Infantile Cerebral and Cerebellar Atrophy Is Associated with a Mutation in the MED17 Subunit of the Transcription Preinitiation Mediator Complex

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Primary microcephaly of postnatal onset is a feature of many neurological disorders, mostly associated with mental retardation, seizures, and spasticity, and it typically carries a grave prognosis. Five infants from four unrelated families of Caucasus Jewish origin presented soon after birth with spasticity, epilepsy, and profound psychomotor retardation. Head circumference percentiles declined, and brain MRI disclosed marked cereberal and cerebellar atrophy with severe myelination defect. A search for a common homozygous region revealed a 2.28 Mb genomic segment on chromosome 11 that encompassed 16 protein-coding genes. A missense mutation in one of them, *MED17*, segregated with the disease state in the families and was carried by four of 79 anonymous Caucasus Jews. A corresponding mutation in the homologous *S.cerevisiae* gene *SRB4* inactivated the protein, according to complementation assays. Screening of *MED17* in additional patients with similar clinical and radiologic findings revealed four more patients, all homozygous for the p.L371P mutation and all originating from Caucasus Jewish families. We conclude that the p. L371P mutation in MED17 is a founder mutation in the Caucasus Jewish community and that homozygosity for this mutation is associated with infantile cerebral and cerebellar atrophy with poor myelination.

Primary microcephaly of prenatal onset is defined by head circumference that is more than three standard deviations (SD) below the age- and sex-adjusted mean and is typically associated with mental retardation. Abnormal tone and seizures are common, but usually there are no additional malformations. Microcephaly of prenatal onset has been linked to disruption of genes that play a role in cell division, chromosome segregation, and centrosome function (reviewed in [1]). Microcephaly of postnatal onset is characterized by postnatal deceleration of head growth, and its differential diagnosis is wide, including acquired insults as well as a long list of neurodevelopmental, neurodegenerative, and neurometabolic disorders. Significant involvement of the cerebellum and brain stem in these conditions is uncommon.

The subjects of this study were five patients, four males and one female from four unrelated Caucasus Jewish families; consanguinity was known in only one of the couples. The pregnancies were uneventful, and the infants were born at term with normal Apgar scores and appropriate weight for gestational age. Of note, normal brain structure and age-appropriate head circumference was noted by ultrasound performed at 22 gestational wks in one patient and at birth in another; head circumferences were within the normal range (32–34 cm) in all of the patients at birth. However, at 4–9 wks of age, swallowing difficulties leading to failure to thrive, jitteriness, poor visual fixation and lack of tracking, truncal arching, and overt seizures became evident. Shown on physical examination were microcephaly, increased muscle tone, clonus, and exaggerated deep tendon reflexes. The patients were not dysmorphic, and there was no indication of other organ involvement. In the ensuing months, there was no acquisition of developmental milestones; the patients suffered from marked spasticity, profound retardation, and occasional seizures; and progressive microcephaly became evident with a head circumference of −6 SD at 9 mo of age. At the time of the report all five patients were alive, aged 5 mo to 15 yrs. EEG disclosed dysmature background with multifocal spike and wave activity at 2–4 mo, hypsarrhythmia at 6–8 mo, and a diffuse slowing of background with bilateral slow, sharp frontotemporal activity at an older age. Brain MRI revealed severe diffuse cerebral and cerebellar atrophy already evident at 3 mo of age (Figure 1). Subsequent imaging scans were striking for poor myelination, cerebral and cerebellar atrophy, small thalami, and a thin brainstem.

Laboratory investigation was normal, including blood count, electrolytes, creatine kinase, thyroid, renal and liver functions, blood gases, lactate, ammonia, amino acids,

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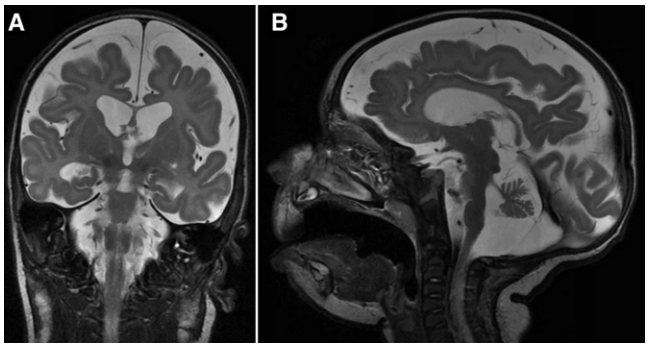
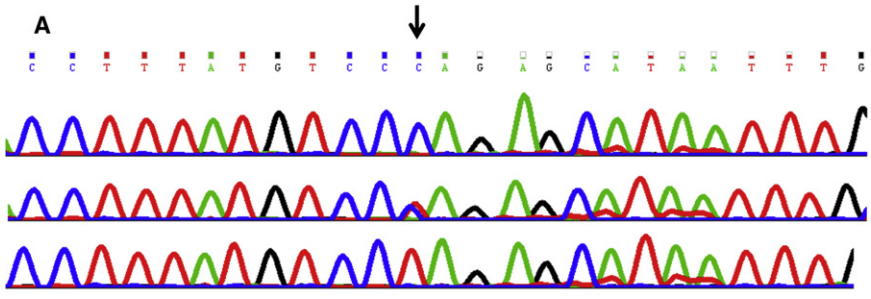


