REPORT

RTTN Mutations Cause Primary Microcephaly and Primordial Dwarfism in Humans

Hanan Shamseldin,[1,10] Anas M. Alazami,[1,10] Melanie Manning,[2,3,10] Amal Hashem,[4] Oana Caluseiu,[5] Brahim Tabarki,[4] Edward Esplin,[3] Susan Schelley,[3] A. Micheil Innes,[6,7] Jillian S. Parboosingh,[6] Ryan Lamont,[6,7] Care4Rare Canada Consortium, Jacek Majewski,[8] Francois P. Bernier,[6,7] and Fowzan S. Alkuraya[1,9,*]

Primary microcephaly is a developmental brain anomaly that results from defective proliferation of neuroprogenitors in the germinal periventricular zone. More than a dozen genes are known to be mutated in autosomal-recessive primary microcephaly in isolation or in association with a more generalized growth deficiency (microcephalic primordial dwarfism), but the genetic heterogeneity is probably more extensive. In a research protocol involving autozygome mapping and exome sequencing, we recruited a multiplex consanguineous family who is affected by severe microcephalic primordial dwarfism and tested negative on clinical exome sequencing. Two candidate autozygous intervals were identified, and the second round of exome sequencing revealed a single intronic variant therein (c.2885+8A>G [p.Ser963*] in *RTTN* exon 23). RT-PCR confirmed that this change creates a cryptic splice donor and thus causes retention of the intervening 7 bp of the intron and leads to premature truncation. On the basis of this finding, we reanalyzed the exome file of a second consanguineous family affected by a similar phenotype and identified another homozygous change in *RTTN* as the likely causal mutation. Combined linkage analysis of the two families confirmed that *RTTN* maps to the only significant linkage peak. Finally, through international collaboration, a Canadian multiplex family affected by microcephalic primordial dwarfism and biallelic mutation of *RTTN* was identified. Our results expand the phenotype of *RTTN*-related disorders, hitherto limited to polymicrogyria, to include microcephalic primordial dwarfism with a complex brain phenotype involving simplified gyration.

The remarkable size and organization of the human cortex is one of the most distinctive human traits. In the recent past, significant progress has been made toward improving the understanding of the molecular basis of human cortex development thanks in large part to the study of Mendelian disorders in which abnormal cortical development can be traced to mutations in individual genes.[1,2] One particularly relevant group of Mendelian diseases in this context is primary microcephalies. These typically autosomal-recessive diseases have allowed an unprecedented window into key mechanisms that control the proliferation of periventricular neuroprogenitors: stem-cell-like cells with the dual capacity to self-renew and differentiate into neurons that migrate to and organize in the cortex in a highly orchestrated manner.[3]

Since the first gene associated with primary microcephaly was identified in 2002,[4] at least 11 more genes have been linked to autosomal-recessive non-syndromic primary microcephaly.[5] One common thread among most of these genes is that they encode components of the centriole and primary cilium, so their functional loss leads to a shift in the balance toward premature differentiation of neuroprogenitors and resulting diminution of the final pool of neurons that will populate the cortex.[6–8] Another mechanism that also emerged from the study of primary-microcephaly-linked genes is impaired DNA-damage repair, which appears to be especially detrimental to neuroprogenitors.[7,9] Clearly, further exploration of locus and allelic heterogeneity of primary microcephalies will provide deeper understanding of the uniqueness of human brain development.

In continuation of our prior efforts to map and characterize causes of primary microcephaly in humans,[9–12] we describe a multiplex consanguineous family who tested negative on clinical exome sequencing. We show that combined autozygome and exome analysis of this family revealed a probably causal non-canonical splicing mutation in *RTTN* (MIM: 610436). This finding informed our reanalysis of another consanguineous family, and as a result, we identified another homozygous *RTTN* mutation. Linkage analysis confirmed linkage to *RTTN*, thus establishing this as a locus associated with this phenotype. Subsequently, through international collaboration, we were able to identify a third family affected by a very similar phenotype and biallelic *RTTN* mutation.

Originally from Yemen, family 1 is a multiplex consanguineous family who was presented to Genetics Services at Stanford Children's Health for evaluation of primary microcephaly and growth deficiency (Figure 1 and Table 1). The index individual (V:2), who was initially seen at 8 years of age, is now 12 years old. He was born

[1]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh 11211, Saudi Arabia; [2]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA; [3]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA; [4]Department of Pediatrics, Prince Sultan Military Medical City, Riyadh 11159, Saudi Arabia; [5]Department of Medical Genetics, University of Alberta, Edmonton, AB T6G 2R3, Canada; [6]Department of Medical Genetics, University of Calgary, Calgary, AB T2N 1N4, Canada; [7]Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB T2N 1N4, Canada; [8]Department of Human Genetics, McGill University, Montreal, QC H3A 0G4, Canada; [9]Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh 11533, Saudi Arabia
[10]These authors contributed equally to this work
*Correspondence: falkuraya@kfshrc.edu.sa
http://dx.doi.org/10.1016/j.ajhg.2015.10.012. ©2015 by The American Society of Human Genetics. All rights reserved.

