

# De Novo Mutations of *RERE* Cause a Genetic Syndrome with Features that Overlap Those Associated with Proximal 1p36 Deletions

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Deletions of chromosome 1p36 affect approximately 1 in 5,000 newborns and are associated with developmental delay, intellectual disability, and defects involving the brain, eye, ear, heart, and kidney. Arginine-glutamic acid dipeptide repeats (*RERE*) is located in the proximal 1p36 critical region. *RERE* is a widely-expressed nuclear receptor coregulator that positively regulates retinoic acid signaling. Animal models suggest that *RERE* deficiency might contribute to many of the structural and developmental birth defects and medical problems seen in individuals with 1p36 deletion syndrome, although human evidence supporting this role has been lacking. In this report, we describe ten individuals with intellectual disability, developmental delay, and/or autism spectrum disorder who carry rare and putatively damaging changes in *RERE*. In all cases in which both parental DNA samples were available, these changes were found to be de novo. Associated features that were recurrently seen in these individuals included hypotonia, seizures, behavioral problems, structural CNS anomalies, ophthalmologic anomalies, congenital heart defects, and genitourinary abnormalities. The spectrum of defects documented in these individuals is similar to that of a cohort of 31 individuals with isolated 1p36 deletions that include *RERE* and are recapitulated in *RERE*-deficient zebrafish and mice. Taken together, our findings suggest that mutations in *RERE* cause a genetic syndrome and that haploinsufficiency of *RERE* might be sufficient to cause many of the phenotypes associated with proximal 1p36 deletions.

Deletions of chromosome 1p36 constitute the most common group of terminal deletions in humans—affecting approximately 1 in 5,000 newborns—and are recognized as an important cause of intellectual disability.<sup>1–3</sup> Additional phenotypes that characterize the 1p36 deletion syndrome (MIM: 607872) include developmental delay, seizures, brain anomalies, vision problems, hearing loss, orofacial clefting, congenital heart defects, cardiomyopathy, renal anomalies, short stature, and a recognizable constellation of dysmorphic features—microbrachycephaly; large, late-closing anterior fontanel; posteriorly rotated, low-set, abnormally shaped ears; straight eyebrows; deep-set eyes; epicanthal folds; midface hypoplasia; a wide and depressed nasal bridge; a long philtrum; and a pointed chin.<sup>4–6</sup> By analyzing the clinical presentations of individuals with terminal 1p36 deletions, Wu et al. determined that haploinsufficiency of genes located distal to marker DIS2870 (chr1:6,289,764–6,289,973; hg19) was sufficient to cause most of these phenotypes.<sup>7</sup> This region

has since been termed the distal or classical 1p36 critical region.<sup>8</sup>

Subsequently, Kang et al. delineated a non-overlapping proximal 1p36 critical region (chr1:8,395,179–11,362,893; hg19) which, when deleted, was associated with a similar constellation of phenotypes—intellectual disability, developmental delay, vision problems, hearing loss, orofacial clefting, congenital heart defects, cardiomyopathy, and short stature.<sup>9</sup> However, these individuals had distinctive facial features—frontal and parietal bossing, low-set, posteriorly-rotated ears, epicanthal folds, anteverted nares, broad eyebrows, and hirsutism.

Animal models have implicated several genes located in the distal and proximal 1p36 critical regions as potential contributors to the neurodevelopmental and structural birth defects associated with the 1p36 deletion syndrome.<sup>8</sup> However, human evidence supporting the role of these genes in the development of specific phenotypes is often lacking. Uncertainty regarding the clinical effects

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<http://dx.doi.org/10.1016/j.ajhg.2016.03.002>

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**Table 1. RERE Sequence Changes Identified in Subjects 1–10**

