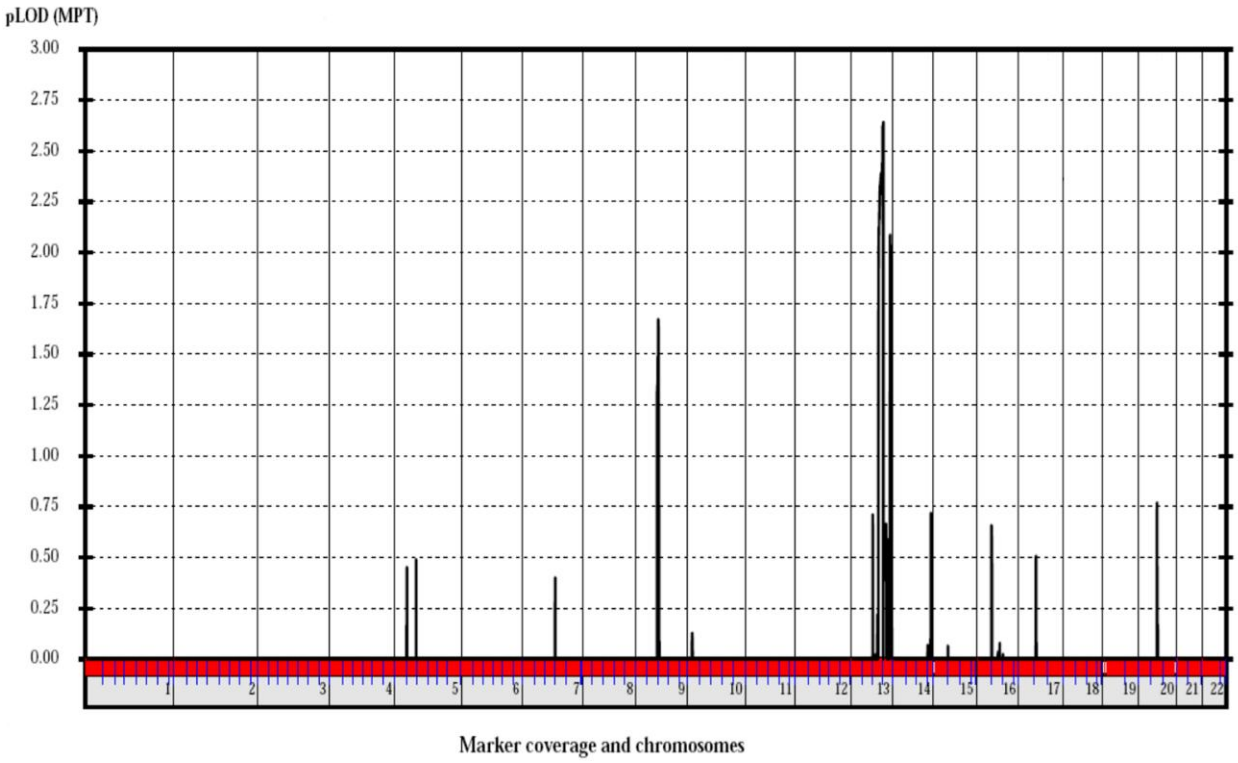

Figure S4. Linkage results for family 2

Multipoint parametric linkage analysis in the family 2 revealed a dominant linkage peak with a LOD score of 2.6409 on chromosome 13.

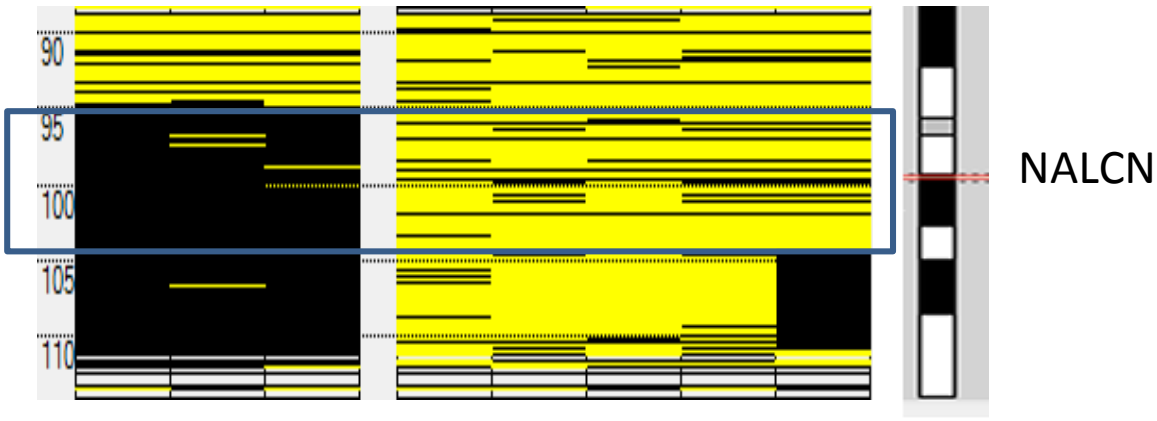

Figure S5. AutoSNPa Results for Family 2

Runs of homozygosity (ROH) blocks generated using genome-wide SNP genotyping calls. ROH profiles were aligned using AutoSNPa software. A single shared homozygosity block on chromosome 13 (Left panel; affected individuals in the family 2) was identified from the analysis of individuals II-1, II-2, and II-3.