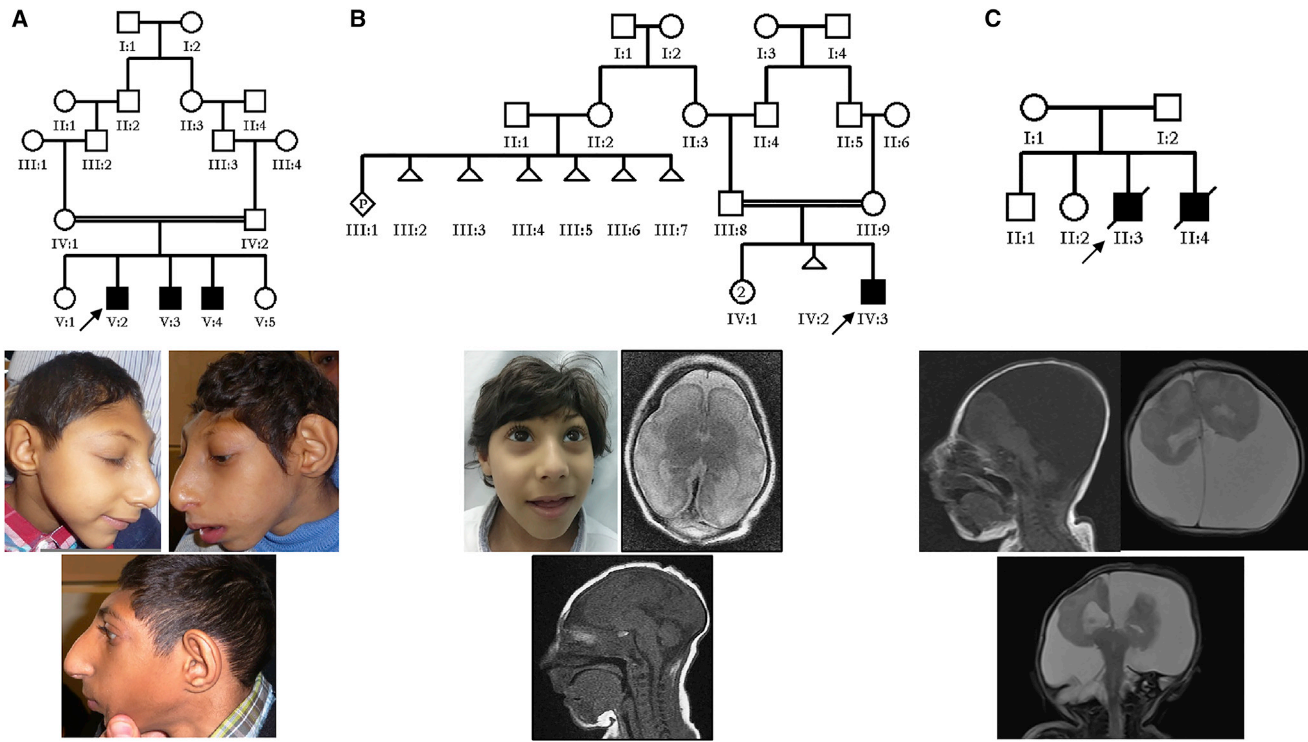

Figure 1. Identification of Three Families Affected by Primary Microcephaly, Lissencephaly, and Dwarfism
(A) Pedigree of family 1 and representative facial photos of the three affected siblings. Note the severe microcephaly and related facial dysmorphism.
(B) Pedigree of family 2 and representative facial photos and brain MRI of the index individual. Note the very small brain volume and lissencephaly.
(C) Pedigree of family 3 and representative brain MRI of II:4. Note the severe cerebral and cerebellar hypoplasia, agenesis of the corpus callosum, reduced sulcation, deformed ventricles, and large cerebrospinal-fluid intensity areas occupying the majority of supratentorial compartments bilaterally.

at home at term, and his birth weight was estimated to be approximately 1 kg (−4.1 SDs). A prior evaluation in Egypt for severe short stature and microcephaly included a brain MRI scan, which reportedly showed microcephaly with few sulcations, bilateral pachygyria, and shallow Sylvain fissures. A renal ultrasound showed a single kidney, and hearing testing showed mild bilateral hearing loss. Genetic testing included a normal karyotype, array comparative genomic hybridization (aCGH), a gene panel for primary microcephaly (*ARFGEF2* [MIM: 605371], *CASC5* [MIM: 609173], *CDK5RAP2* [MIM: 608201], *CDK6* [MIM: 603368], *CENPJ* [MIM: 609279], *CEP135* [MIM: 611423], *CEP152* [MIM: 613529], *CEP63* [MIM: 614724], *MCPH1* [MIM: 607117], *MED17* [MIM: 603810], *NDE1* [MIM: 609449], *PHC1* (MIM: 602978], *PNKP* [MIM: 605610], *SLC25A19* [MIM: 606521], *STAMBP* [MIM: 606247], *STIL* [MIM: 181590], *WDR62* [MIM: 613583], and *ZNF335* [MIM: 610827]), and whole-exome sequencing by a major reference clinical laboratory. He sat up at 18 months of age and walked at 24 months. At the age of 12 years, he has no words. His parents are healthy second cousins from Yemen. They have two healthy daughters and two sons who, like the eldest brother, are affected by profound short stature, microcephaly, and severe intellectual disability. The father has a healthy daughter from a prior nonconsanguineous relationship. The index individual's growth parameters at the age of 12 years are a height of 111 cm ($Z = -5.6$), weight of 14.8 kg ($Z = -8.1$), and head circumference of 34.2 cm ($Z = -8.2$). He has microcephaly-related craniofacial dysmorphism and brisk reflexes. An 11-year-old brother (V:3) has a sacral lesion cephalad to the gluteal crease without abnormality of the spinal cord, a right pelvic ectopic kidney, hypospadias, and an undescended testis. At 10 years of age, his height was 95.5 cm ($Z = -7.2$), his weight was 10.8 kg ($Z = -12.6$), and his head circumference was 34 cm ($Z = -8.2$). A 6-year-old brother (V:4) also has a sacral lesion cephalad to the gluteal crease. His has a height of 91.3 cm ($Z = -5$), weight of 9 kg ($Z = -10.2$), and head circumference of 34.5 cm ($Z = -8.2$).