Subject No.	Sequence Changes in RERE <sup>a</sup>	PolyPhen-2	SIFT	MutationTaster	Alleles in Exome Variant Server	Alleles in ExAC Browser
1	c.3466 G>A, p.Gly1156Arg	probably damaging	damaging	disease causing	none	2/66,082 European; 2/120,478 total <sup>b</sup>
2	c.4313_4318dupTCCACC, p.Leu1438_His1439dup	NA	NA	polymorphism	none	none
3	c.3785C>G, p.Pro1262Arg	probably damaging	damaging	disease causing	none	none
4	c.4293C>G, p.His1431Gln	probably damaging	damaging	disease causing	none	none
5	c.4293C>A, p.His1431Gln	probably damaging	damaging	disease causing	none	none
6	c.3122delC, p.Pro1041Lysfs*40	NA	NA	disease causing	none	none
7	c.1411G>A, p.Val471Ile	possibly damaging	tolerated	disease causing	1, African American	none
8	c.1104delA, p.Leu369Cysfs*16	NA	NA	NA <sup>c</sup>	none	none
9	c.2249_2270dup p.Thr758Serfs*36	NA	NA	disease causing	none	none
10 <sup>d</sup>	c.2278 C>T, p.Gln760*	NA	NA	disease causing	none	none

NA, not applicable.

<sup>a</sup>Based on *RERE* transcript variant 1 [RefSeq: NM\_012102.3].

<sup>b</sup>Alleles found only in the heterozygous state.

<sup>c</sup>Change affects the last nucleotide of an exon.

<sup>d</sup>Subject 10 was previously reported by Krumm et al. as subject 11654.p1.<sup>18</sup>

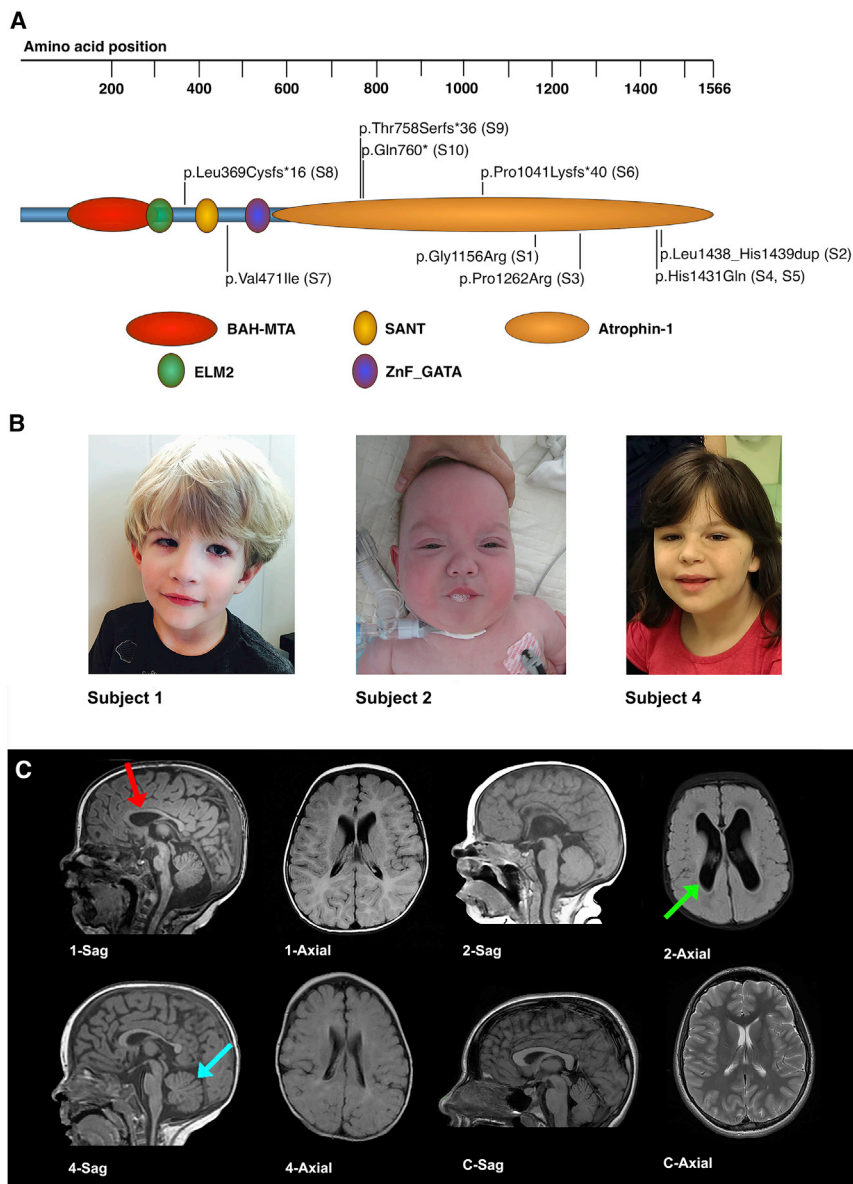
caused by haploinsufficiency of specific genes makes it difficult for physicians to provide prognostic information to families and to generate individualized care plans for their patients solely on the basis of the location and extent of their 1p36 deletions.