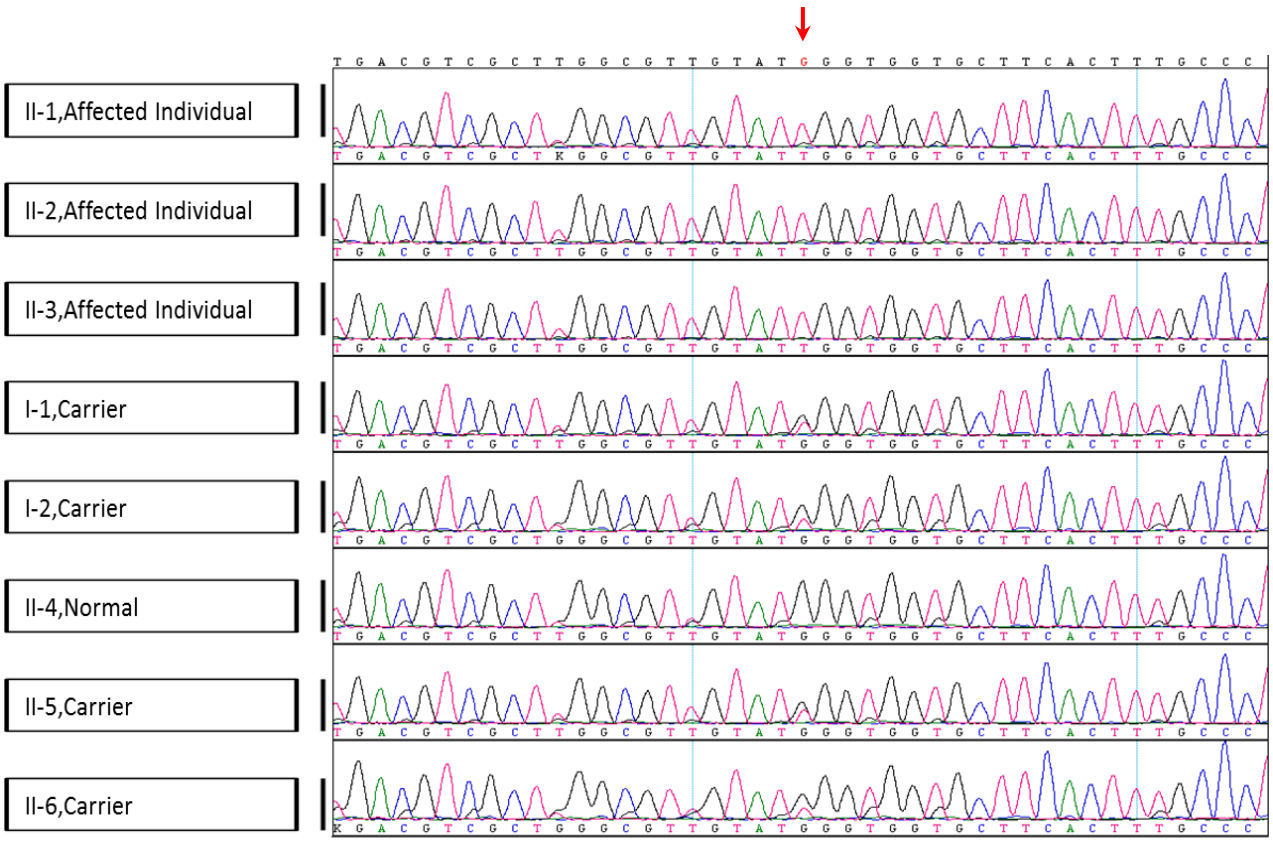

Figure S6. Chromatogram showing c.G3860T; pW1287L missense mutation in family 2.

Sequencing chromatogram from all the available individuals in the family indicated segregation of c.G3860T in *NALCN* in this consanguineous Saudi family.