Because of the negative results on clinical exome sequencing and the microcephaly panel, we hypothesized that the cause of disease in this family is a mutation that was missed in an undescribed primary-microcephaly-associated gene. Therefore, we recruited all family members after obtaining written informed consent under a research protocol approved by King Faisal Specialist Hospital and Research Center research advisory council no. 2080006. Blood was collected in EDTA and PAXGene tubes for DNA and RNA extraction, respectively.

Table 1. Clinical Characteristics of Three Families Affected by *RTTN*-Related Microcephalic Primordial Dwarfism

	Family 1			Family 2	Family 3	
	V:2	V:3	V:4	IV:3	II:3	II:4
Gender	male	male	male	male	male	male
Age	12 years	11 years	6 years	5.5 years	newborn	newborn
Ethnicity	Arab	Arab	Arab	Arab	European	European
Head circumference (SD)	−8.2	−8.2	−8.1	−11.3	−5	−4.5
Length (SD)	−5.6	−7.2	−5	−4.1	−5	−6
Weight (SD)	−8.1	−12.6	−10.2	−2.8	−4	−6
MRI	severe microcephaly with few sulcations, bilateral pachygyria, and shallow Sylvain fissures	–	–	severe microcephaly with simplified gyration	severe microcephaly with severe cerebral and cerebellar hypoplasia, incomplete separation of the cerebral hemispheres, dysgenesis of the corpus callosum, multiple areas of lissencephaly and/or pachygyria and polymicrogyria, and multiple subependymal gray-matter heterotopias	severe microcephaly with severe cerebral and cerebellar hypoplasia, agenesis of the corpus callosum, reduced sulcation, deformed ventricles, and large CSF intensity areas occupying the majority of supratentorial compartments bilaterally
Others	single kidney, hearing loss	sacral lesion cephalad to the gluteal crease without abnormality of the spinal cord, right pelvic ectopic kidney, hypospadias, and undescended testis	sacral lesion cephalad to the gluteal crease	–	joint contractures, death at 2 months	joint contractures, cryptorchidism, duodenal atresia, death at 17 days

The following abbreviation is used: CSF, cerebrospinal fluid.

Mapping of all autozygous intervals per genome (autozygome) was as described before.[13,14] In brief, genome-wide genotyping was performed on the Axiom SNP Chip platform according to the manufacturer's (Affymetrix) protocol, and then AutoSNPa was used for mapping runs of homozygosity (ROHs) of $\geq$2 Mb as surrogates of autozygosity given the parental consanguinity.[15] Autozygome analysis of family 1 revealed multiple ROHs shared by the three affected siblings; however, only two ROHs (chr18: 66,721,256–76,152,320 and chr11: 126,435,800–133,228,400; Figure S1) were exclusive to them. These two loci do not overlap any of the previously described primary-microcephaly-associated loci, which suggests that one of them represents a previously undescribed primary-microcephaly-associated locus. Therefore, we proceeded with whole-exome sequencing.

DNA samples were randomly fragmented by Covaris for generating DNA fragments with a base pair peak of 150–200 bp. Adapters were ligated to both ends of the resulting fragments. The adaptor-ligated templates were purified by the Agencourt AMPure SPRI beads, and fragments with an insert size of about 250 bp were excised. Extracted DNA was amplified by ligation-mediated PCR (LM-PCR), purified, and hybridized to the SureSelect Biotinylated RNA Library (BAITS) for enrichment. Hybridized fragments were bound to the streptavidin beads, whereas non-hybridized fragments were washed out after 24 hr. Captured LM-PCR products were subjected to the Agilent 2100 Bioanalyzer for estimating the magnitude of enrichment. The captured library was then loaded on a HiSeq 2000 platform and run until at least 50-fold coverage was achieved. Raw image files were processed by Illumina Pipeline v.1.6 for base calling with default parameters, and the sequences of each individual were generated as 90 bp paired-end reads. The Burrows-Wheeler Aligner was used to align the clean reads to the human reference genome (UCSC Genome Browser). SNPs and indels were detected by SAMtools. Variants were filtered as follows: homozygous → within autozygome → novel or rare (minor allele frequency $<$ 0.001 according to 1000 Genomes and the ExAC Browser) (Figure 2).