Arginine-glutamic acid dipeptide repeats (*RERE* [MIM: 605226]) is located in the proximal 1p36 critical region.<sup>8,9</sup> *RERE* encodes a widely-expressed nuclear receptor coregulator that positively regulates retinoic acid signaling.<sup>10–12</sup> Data available from the Exome Aggregation Consortium (ExAC) Browser suggests that *RERE* has a high probability of loss-of-function intolerance (pLI = 1.0) given that 47.5 loss-of-function variants were expected based on gene size and GC content but only three were observed. Data from zebrafish and mouse models suggest that haploinsufficiency of *RERE* might contribute to the intellectual disability, developmental delay, structural brain anomalies, vision problems, hearing loss, congenital heart defects, cardiomyopathy, and renal anomalies seen in individuals with 1p36 deletions.<sup>13–17</sup> However, the exact role that *RERE* deficiency plays in 1p36 deletion syndrome, and more generally in human disease, remains unclear.

Here, we describe ten individuals with neurodevelopmental phenotypes and congenital anomalies who carry rare, putatively deleterious, sequence changes in *RERE*. Clinical data were obtained through written informed consent. In all cases, the procedures followed were in accordance with the ethical standards of the respective institution's committee on human research and were in keeping with national standards. *RERE* sequence changes were identified in nine of these individuals (subjects

1–9) by clinically-based exome sequencing performed in CLIA- or ISO15189-certified laboratories and confirmed by Sanger sequencing. Changes in subject 10 were identified by the Simons Foundation Autism Research Initiative (SFARI) and reported in SFARI Base—a central database of clinical and genetic information about families affected by autism and other neurodevelopmental disorders. In all cases where parental DNA samples were available, these changes were found to be de novo. The clinical phenotypes of these individuals and a description of their *RERE* sequence changes—including in silico prediction of effects on protein function via PolyPhen-2, SIFT, and MutationTaster and allele frequencies in control populations reported in the NHLBI Exome Variant Server and the ExAC Browser—are summarized below and in Table 1, Tables S1 and S2, Figure 1, and the Supplemental Note.

Subject 1 is a 3-year-old male of European descent who carries a de novo c.3466G>A, p.Gly1156Arg missense change in *RERE* (GenBank: NM\_012102.3). He was noted to have intrauterine growth retardation, was born prematurely at 35 weeks of gestation, and was small for gestation age with a birth weight of 1.64 kg (<3<sup>rd</sup> percentile). Multiple regions of infarction were found in the placenta. His clinical features include global developmental delay, mild spastic quadriplegia, dysarthric speech, swallowing difficulties, bilateral optic colobomas, optic nerve hypoplasia, unilateral microphthalmia, anisometropia, mild sensorineural hearing loss, micrognathia, a ventricular septal defect (VSD), a patent foramen ovale, hypospadias with penile chordee, and gastroesophageal reflux disease (GERD). A brain MRI obtained at 7 months of age revealed



**Figure 1. Molecular Changes and Selected Clinical Findings for Individuals with Putatively Deleterious Changes in RERE**

(A) The predicted locations of domains within RERE are presented along with the locations of the RERE changes seen in subjects 1–10.

