Supplementary Information

Supplementary Note - Clinical Data

Family LIS-900 was evaluated by one of the authors (LFS) and includes 2 affected daughters, an affected son and an unaffected daughter of consanguineous parents of Mexican ancestry. The clinical information was initially presented as an abstract in 2000. The eldest child, LIS-902, presented as a twelve year old girl with severe developmental delay who could not walk or talk. She had suffered a difficult delivery with use of forceps. Birth weight was 2.75kg (10^{th} percentile) and length was 50 cm (58^{th} percentile) and head circumference was not available. Generalized tonic clonic seizures began at nine months of age and were controlled by valproic acid. Her development was extremely delayed with head control not noted until two years of age and she has never sat, crawled or developed speech. Her younger brother, LIS-901, was seven years old at the time of evaluation. He was the product of a normal pregnancy although he was delivered by Caesarean section for failure to progress. Birth measurements included a head circumference of 32 cm (4^{th} percentile), weight of 2.98kg (18^{th} percentile) and length of 51cm (62^{nd} percentile). He walked independently by age 3-4 y and at the age of seven his speech was limited to several words. The youngest child (LIS-903) was born prematurely at 34 wks gestation following premature rupture of membranes and repeat Caesarean section. Birth weight was 1.7kg (-3.2SD) and head circumference was 26.5 cm. At 10 months (~8months adjusted) her head circumference was 37cm (-5.4SD). Her early psychomotor development was relatively normal but there were later delays. By

age 3 she was walking but had no speech. Neurologic exam revealed generalized hypertonia and hyperreflexia. Patients LIS-901 and LIS-903 did not have seizures. All three patients had a normal karyotype. All three children showed a low sloping forehead and mild dysmorphic features.

Patient LIS-903's brain showed microcephaly with diffuse simplification of the gyral pattern (oligogyria) without ventricular enlargement (Fig. 2b and 2g). The cortex was thin, but tended to be thicker in posterior frontal and parietal cortex, where there was a suggestion of subcortical heterotopia raising the possibility of abnormal neuronal migration. The corpus callosum was thin, whereas the cerebellum and brainstem were relatively preserved. Patient LIS-902's brain MRI showed microcephaly and gyral simplification accompanied by additional abnormalities (enlarged lateral ventricles and relatively smaller right hemisphere, not shown) although her clinical history raised the suspicion for superimposed anoxic birth injury. MRI imaging was not available for LIS-901, but head CT showed microcephaly and simplified gyral pattern.

Pedigree LIS-2600 includes first-cousin parents and two affected offspring: an affected male child (LIS-2602) and a pregnancy that was terminated at 27 week with prenatal microcephaly (LIS-2601), and was previously described in detail (Sergi et al, Prenatal Diagnosis 2000; 20:505-509). The first child, LIS-2602, was the product of a pregnancy that was normal until the 25th week, when ultrasound showed retardation of head growth and microcephaly. Delivery was spontaneous at term, with Apgar scores of 9-10-10, birth weight of 2,960 g (25th percentile), crown-heel-length (CHL) of 49 cm (25th percentile)

but head circumference of only 30 cm (<<3rd percentile). There was microcephaly with a low, sloping forehead, prominent occiput, broad and prominent nasal bridge, widely set eyes and small fontanels. Development was severely delayed: at 12 months he could grasp objects and sit, and at 18 months he could move by himself by sliding but not crawl or pull up to stand. Verbal expression was limited. At age of 2 3/4 years, head circumference was 42 cm <<3rd percentile), body weight was 13 kg (25th percentile), and body length was 90 cm (3rd – 10th percentile). During a second pregnancy, prenatal ultrasound revealed microcephaly and abnormal gyral pattern in the fetus, and the pregnancy was terminated at 27 weeks. Pathological analysis of the aborted fetus confirmed microcephaly with abnormal gyral development.

MRI of the brain for LIS-2602 demonstrated microcephaly, with polymicrogyria and volume loss worse on the left than the right, in the left hemisphere, and simplified gyral pattern on the right. The corpus callosum had an incomplete genu and small splenium. The cerebellum and brainstem appeared relatively preserved.