Because no causal mutation had been identified by clinical exome sequencing in family 1, we reckoned that an unusual mutation might have been missed within the two critical loci. Indeed, our second round of exome sequencing revealed no novel coding or canonical splicing

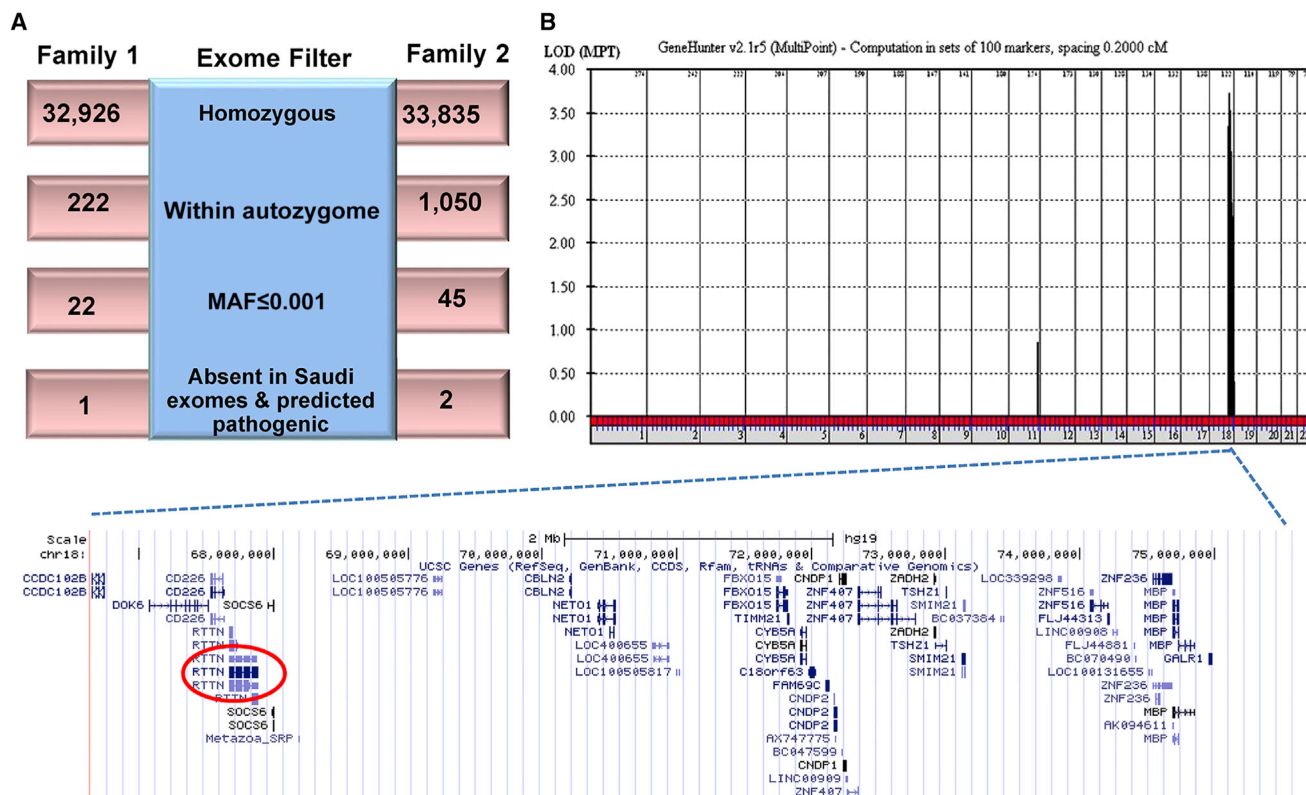

Figure 2. Identification of a Primary-Microcephaly-Associated Locus Defined by *RTTN* Mutations
(A) An exome filtering scheme of the two studied families shows that *RTTN* is the only commonly mutated gene.
(B) Linkage analysis of the two families revealed a linkage peak (minimum LOD score of 3.9) that spans *RTTN* (circled in red), as shown below. Note that this is a minimum LOD score because we inputted family 1 as first rather than second cousins for computational limitation.

variants therein. However, in the second locus, we did identify an intronic variant (c.2885+8A>G [GenBank: NM_173630.3]) in exon 23 of *RTTN*. As predicted in silico, this variant creates a cryptic donor site, and RT-PCR using RNA from blood showed retention of the intervening 7 bp of intron 23 (Figures 3 and S2). This introduces a premature stop codon in the transcript (p.Ser963*). This variant is absent in 1000 Genomes, the ExAC Browser, and 650 in-house ethnically matched exomes, and it segregates fully with the family's phenotype in an autosomal-recessive fashion.