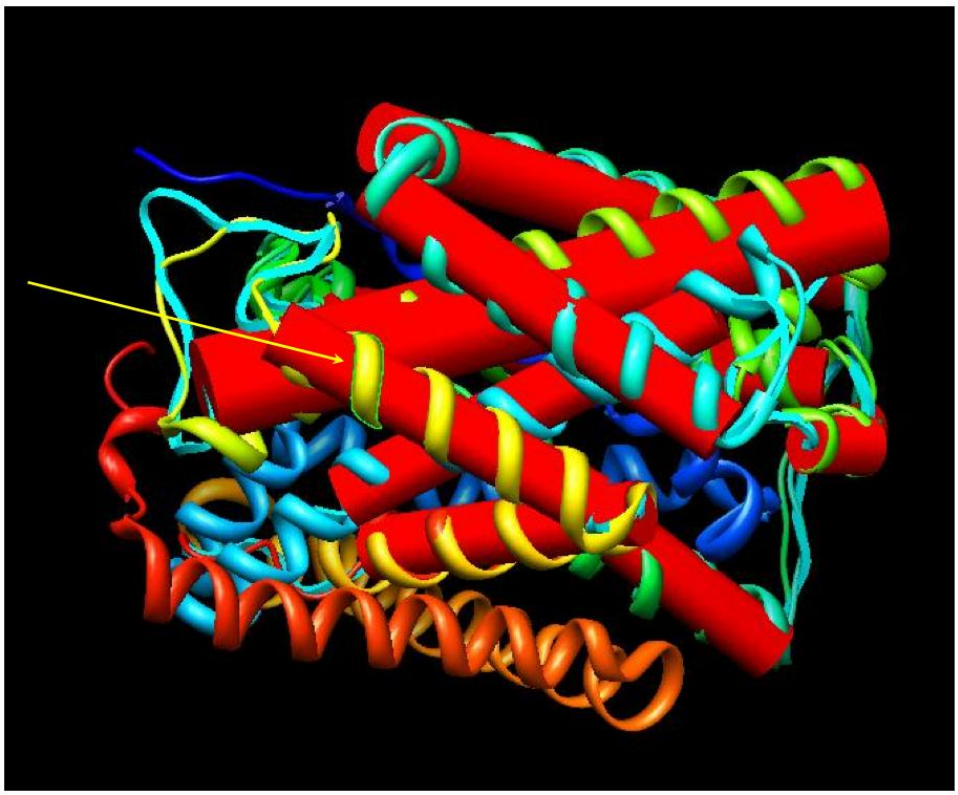

Figure S7. Location of p.W1287L on 3-D structure

The three-D structure image was generated based on UniProt ID:Q8IZF0 and location of p.W1287L of *NALCN* depicted on the structure using Chimera software.

Table S1. Detailed clinical and demographic information of the affected individuals with the *NALCN* defect

	FAMILY 1			FAMILY 2		
Individuals	**IV-1**	**V-1**	**V-2**	**II-1**	**II-2**	**II-3**
Age (years)	7 years, 3 months	7 years,1 month	4 years, 4 months	17 years, 6 months	16 years	9 years, 4months
Gender	Male	Male	Male	Female	Female	Female
Demographics/ Geographical Information	Central Region	Central Region	Central Region	North Region	North Region	North Region
Age at presentation	Birth	Birth	Birth	Birth	Birth	Birth
Birth & Neonatal History	Cesarean Section due to Fetal Distress Bw-2.6 Kg Meconium Stain Floppy Transient Thrombocytopenia Feeding difficulties-Poor sucking	Normal Delivery BW 4 Kg Floppy Feeding difficulties-Poor sucking	C-Section due to previous one. Bw-2.5 Kg Floppy Meconium Aspiration Suspected Hirsprung Disease Ileostomy at three days of age Feeding difficulties-Poor sucking	Normal Delivery BW 2.8 kg Floppy Feeding difficulties-Poor sucking	Normal Delivery BW 3 Kg Floppy Feeding difficulties-Poor sucking	C-Section due to previous C/S 2 weeks in the hospital Due to meconium aspiration BW 2.8 kg Floppy
Growth History	Normal	Initial Failure to Thrive followed by normal growth	Initial Failure to Thrive followed by Normal Growth	Normal	Normal	Normal
Current Growth Parameters (Weight, Height, Head Circumference)	Normal	Normal	Normal	Normal	Normal	Normal
Vision	Normal Bilateral Squint	Severe anisometropia	Normal	Normal	Normal	Normal Bilateral Squint
Hearing	Normal	Normal	Normal	Normal	Normal	Normal
Dysmorhic Featires	Broad forehead Triangular Face Brachycephaly, Large low set ears Prominent slender nose Smooth Philtrum Thin upper lips Fetal pads	Broad forehead Triangular Face Brachycephaly, Large low set ears Prominent slender nose Smooth Philtrum Thin upper lips Fetal pads	Broad forehead Triangular Face Brachycephaly, Large low set ears Prominent slender nose Smooth PhiltrumThin upper lips Fetal pads	Broad forehead Triangular Face Smooth Philtrum Prominent slender nose Thin upper lips	Broad forehead Triangular Face Smooth Philtrum Prominent slender nose Thin upper lips	Broad forehead Triangular Face Prominent slender nose Smooth Philtrum Thin upper lips