Figure 1. Brain MRI at 3 Months of Age
Coronal (A) and midsagittal (B) T2-weighted magnetic resonance images illustrating pronounced cerebral and cerebellar atrophy.

isoelectrofocusing of transferrins, very long chain fatty acids, biotinidase activity and carnitine levels in plasma, and organic acids in urine. Muscle histochemistry and the activity of the five enzymatic complexes of the mitochondrial respiratory chain were normal.

In order to localize the mutated gene, we performed homozygosity mapping, using the GeneChip Human Mapping 250K Nsp Array of Affymetrix in the samples of three patients, as previously described.[2] Informed consent was granted by the parents, and the study was approved by the Hadassah and Sheba ethical review committees. A single homozygous region, spanning 2.28 Mb on chromosome 11 (SNP markers rs12363947–rs333027, corresponding to 91.94–94.22 Mb) (positions according to the March 2006 release of the human genome assembly, hg18), was shared by the three patients, and the genotype of the 254 SNP markers that are included in this region was identical. Notably, the three patients did not share any other genomic region. The common genomic segment encompassed 16 protein-coding open reading frames. These genes were prioritized according to their expression and function (GeneDistiller),[3] and the sequence of the coding exons and the flanking intronic regions of *MRE11A* (MIM 600814), *PANX1* (MIM 608420), *MED17* (MIM 603810) and *TAF1D* (MIM 612823) was determined. No mutation was identified in *MRE11A*, *PANX1*, or *TAF1D*; however, in *MED17*, which spans 12 exons encoding 651 amino acids, a single mutation was detected in exon 7, c.1112 T>C (Figure 2) (GI: 18089269), which is predicted to result in the substitution of leucine at codon 371 by proline (p.L371P). The five patients were homozygous, and seven parents and three of five available siblings were heterozygous for the mutation. We then screened *MED17* in additional patients with similar clinical and radiologic findings and identified four more patients, all homozygous for the p.L371P mutation and all originating from Caucasus Jewish families. A maximum LOD score of ~2.4 was obtained for the p.L371P mutation with zero recombination via the SuperLink program for the families of the nine Caucasus Jewish patients. In order to estimate the prevalence of the mutation among Jews of Caucasus origin, we determined the sequence of exon 7 of *MED17* in the DNA samples of 79 anonymous Caucasus and Bukharin Jews who requested premarital or prenatal genetic screening. Four of the 79 anonymous individuals carried the mutation; no carriers were detected among 110 anonymous Ashkenazi Jews and 113 anonymous individuals of Arab Moslem origin. On the basis of the identical SNP haplotype in the three unrelated patients who were subjected to the 250K SNP analysis, the presence of homozygosity for the mutation in nine patients of the same ethnic origin, and the considerable prevalence of a carrier state among healthy individuals of this ethnicity, we believe that p.L371P is a founder mutation in the Caucasus Jewish community.

The transcription of protein-coding genes is initiated by the binding of a ~2 MDa preinitiation complex to the promoter DNA. This complex consists of transcription factors, the Mediator complex, and RNA polymerase II. The Mediator complex, itself assembled of ~30 subunits, is regarded as a bridge to convey information from gene-specific regulatory proteins to the basal RNA polymerase II transcription machinery (reviewed in [4]). MED17 is a central component of Mediator, required for the structure



Human	D	H	L	Y	V	L	E	H	N	L	H	L	L
Danio Rerio	D	H	L	Y	V	L	E	H	N	L	H	Q	L
D. Melanogaster (TRAP80)	D	H	D	H	V	L	E	H	S	L	H	Q	L
S. Cereviciae (SRB4)	K	R	A	N	L	M	L	V	M	L	R	L	L

Figure 2. The c.1112T>C (p.L371P) Mutation in *MED17*
(A) Exon 7 of *MED17*. The mutated nucleotide is indicated by an arrow. The patient, an obligate heterozygote, and a control sample are shown in the upper, middle, and lower lanes, respectively.
(B) Amino acid conservation at and around the mutated codon (asterisk) site.