The above findings suggest that mutations in *RTTN* can cause severe primary microcephaly with growth retardation. In order to confirm these results, we queried our internal dataset of matching phenotypes with available exome data and identified a second mutation likely to be causal in family 2. The index individual in family 2 (IV:3) is a 5.5-year-old boy who was born via Cesarean section at 34 weeks of gestation because of severe intrauterine growth retardation (Figure 1 and Table 1). Birth growth parameters were as follows: weight 1,590 g (Z = −2), length 38 cm (Z = −3.5), and head circumference 25 cm (Z = −4.7). His motor development was only mildly delayed (he sat at 9–10 months, crawled at 11 months, and took his first independent steps at 16 months). However, he had noticeable delay in cognitive and speech development. At 3 years of age, he could say only one to two words and was unable to identify more than two body parts. Currently, his speech is still limited to two to three words, and he cannot identify more than three body parts. His parents are healthy Saudi Arabian first cousins with two healthy daughters and one first-trimester abortion. His growth parameters at 3 years of age were as follows: weight 9 kg (Z = −3.9), length 75.2 cm (Z = −5.5), and head circumference 34.5 cm (Z = −9.2). At 5.5 years of age, his weight of 13.6 kg (Z = −2.8), length of 92.1 cm (Z = −4.1), and head circumference of 36 cm (Z = −11.3) indicated continued microcephalic dwarfism. He had typical microcephaly-related craniofacial dysmorphism (Figure 1) and increased tone. The rest of his dysmorphology and neurology examination was normal. His evaluation included a normal regular and molecular karyotype. Brain MRI revealed severe microcephaly with simplified gyration, and a 3D computed-tomography scan revealed secondary craniosynostosis (Figure 1).

Our initial analysis of the family 2 index individual's exome revealed a shortlist of two variants: c.3190A>C (p.Lys1064Gln) (GenBank: NM_173630.3) in *RTTN* and c.350A>C (p.Ser117Tyr) (GenBank: NM_001163149) in *ETV1* (Figure 2). However, reanalysis of the exome in light

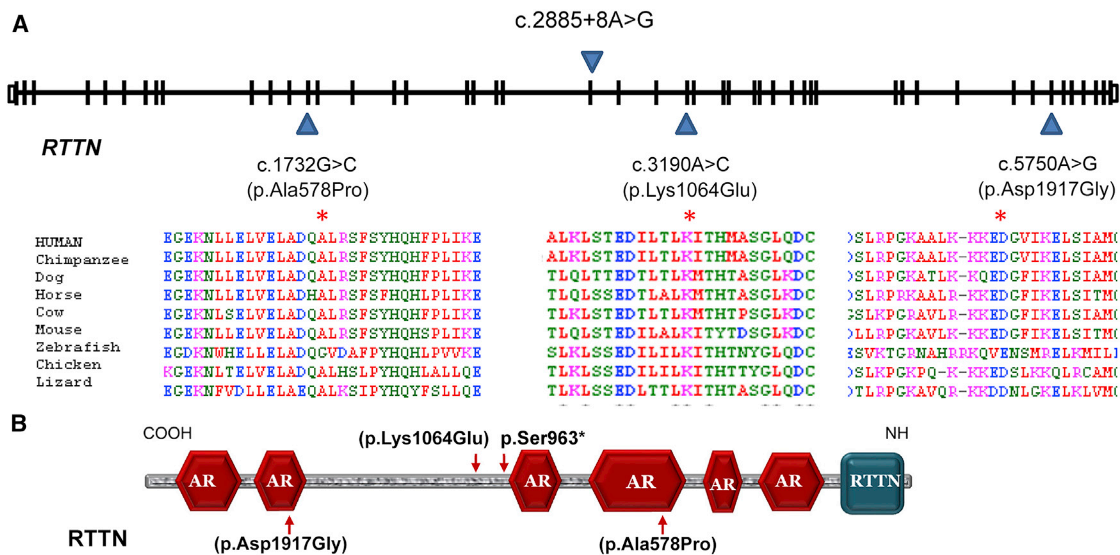

Figure 3. Identification of Four Mutations in *RTTN*

(A) Schematic of *RTTN* shows the four variants on the corresponding genic regions. Multispecies alignment is shown to highlight the strong conservation of the three missense variants.

(B) Schematic of RTTN shows the four variants on the corresponding domains.

of our identification of *RTTN* as a candidate gene for microcephalic primordial dwarfism highlighted the *RTTN* variant (c.3190A>C) as the most likely causal variant. The affected lysine residue is invariant in humans (as revealed by 1000 Genomes, the ExAC Browser, and our in-house exomes), is predicted to be highly pathogenic (PolyPhen score = 0.992; CADD score = 22.7), and is conserved down to zebrafish (Figure 3). Sanger sequencing confirmed that it is homozygous in the index individual and heterozygous in the parents and the two healthy sisters. Reassuringly, combined linkage analysis of the two families generated a single significant linkage peak delimited by rs593385 and rs12959637 (chr18: 66,644,930–75,682,619), spanning *RTTN*, on chromosome 18 (Figure 2).