(B) Craniofacial changes noted in subjects. Subject 1, at 3 years of age, presents with bilateral optic colobomas, unilateral microphthalmia, low-set ears, and micrognathia. Subject 2, at 11 months of age, presents with a unilateral iris coloboma and simple ears. Subject 4, at 9 years of age, presents with deep-set eyes, epicanthal folds, and abnormal ears.

(C) Sagittal (Sag) and axial brain MRI scans of subjects 1, 2, and 4 demonstrating characteristic findings. Subject 1, at 7 months of age, had a thin corpus callosum (red arrow), a small anterior vermis, a small pons with a ventral cleft at the ponto-medullary junction, delayed myelination, and severely decreased white matter volume. Subject 2, at 4 months of age, had a thin corpus callosum, ventriculomegaly (green arrow), incompletely folded hippocampi, and severely diminished white matter volume. Subject 4, at 1 year and 2 months of age, had a thin corpus callosum, a diminished cerebellar vermis with a deep fissures (blue arrow), and significantly diminished white matter volume. For comparison, we have shown a mid-sagittal T1-weighted image showing a normal sized corpus callosum and intact cerebellar vermis and an axial T2-weighted image showing normal sized ventricles without increased extra-axial space.

a thin corpus callosum, small anterior vermis, small pons with a ventral cleft at the ponto-medullary junction, delayed myelination, and severely decreased white matter volume (Figure 1).

Subject 2 is a 15-month-old male of European descent who carries a de novo c.4313\_4318dupTCCACC, p.Leu1438\_His1439dup change in *RERE*. This change results in the addition of a leucine and histidine to an already histidine-rich compositional bias region of RERE (UniProt: Q9P2R6). This type of change is not amenable to evaluation by PolyPhen-2 or SIFT, and MutationTaster predicts this change to be a polymorphism. However, this duplication—like the amino acid change in subject 1—is found in a conserved region of the atrophin domain of *RERE*. He was also found to have a de novo c.4211G>A, p.Arg1404His (GenBank: NM\_001252100.1) change in kinesin family member 21B (*KIF21B* [MIM: 608322]) and a de novo c.1433C>T, p.Pro478Leu (GenBank: NM\_170744.4) change in unc-5

netrin receptor B (*UNC5B* [MIM: 607870]). Both of these changes were predicted to be probably damaging by PolyPhen-2, damaging by SIFT, and disease causing by MutationTaster. *KIF21B* encodes a member of the kinesin family of proteins that acts as a plus-end-directed microtubule motor, participating in transport of cellular cargo in neurons as well as in other cell types.<sup>19</sup> Variants in *KIF21B* have been associated with multiple sclerosis and ankylosing spondylitis in genome-wide association studies.<sup>20,21</sup> The UNC5 family of proteins plays a critical role in a number of cellular processes, including axonal guidance, angiogenesis, and apoptosis. However, mutations in *UNC5B* have not been associated with a genetic disorder in humans.<sup>22</sup>

Subject 2 was born prematurely at 30 weeks and 6 days of gestation via C-section for symmetric intrauterine growth retardation and fetal distress. His birth weight was 0.94 kg (~3<sup>rd</sup> percentile). His clinical features include developmental delay, post-natal growth retardation, short stature with a height of 64 cm at 15 months (<1<sup>st</sup> percentile), microcephaly with an occipital frontal circumference

(OFC) of 42.5 cm at 15 months (<1<sup>st</sup> percentile), a unilateral iris coloboma, choanal atresia, a VSD, a patent ductus arteriosus, unilateral multicystic kidney, and cryptorchidism. A brain MRI obtained at 4 months revealed a thin corpus callosum, ventriculomegaly, incompletely folded hippocampi, and severely diminished white matter volume. This subject was initially presumed to have CHARGE syndrome. However, *CHD7* sequencing and deletion and duplication analysis revealed no pathologic variants.