Pedigree LIS-2500 includes one female affected offspring (LIS-2501) and an older healthy sister of healthy first cousin parents from Turkey. LIS-2501 was evaluated clinically by MT and came to medical attention for concerns of microcephaly. She was last evaluated at 7 months of age. History includes delivery at term with birth weight of 3.5kg (50th percentile), length of 51cm (75th percentile) and head circumference of 31cm (-2.6SD) with normal newborn reflexes. At approximately age 2 months, head circumference was 33.5cm (-4.0SD) and at age 5 months the head circumference was

36.5cm (-5.3SD) with weight of 6.5kg (25-50th percentile)and height of 63cm (~50th percentile). A high arched palate was noted. No clinical seizures were reported by 7 months of age but an EEG showed left central-parietal sharp waves. MRI evaluation showed that the cerebellum and brainstem were large in proportion to a microcephalic cerebrum with markedly diminished sulcation. Imaging was not optimal but there was evidence of bilateral band heterotopia in the posterior frontal and parietal lobes and a thin, hypogenetic corpus callosum. Family history includes a female maternal first cousin with seizures and paternal grandmother with a small head (no head measurements or other clinical details available).

Pedigree MC-1600 includes an affected male child (MC-1601), the offspring of healthy first cousin parents also from Turkey. The child was evaluated by one of the authors (MT) at 4 years of age. His birth weight was 3,300 g (25-50th percentile). He first came to medical attention in infancy by 6 months old following a febrile illness when microcephaly and developmental delay were noted. He developed generalized tonic clonic seizures at 1.5 years old, treated by valproic acid and carbamazepine. He sat at 9 months, walked by age 1.5 years and had intelligible single words by age 3. Physical findings included a 3 x 4 cm hypopigmented macule on the right lumber area and 3-4 additional hypopigmented spots on the upper extremities. The MRI showed a cerebellum and brainstem slightly large compared to the cerebrum and corpus callosum that was complete but abnormally thin, especially at the splenium. The immediate family history includes 2 unaffected older sisters.

An additional Turkish pedigree is MC-1400 which includes an affected female only child (MC-1403) with healthy first cousin parents. She was seen by MT at about one year of age following a prenatal diagnosis of microcephaly noted at 7 months gestation. At age one year her head circumference was 34.5cm (-8.5SD) and no seizures or dysmorphic features were noted. While early milestones were on target, motor delays were noted. MRI for this individual showed roughly proportionate cerebellum, brainstem and cerebrum. There was evidence of band heterotopia in the posterior frontal and parietal lobes and a complete but very thin corpus callosum.

The family PH-16900 includes multiple children clinically evaluated by one of the authors (MM). The parents are from Saudi Arabia and are paternal first cousins (the fathers are brothers) as well as maternal first cousins once removed. The eldest affected individual (PH-16901) was 15y 3m at evaluation. Prenatal history included pregnancy complicated by maternal flu-like illness with fever at four months gestation and again at 7-8 months gestation. The term birth was spontaneous and vaginal. There was some concern in the newborn period for delays but no regressions were noted. He walked at 21 months and parents reported he occasionally spoke in sentences. This individual had surgery for an inguinal hernia and was hospitalized periodically until age 9 for headaches with vomiting. Measurements at the visit included height of 159 cm (10^{th} percentile), weight of 50.4 kg (21^{st} percentile) and head circumference of 47cm (-5.3SD). He had constant drooling, normal facial, tongue and eye movements and normal muscle bulk, tone and deep tendon reflexes with no abnormal movements. Examination of extremities revealed reduced extension of both elbows, bilateral hallux valgus and pes planus and the