On the basis of the above results, we initiated a matchmaking query as described before[16] and identified a Canadian family affected by similar clinical features and biallelic mutation of *RTTN*. Family 3 was ascertained during this couple's third pregnancy, when at 12 weeks of gestation, concerns regarding possible intracranial and facial anomalies were noted. By 17 weeks of gestation, the fetus showed severe growth restriction characterized by microcephaly, agenesis of the corpus callosum, cerebellar hypoplasia, a sloping forehead, prominent eyes, micrognathia, and bilateral clubfeet. A baby boy (II:3) was delivered at term, and he weighed only 1,150 g (Z = −4) and had a length of 31.5 cm (Z = −5) and head circumference of 24 cm (Z = −5). Clinically, he had severe microcephaly, a sloping forehead, and a high broad nasal bridge. He had multiple joint contractures, and he was clinically diagnosed with microcephalic osteodysplastic primordial dwarfism type 1. His postnatal course was characterized by severe failure to thrive, and he died at 2 months of age of a cardiorespiratory arrest. His MRI showed multiple abnormalities, including severe microcephaly and severe cerebral and cerebellar hypoplasia. There was incomplete separation of the cerebral hemispheres, dysgenesis of the corpus callosum, an abnormal ventricular system, and a large posterior cyst, but an interhemispheric fissure and a falx cerebri were present. There were multiple areas of lissencephaly and/or pachygyria and polymicrogyria and multiple subependymal gray-matter heterotopias. This family had a recurrence in their fourth pregnancy, in which the fetus presented similarly with severe prenatal-onset growth restriction characterized by microcephaly, a sloping forehead, and abnormal intracranial anatomy. This boy (II:4) was delivered at 37 weeks of gestation with birth parameters as follows: weight 860 g (Z = −6), length 34.5 cm (Z = −6), and head circumference 24 cm (Z = −4.5). His appearance was similar to that of his brother, but additionally he was born with cryptorchidism and required urgent surgical intervention for duodenal atresia. His MRI showed microcephaly with gross brain parenchymal abnormalities affecting both cerebral hemispheres and the cerebellum (which were significantly small in size), agenesis of the corpus callosum, reduced sulcation, deformed ventricles, and large cerebrospinal-fluid intensity areas occupying the majority of supratentorial compartments bilaterally (Figure 1 and Table 1). He died at 19 days of age. Sequencing of *RNU4ATAC* was normal, as was aCGH testing. Whole-exome sequencing as previously described[17] of the first sibling identified biallelic *RTTN* mutations affecting highly conserved amino acids encoded by exons 13 and 43 (c.1732G>C [p.Ala578Pro] and c.5750A>G [p.Asp1917Gly]). Both mutations were predicted in silico to be pathogenic (c.1732G>C: SIFT score 0.01 and CADD score 28.6; c.5750A>G: SIFT score 0.03 and CADD score 27.1), and

Sanger sequencing confirmed that they are present in both affected children.

RTTN encodes rotatin, and its mouse ortholog was found to be truncated or completely absent in two mouse lines that have embryonic lethality and abnormal development of left-right asymmetry, hence the name "no turning," or "nt," mutant.[18,19] Mechanistic studies revealed that RTTN most likely acts upstream of most known left-right asymmetry proteins, e.g., nodal, lefty, and PITX2, the localization of which is either completely lost or abnormally changed to being bilateral instead of unilateral in the lateral plate mesoderm.[18] The localization of its *Drosophila* ortholog to the basal body suggests that the mechanism of *RTTN*-related left-right asymmetry is caused by abnormal cilia, as later revealed by *RTTN*-knockdown experiments that clearly showed abnormal shortening of the cilia.[20,21] Cilia are known to play a critical role in the creation of the nodal flow, which in turn is thought to be a primary cue that breaks the early left-right symmetry, and left-right asymmetry is a classical feature of primary ciliary dyskinesia.[22,23]

In 2012, two missense mutations in *RTTN* were reported in two families affected by an apparently autosomal-recessive form of polymicrogyria, a brain anomaly that falls under the category of abnormal neuronal organization in the human cortex.[20,24] This was a surprising finding because the ciliary nature of RTTN would predict an effect at the level of proliferation or migration of neuroprogenitors or differentiated neurons, respectively, as shown for other ciliary proteins. Indeed, those investigators demonstrated that primary fibroblasts from affected individuals with *RTTN*-related polymicrogyria displayed variable ciliary defects in the form of short and dysmorphic (bulbous tip) cilia.[20]

Our results seem to address a gap in our understanding of the developmental role of *RTTN* by demonstrating that it represents a locus associated with microcephalic primordial dwarfism, consistent with its role in the basal body and cilia. We propose that the rather limited phenotype associated with the two missense mutations reported in 2012 might have been allele specific and that the true clinical spectrum of *RTTN*-related phenotypes entails microcephalic primordial dwarfism and a complex brain phenotype that encompasses simplified gyration and other malformations. This expanded phenotypic spectrum suggests a role for RTTN in cellular proliferation and neuronal migration, which we hope will be the subject of future research. Interestingly, although family 1 appears to have a more severe mutation (truncation) than do families 2 and 3 (missense), the striking similarity in phenotype suggests that the missense mutations most likely exert a similarly detrimental effect on the actual protein. On the other hand, the uniformly lethal outcome of complete loss of *Rttn* in mice suggests that even the truncating mutation might have retained some function and that a truly null mutation might lead to early embryonic lethality in humans.