Subject 3 is a 2-year-old Hispanic male who carries a de novo c.3785C>G, p.Pro1262Arg missense change in *RERE* that was found to be probably damaging by PolyPhen-2, damaging by SIFT, and disease causing by MutationTaster. Pregnancy was complicated by polyhydramnios secondary to duodenal atresia. His other clinical features include post-natal growth retardation with a weight of 10.3 kg (1<sup>st</sup> percentile), a height of 82 cm (2<sup>nd</sup> percentile), and an OFC of 46.4 cm (4<sup>th</sup> percentile) at 2 years and 4 months of age, developmental delay, hypotonia, staring spells, dysmorphic features, unilateral Peter anomaly, bilateral iris abnormalities, a VSD, bilateral hydronephrosis with grade 2–4 vesicoureteral reflux, an annular pancreas, and syndactyly. A brain MRI obtained at 1 year and 6 months showed a thin corpus callosum, deep primary fissure of the cerebellar vermis, ventriculomegaly, and severely diminished white matter volume.

Subject 4 is a 9-year-old female of European descent who carries a de novo c.4293C>G, p.His1431Gln missense change in *RERE* that was found to be probably damaging by PolyPhen-2, damaging by SIFT, and disease causing by MutationTaster. She was also found to be mosaic for a de novo c.2464G>A, p.Gly822Ser (GenBank: NM\_021007.2) change in sodium channel, voltage-gated, type II alpha subunit (*SCN2A* [MIM: 182390]). This variant is present in 14/95 reads for 14.7% mosaicism with a 95% confidence interval of 8.3%–23.5%. This *SCN2A* change is predicted to be probably damaging by PolyPhen-2, damaging by SIFT, and disease causing by MutationTaster. Mutations in *SCN2A* have been implicated in two autosomal-dominant disorders: epileptic encephalopathy, early infantile, 11 (MIM: 613721) and seizures, benign familial infantile, 3 (MIM: 607745). Her clinical features included hypotonia, moderate intellectual disability, severe behavioral issues and seizures, dysmorphic features, GERD, developmental hip dysplasia, and severe vesicoureteral reflux requiring surgery. A brain MRI obtained at 1 year and 2 months showed a thin corpus callosum, a diminished cerebellar vermis with deep fissures, and significantly diminished white matter volume—findings not reported in prior studies of *SCN2A*.<sup>23,24</sup>

Subject 5 is a 12-year, 8-month-old Dutch male (reported previously by Bosch et al. as patient 22) with a de novo c.4293C>A change in *RERE* that results in the same amino acid change, p.His1431Gln, caused by the c.4293C>G change documented in subject 4.<sup>25</sup> His clinical features include severe developmental delay, seizures, feeding problems, blepharophimosis, strabismus, bilateral optic atro-

phy, cortical visual impairment, dysmorphic features, GERD, and pyloric hypertrophy requiring surgical intervention. A brain MRI obtained at 3 years and 6 months of age showed an abnormal corpus callosum with blunting of the rostrum, mild to moderate ventriculomegaly, a small cerebellar vermis, and globally diminished white matter volume.

Subject 6 is a 6-year-old adopted female of mixed European and Hispanic descent with a c.3122delC, p.Pro1041Lysfs\*40 frameshift change in *RERE*. Parental DNA is not available. Given that this change occurs in the 17<sup>th</sup> of 21 *RERE* coding exons, it might trigger nonsense-mediated mRNA decay. Pregnancy and birth history are limited, but there was reported alcohol use during the pregnancy. Her family history is positive for intellectual disability and mental illness in her biological mother and intellectual disability in both of her maternal grandparents and a maternal half-uncle. Her clinical features include developmental delay, hypotonia, behavior problems, dysmorphic features, lumbar lordosis, and macrocephaly with an OFC of 54.1 cm (>98<sup>th</sup> percentile).