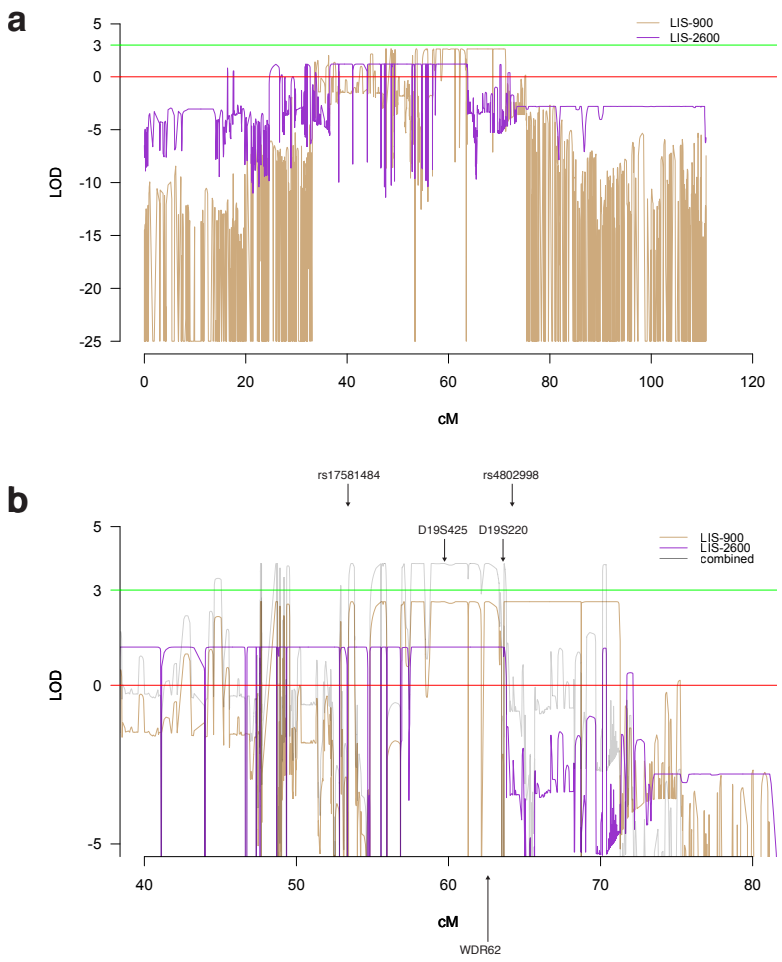
second toe overlapped the first toe bilaterally. The MRI for this male individual showed a disproportionately large cerebellum and brain stem with a simplified gyral pattern for the cortex and a largely normal corpus callosum. Two younger siblings, a female (PH-16902) and male (PH-16903) had more severe clinical presentations and MRI imaging findings. The younger sister (PH-16902) was age 8 years 6 month at evaluation. Poor fetal movements and growth were noted by 7 months gestation. Birth was post-term, induced and described as difficult. The neonatal period was complicated by poor feeding and a patchy skin infection. She was on asthma medication for episodes of shortness of breath. At evaluation measurements included height of 114cm (-2.8SD), weight of 16.9kg (-2.9SD) and head circumference of 40cm (-9.8SD). The child was unable to sit, and had no words. Neurological exam revealed spastic quadriparesis with increased tone and decreased movements in all four extremities. No digital anomalies were noted. Like PH-16901, MRI showed a disproportionately large cerebellum and brainstem when compared to the cerebrum as well as diffuse polymicrogyria with a narrow right temporo-parietal open lip schizencephaly. The corpus callosum was dysmorphic with a thick body and a small genu. The affected younger brother (PH-16903) was age 6.5 years at evaluation. Pre- and perinatal history included prenatal microcephaly detected on ultrasound but an otherwise uncomplicated pregnancy with normal movements and a term, spontaneous vaginal birth. Like his sister he occasionally had shortness of breath. His measurements were height of 98cm (-4.0SD), weight of 12.2kg (-4.0SD) and head circumference of 39.5cm (-9.2SD). He had somewhat better development than his older sister as he could babble, roll and tear papers. He also had spastic quadriparesis. Digits and extremities were normal and he had a hyperpigmented

skin lesion on the center of his chest extending up over the right shoulder. This individual's MRI revealed a disproportionately large brain stem and cerebellum, widespread polymicrogyria, and right temporo-parietal open lip schizencephaly. Another female in the family (PH-16907) was 12 years and 3 months at evaluation by MM. Although her MRI and health were largely normal, there were parental concerns centered around speech acquisition and articulation, difficulty with attention and extreme shyness. Her mother had a flu-like illness at about 4 months gestation but the pregnancy was normal with a vaginal, spontaneous term birth. She had febrile seizures in childhood that stopped by age 7 and she was otherwise generally healthy with occasional headaches. Her measurements were height of 145.3cm (16^{th} percentile); weight of 37.5kg (21^{st} percentile) and head circumference of 54 cm (76^{th} percentile). Her skin findings included a linear area of hyperpigmentation from the right shoulder to the right elbow. The speech evaluation done around the same time of the genetics evaluation confirmed difficulties with motor planning for speech production with a recommendation for speech therapy. Affected family members underwent extensive testing that failed to detect underlying causes for their condition. Normal screening included karyotype, FISH for Williams syndrome, Velocardiofacial Syndrome, and Miller Dieker, subtelomere probes, urine organic acids, peroxisomal diseases, congenital disorders of glycosylation, and serum amino acids.

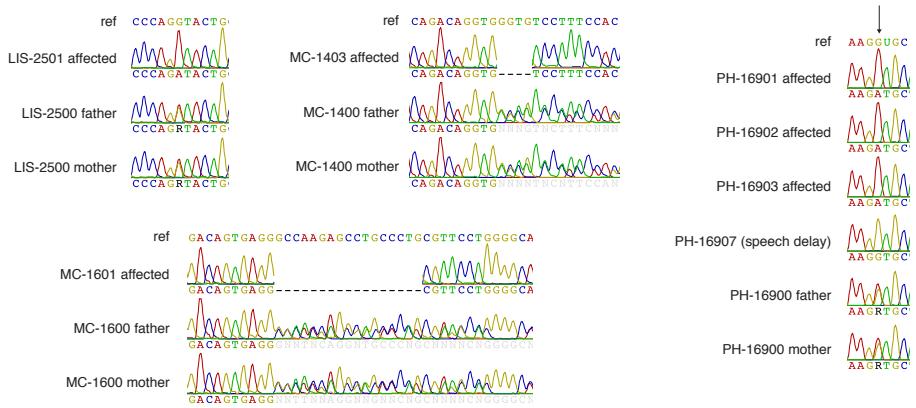
Supplementary Movie 1. Microcephaly, simplified gyri, polymicrogyria, and schizencephaly in a patient with a WDR62 mutation.