Another interesting phenotypic aspect in the three studied families is their severe growth retardation. This is of particular interest because we have previously proposed the continuum of phenotype between primary microcephaly and microcephalic primordial dwarfism on the basis of our experience with *CENPJ* (MIM: 609279), and this notion was later confirmed in other primary-microcephaly-associated genes, e.g., *CEP152* (MIM: 613529).[25–27] Therefore, it appears that *RTTN* is another example of a gene in which mutations can cause both isolated primary microcephaly and microcephalic primordial dwarfism.

In conclusion, we show that *RTTN* is a gene that is mutated in severe primary microcephaly with associated growth deficiency and complex brain malformations including simplified gyration. Our finding of a non-canonical splicing mutation that evaded detection by a clinical exome laboratory is a reminder that commonly used filters can miss causal mutations and that intronic mutations that are deeper than the consensus ± 1 and ± 2 splicing sites should be considered in individuals who test negative on clinical exome sequencing.[28,29]

Supplemental Data

Supplemental Data include two figures and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2015.10.012.

Consortia

The members of the Care4Rare Canada Gene Discovery Steering Committee are Kym Boycott, Alex MacKenzie, Jacek Majewski, Michael Brudno, Dennis Bulman, and David Dyment.

Acknowledgments

We thank the families for their enthusiastic participation and Salma Wakil, Dorota Monies, and the genotyping and sequencing core facilities at the King Faisal Specialist Hospital and Research Center for their invaluable help. This work was supported by a King Salman Center for Disability Research Grant (to F.S.A.). Part of this work was performed by the Care4Rare Canada Consortium (funded by Genome Canada), the Canadian Institutes of Health Research, the Ontario Genomics Institute, Ontario Research Fund, Genome Quebec, and Children's Hospital of Eastern Ontario Research Foundation. We acknowledge the contribution of the high-throughput sequencing platform of the McGill University and Genome Quebec Innovation Centre.

Received: August 9, 2015
Accepted: October 20, 2015
Published: November 19, 2015

Web Resources

The URLs for data presented herein are as follows:

BWA, http://bio-bwa.sourceforge.net/
CADD, http://cadd.gs.washington.edu/score

ExAC Browser, http://exac.broadinstitute.org
OMIM, http://www.omim.org
PolyPhen-2, www.genetics.bwh.harvard.edu/pph2/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/
SIFT, http://sift.jcvi.org/www/SIFT_seq_submit2.html
UCSC Genome Browser, http://genome.ucsc.edu/