Hypotonia	Severe Since Birth	Severe Since Birth	Severe Since Birth	Mild-moderate and improved with age	Mild-moderate and improved with age	Mild-moderate and improved with age
Motor Milestones	Cannot Sit without support, stand or walk	Cannot Sit without support, stand or walk	Cannot Sit without support, stand or walk	Sat at 18 months/Walk at 3 and a half years.	Sat at one year/Walk at 3 yearrs	Sat at 10 months/Walk at 2 years
Speech	Babbles Only	Babbles Only	Babbles only	Single wordby 18 months /combined 2 words by 4yrs. Severe receptive and expressive language delay	Single word by 2 years/combined 2 words by 8yrs. Severe receptive and expressive language delay	Single word by 1 yr./combined 2 words 3 yrs.
Cognitive function	Severe Delay/IQ not assessable. Vineland Adaptive Behavior Scale performed at 6 years and 7 months, revealed communication skills at 1 month level, daily living skills at 6 months, social skills at 1 month and motor skills at 1 month	Severe Delay/IQ not assessable.	Severe Delay/IQ not assessable	IQ (45/100)	. IQ (42/100)	IQ(43/100)
Other Medical /Surgical Problems	Constipation Gastroesophageal reflux Recurrent aspirtaion Pneumonia Positional posterior plagiocephaly Bilateral orchidopexy Inguinal hernia repair Esotropia bilateral medial rectus muscle recession)	Constipation Gastroesophageal reflux Recurrent aspirtaion pneumonia Sepsis with Klepsiella Pnuemonae GT-Tube insertion and fundoplication	Constipation Positional posterior plagiocephaly bilateral orchidopexy	Constipation Seizure disorder (Onset 7yrs) Controlled with monotherapy Hyperactive	Constipation Seizure disorder (Onset 7yrs) Controlled with monotherapy Hyperactive Hyperpigmentation	Constipation Seizure disorder (Onsent 3 and a half years) Controlled with monotherapy Hyperpigmentation
Metabolic Screen	Acylcarnitines Normal/Organic acids Normal/Creatine Metabolism Panel	Acylcarnitines Normal/Organic acids Normal Normal Serum Lactic acid	Acylcarnitines Normal/Organic acids Normal/Creatine Metabolism Panel	Acylcarnitines Normal Organic acids Normal.	Acylcarnitines Normal Organic acids Normal. VLCFA Normal Serum Biotinidase Normal	Acylcarnitines Normal Organic acids Normal Creatine Metabolism Panel Normal Normal Serum Lactic

	Normal Normal Serum Lactic acid		Normal Normal Serum Lactic acid			acid. Isoelectric focusing for Transferrin Normal
Imaging	Normal Brain MRI	MRI brain (3 yrs. 9 months) revealed bilateral white matter abnormalities most prominent in the parietal regions in a fairly symmetric appearance. MRI brain (5 yrs 3 months) revealed interval progression of the parietal periventricular and frontal corona radiata and centrum semiovale increased signal intensity. PET-CT brain done (5 yrs 9 months) revealed diffuse hypo metabolism involving bilateral parieto-occipital regions, bilateral thalami and cerebellum	Small bony posterior fossa with fullness and crowding in the posterior fossa. Otherwise Normal 3 D-CT skull evaluation revealed brachycephaly but no craniosynostosis Skeletal survey normal	Normal Brain MRI Prominent temporal horn of the left lateral ventricle with relatively smaller-sized left hippocampus	Normal Brain MRI	Normal Brain MRI
Karyotype	Normal Male	Normal Male	Normal Male	Normal Female	--	Normal Female
Molecular Karyotype	Malignant CNVs were not observed in the linkage interval	Malignant CNVs were not observed in the linkage interval	Malignant CNVs were not observed in the linkage interval	Malignant CNVs were not observed in the linkage interval	Malignant CNVs were not observed in the linkage interval	Malignant CNVs were not observed in the linkage interval
Other Studies	Muscle biopsy microscopic examination, histochemical staining and electron microscopy were negative with no evidence of pathology. MECP2 Gene sequencing Negative		EEG, BSAEP and VEPs normal.	EEG- generalized intermittent slow activity with slow background activity.	EEG- Nonspecific intermittent irregularities over both temporal r egions. No Epileptiform discharges	EEG-No Epileptiforn Discharges