T1-weighted MRI sequence of a patient (PH-16903) with a homozygous V65M mutation, demonstrating microcephaly, simplified gyri, relatively preserved brain stem and cerebellum, widespread polymicrogyria, and right temporo-parietal open lip schizencephaly.

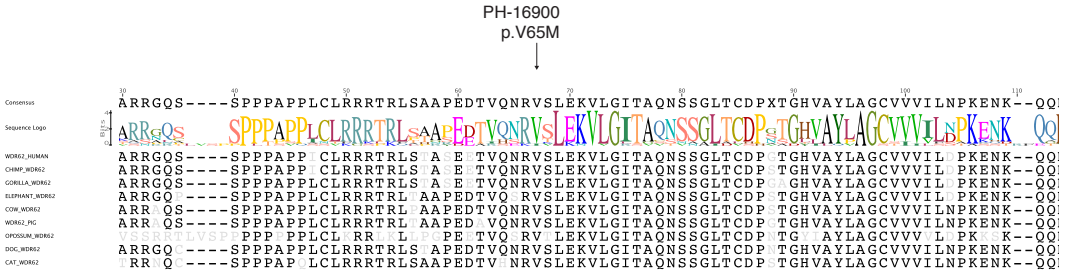
Supplementary Figure 1. Homozygosity mapping in LIS-900. Plotted in red are all homozygous blocks > 1 cM in a single affected child (top), and common to all three affected children (bottom), from the LIS-900 family. After exclusion of artifactual IBD spanning centromeres, the largest candidate interval shared by all affected members of LIS-900 (cyan) corresponds to the 19q13 locus.



Supplementary Figure 2. Multipoint LOD scores for LIS-900 and LIS-2600 on chromosome 19. (a) Linkage analysis was performed on Affy 5.0 SNP data for LIS-900 and LIS-2600. A combined maximum multipoint LOD score of 3.84 was obtained at 19q13. (b) Detail of linkage peak on chr19q13, showing LOD peak in relation to *WDR62*, a 7.5 Mb candidate interval (rs17581484 to rs4802998) implicated by homozygosity mapping in LIS-900 and LIS-2600, and a smaller candidate subregion (D19S425 to D19S220) suggested by homozygosity analysis using microsatellite markers in LIS-900, LIS-2600, PH-16900, MC-1400, and MC-1600 (data not shown). The larger interval was chosen for exonic capture and high throughput sequencing.



Supplementary Figure 3. *WDR62* mutations in four additional families with microcephaly, simplified gyri, and diverse cortical malformations. Sanger sequencing traces demonstrate that patients from families LIS-2500, MC-1400, MC-1600, and PH-16900 bear homozygous mutations in *WDR62* and confirm segregation with disease within the family. In the case of PH-16900, all three children affected with microcephaly and developmental delay were homozygous for the c.193G>A change, and parents are heterozygous. A fourth child, PH-16907, suffered from mild speech delay and articulation issues but did not have microcephaly, and was homozygous for the wildtype allele.



Supplementary Figure 4. Sequence conservation of the V65 residue in WDR62 orthologs. Alignment of vertebrate WDR62 homologues demonstrates complete conservation of the V65 residue affected in PH-16900, arguing for pathogenicity of this change.

Supplementary Table 1. Microcephaly Collaborative Members

Member	Institution
Abdulsalam Abu-Libdeh	Makassed Islamic Charitable Hospital, Jerusalem, Israel
Dianne Abuelo	Rhode Island Hospital, USA
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Lihadh Al-Gazali	United Arab Emirates University, United Arab Emirates
Isphana Al Khatib	Al Noor Training Centre for Children with Special Needs, United Arab Emirates
Kerry Baldwin Jedele	Gundersen Lutheran Medical Center, USA
Lina Basel-Vanagaite	Rabin Medical Center, Israel
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Member	Institution
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Ingrid Scheffer	Austin Hospital, Australia
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Ruthie Shenhav	Rabin Medical Center, Israel
Elliott Sherr	University of California at San Francisco, USA
Janet Soul	Children's Hospital Boston, USA
Joan Stoler	Children's Hospital Boston, USA
Rachel Straussberg	Tel Aviv University, Israel
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