References

1. Manzini, M.C., and Walsh, C.A. (2011). What disorders of cortical development tell us about the cortex: one plus one does not always make two. Curr. Opin. Genet. Dev. *21*, 333–339.
2. Bae, B.-I., Tietjen, I., Atabay, K.D., Evrony, G.D., Johnson, M.B., Asare, E., Wang, P.P., Murayama, A.Y., Im, K., Lisgo, S.N., et al. (2014). Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. Science *343*, 764–768.
3. Lehtinen, M.K., and Walsh, C.A. (2011). Neurogenesis at the brain-cerebrospinal fluid interface. Annu. Rev. Cell Dev. Biol. *27*, 653–679.
4. Jackson, A.P., Eastwood, H., Bell, S.M., Adu, J., Toomes, C., Carr, I.M., Roberts, E., Hampshire, D.J., Crow, Y.J., Mighell, A.J., et al. (2002). Identification of microcephalin, a protein implicated in determining the size of the human brain. Am. J. Hum. Genet. *71*, 136–142.
5. Faheem, M., Naseer, M.I., Rasool, M., Chaudhary, A.G., Kumosani, T.A., Ilyas, A.M., Pushparaj, P., Ahmed, F., Algahtani, H.A., Al-Qahtani, M.H., and Saleh Jamal, H. (2015). Molecular genetics of human primary microcephaly: an overview. BMC Med. Genomics *8* (*Suppl 1*), S4.
6. Yang, Y.J., Baltus, A.E., Mathew, R.S., Murphy, E.A., Evrony, G.D., Gonzalez, D.M., Wang, E.P., Marshall-Walker, C.A., Barry, B.J., Murn, J., et al. (2012). Microcephaly gene links trithorax and REST/NRSF to control neural stem cell proliferation and differentiation. Cell *151*, 1097–1112.
7. Gilmore, E.C., and Walsh, C.A. (2013). Genetic causes of microcephaly and lessons for neuronal development. Wiley Interdiscip. Rev. Dev. Biol. *2*, 461–478.
8. Bettencourt-Dias, M., Hildebrandt, F., Pellman, D., Woods, G., and Godinho, S.A. (2011). Centrosomes and cilia in human disease. Trends Genet. *27*, 307–315.
9. Awad, S., Al-Dosari, M.S., Al-Yacoub, N., Colak, D., Salih, M.A., Alkuraya, F.S., and Poizat, C. (2013). Mutation in PHC1 implicates chromatin remodeling in primary microcephaly pathogenesis. Hum. Mol. Genet. *22*, 2200–2213.
10. Alkuraya, F.S., Cai, X., Emery, C., Mochida, G.H., Al-Dosari, M.S., Felie, J.M., Hill, R.S., Barry, B.J., Partlow, J.N., Gascon, G.G., et al. (2011). Human mutations in NDE1 cause extreme microcephaly with lissencephaly [corrected]. Am. J. Hum. Genet. *88*, 536–547.
11. Alazami, A.M., Patel, N., Shamseldin, H.E., Anazi, S., Al-Dosari, M.S., Alzahrani, F., Hijazi, H., Alshammari, M., Aldahmesh, M.A., Salih, M.A., et al. (2015). Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. Cell Rep. *10*, 148–161.
12. Hu, W.F., Pomp, O., Ben-Omran, T., Kodani, A., Henke, K., Mochida, G.H., Yu, T.W., Woodworth, M.B., Bonnard, C., Raj, G.S., et al. (2014). Katanin p80 regulates human cortical development by limiting centriole and cilia number. Neuron *84*, 1240–1257.
13. Alkuraya, F.S. (2010). Autozygome decoded. Genet. Med. *12*, 765–771.
14. Alkuraya, F.S. (2012). Discovery of rare homozygous mutations from studies of consanguineous pedigrees. Curr. Protoc. Hum. Genet. *Chapter 6*, Unit 6.12.
15. Carr, I.M., Flintoff, K.J., Taylor, G.R., Markham, A.F., and Bonthron, D.T. (2006). Interactive visual analysis of SNP data for rapid autozygosity mapping in consanguineous families. Hum. Mutat. *27*, 1041–1046.
16. Faden, M., AlZahrani, F., Mendoza-Londono, R., Dupuis, L., Hartley, T., Kannu, P., Raiman, J.A., Howard, A., Qin, W., Tetreault, M., et al.; Care4Rare Canada Consortium (2015). Identification of a Recognizable Progressive Skeletal Dysplasia Caused by RSPRY1 Mutations. Am. J. Hum. Genet. *97*, 608–615.
17. Bernier, F.P., Caluseriu, O., Ng, S., Schwartzentruber, J., Buckingham, K.J., Innes, A.M., Jabs, E.W., Innis, J.W., Schuette, J.L., Gorski, J.L., et al.; FORGE Canada Consortium (2012). Haploinsufficiency of SF3B4, a component of the pre-mRNA spliceosomal complex, causes Nager syndrome. Am. J. Hum. Genet. *90*, 925–933.
18. Chatterjee, B., Richards, K., Bucan, M., and Lo, C. (2007). Nt mutation causing laterality defects associated with deletion of rotatin. Mamm. Genome *18*, 310–315.
19. Faisst, A.M., Alvarez-Bolado, G., Treichel, D., and Gruss, P. (2002). Rotatin is a novel gene required for axial rotation and left-right specification in mouse embryos. Mech. Dev. *113*, 15–28.
20. Kheradmand Kia, S., Verbeek, E., Engelen, E., Schot, R., Poot, R.A., de Coo, I.F., Lequin, M.H., Poulton, C.J., Pourfarzad, F., Grosveld, F.G., et al. (2012). RTTN mutations link primary cilia function to organization of the human cerebral cortex. Am. J. Hum. Genet. *91*, 533–540.
21. Stevens, N.R., Dobbelaere, J., Wainman, A., Gergely, F., and Raff, J.W. (2009). Ana3 is a conserved protein required for the structural integrity of centrioles and basal bodies. J. Cell Biol. *187*, 355–363.
22. Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M., and Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell *95*, 829–837.
23. Okada, Y., Takeda, S., Tanaka, Y., Izpisúa Belmonte, J.C., and Hirokawa, N. (2005). Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. Cell *121*, 633–644.
24. Chang, B., Walsh, C.A., Apse, K., and Bodell, A. (2007). Polymicrogyria Overview. GeneReviews. http://www.ncbi.nlm.nih.gov/books/NBK1329/.
25. Al-Dosari, M.S., Shaheen, R., Colak, D., and Alkuraya, F.S. (2010). Novel CENPJ mutation causes Seckel syndrome. J. Med. Genet. *47*, 411–414.
26. Klingseisen, A., and Jackson, A.P. (2011). Mechanisms and pathways of growth failure in primordial dwarfism. Genes Dev. *25*, 2011–2024.
27. Alkuraya, F.S. (2015). Primordial dwarfism: an update. Curr. Opin. Endocrinol. Diabetes Obes. *22*, 55–64.
28. Yavarna, T., Al-Dewik, N., Al-Mureikhi, M., Ali, R., Al-Mesaifri, F., Mahmoud, L., Shahbeck, N., Lakhani, S., AlMulla, M., Nawaz, Z., et al. (2015). High diagnostic yield of clinical exome sequencing in Middle Eastern patients with Mendelian disorders. Hum. Genet. *134*, 967–980.
29. Ben-Omran, T., Alsulaiman, R., Kamel, H., Shaheen, R., and Alkuraya, F.S. (2015). Intrafamilial clinical heterogeneity of CSPP1-related ciliopathy. Am. J. Med. Genet. A. *167*, 2478–2480.