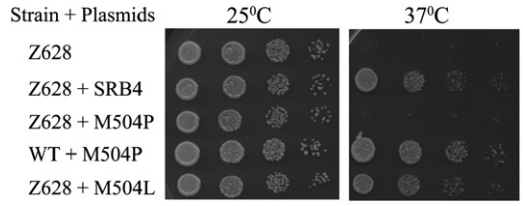


Figure 3. SRB4-M504P, Corresponding to Human MED17-L371P, Cannot Complement the Depletion of Active SRB4 in Yeast

The wild-type (Z579) and a mutant expressing temperature-sensitive ts-SRB4 (Z628) were transformed with the indicated plasmids. Cultures were serially diluted at 10-fold intervals, and 10 μl of each was spotted onto YPD plates, which were incubated at 25°C (permissive temperature, left panel) or 37°C (restrictive temperature, right panel) for 2 days.

and integrity of the complex; the elimination of its homolog in yeast (SRB4) and *Drosophila* (TRAP80) has resulted in reduction of transcriptional activation and lethality of the mutants.[5,6] On the basis of the functional homology and the relatively high conservation around the mutation site, we used a yeast SRB4 conditional depletion mutant to examine the potential pathogenicity of the p.L371P mutation. To this end, we mutated Met504 (which corresponds to Leu371 in the human MED17 subunit) of the yeast SRB4 to proline (M504P). The experimental setup was based on the yeast strain Z628 (ts-SRB4), which expresses a temperature-sensitive mutant of SRB4, thus enabling the strain to grow at 25°C but not at 37°C.[5] Transformations were conducted with yeast SRB4, rather than human MED17, because of its overall low amino acid sequence conservation. Transformation of the ts-SRB4 strain with a plasmid encoding the wild-type SRB4 fully complemented the ts-SRB4 activity (Figure 3). In contrast, the mutant of interest, SRB4-M504P, did not complement the ts-SRB4 activity, indicating that it was functionally inactive. Notably, the expression of SRB4-M504P in a wild-type background did not affect growth, indicating that M504P is a recessive mutation. As a control, we also mutated methionine 504 of SRB4 to leucine, the human residue at this position (M504L); as expected, this mutant fully complemented the ts-SRB4 activity (Figure 3). Together, these results underscore the importance of the 504 residue (codon 371 in human) and the unfavorable functional consequences of its replacement by proline.

Until now, defects in two Mediator subunits have been associated with disease in human. Charcot-Marie-Tooth disease type 2B2 (MIM 605589), an axonal neuropathy transmitted in an autosomal-recessive manner, is caused by a missense mutation in *MED25* (MIM 610197),[7] and two x-linked dysmorphic mental retardation syndromes, Opitz-Kaveggia (FG) syndrome (MIM 305450) and Fryns-Lujan syndrome (MIM 309520), were associated with mutations in *MED12* (MIM 300188).[8,9] The organ-specific involvement in defects of each of the three subunits, MED12, MED17, and MED25, could probably be explained by a recent observation in rodent fibroblasts, where MED17 was shown to be essential for the expression of only a minority of the genes. The recruitment of RNA polymerase II to gene promoters and the initiation of transcription were maintained even when the level of MED17 was nearly abolished.[10] In view of the seemingly normal prenatal development and the prominent white matter involvement postnatally, it is tempting to speculate that the *MED17* mutation interferes with the expression of the gene(s) involved with oligodendrocyte development, a process that in human commences only after birth.

The Jewish community in the Caucasus region is believed to originate from today's southern Iran. The Caucasus Jews were genetically isolated for more than 2500 yrs by their language and religious practice. Most members of this community immigrated to Israel in 1970–1990. Still, marriage within the community is preferred, currently approaching 45% (J. Zlotogora, personal communication). The fact that three of the four families denied consanguinity and the relatively small size of the common homozygous genomic region attest to the ancient nature of the causative mutation. Although the number of anonymous controls is small, our results suggest that a carrier state for the *MED17* p.L371P mutation is of considerable prevalence within this ethnic group. Screening for the mutation is warranted among Caucasus Jewish couples, which would enable a better estimation of the carrier rate and identify couples at risk.

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Web Resources

The URLs for data presented herein are as follows:

GeneDistiller, http://www.genedistiller.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
SUPERLINK, http://bioinfo.cs.technion.ac.il/superlink-online/

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