Subject 7 is an 11-year-old Dutch male who carries a de novo c.1411G>A, p.Val471Ile missense change in *RERE* that was estimated to be possibly damaging by PolyPhen-2 and disease causing by MutationTaster. He was also found to have a compound heterozygous pair of mutations in fibrous sheath interacting protein 2 (*FSIP2* [MIM: 615796]): c.2602del, p.Ser868Hfs\*12 and c.4583C>G, p.Thr1528Arg (GenBank: NM\_173651.2). The c.2602del deletion is inherited from the father, and the c.4583C>G missense mutation is inherited from the mother and is a previously reported variant, rs192957612. This gene encodes for a protein localized to the sperm flagellum and is not associated with any known human disease.<sup>26</sup> The subject was born prematurely at 34 weeks of gestation and weighed 2.24 kg (~5<sup>th</sup> percentile). This twin pregnancy was complicated by maternal hypertension for which the mother used medication. The subject has an older sister who is healthy, but his twin sister was recently diagnosed with KBG syndrome caused by a de novo c.3382\_3383delGA, p.Asp1128Glnfs\*41 (GenBank: NM\_001256182.1) change in ankyrin repeat domain-containing protein 11 (*ANKRD11* [MIM: 611192]), which was not found in this individual.<sup>27</sup> His clinical features include developmental delay, hypotonia, ADHD, mild intellectual disability, growth retardation, dysmorphic features, and a cleft lip. A brain MRI obtained at 7 years, 9 months of age showed an abnormal corpus callosum with blunting of the rostrum, a small anterior commissure, and diminished white matter volume.

Subject 8 is a 10-year-old male of European descent who carries a c.1104delA, p.Leu369Cysfs\*16 change in *RERE*. This variant was not present in his mother, but a paternal DNA sample could not be obtained for testing. Because this change occurs in the last nucleotide of the 9<sup>th</sup> of 21 *RERE* coding exons, it might trigger nonsense-mediated mRNA decay. Pregnancy was complicated by anemia, a vaginal infection, and maternal smoking and alcohol

**Table 2. Overlapping Features Seen in Our Subjects, Individuals with Isolated 1p36 Deletions that Include *REER*, and Zebrafish and Mice Models**

Phenotype	Subjects 1–10 Documented with Phenotype (% and No.)	Individuals with Isolated 1p36 Deletions Involving <i>REER</i> Documented with Phenotype (% and No.)	Related Phenotype Documented in <i>Rere</i> -Deficient Zebrafish? <sup>13,17</sup>	Related Phenotype Documented in <i>REER</i> -Deficient Mice? <sup>14,15</sup>
Neurologic problems	100% (10/10)	97% (30/31)	no	no
CNS anomalies	88% (7/8)	29% (9/31)	no	yes
Ophthalmologic abnormalities	40% (4/10)	42% (13/31)	yes	yes
Hearing loss	10% (1/10)	26% (8/31)	yes	yes
Orofacial clefting	10% (1/10)	16% (5/31)	no	no
Congenital heart defects	40% (4/10)	71% (22/31)	no	yes
Genitourinary anomalies	50% (5/10)	13% (4/31)	no	yes

consumption until eight weeks of gestation, when the pregnancy was recognized. He was born prematurely at 36 weeks of gestation. His clinical features included fine motor and speech delay, autism spectrum disorder, and dysmorphic features. A brain MRI ordered for developmental delay was normal.

Subject 9 is a 7-year-old Dutch male who carries a de novo c.2249\_2270dup, p.Thr758Serfs\*36 frameshift change in *REER*. This mutation was predicted to be disease causing by MutationTaster. Pertinent findings in his family history include a younger sister with autism, a paternal uncle who died of Duchenne muscular dystrophy, and a second paternal uncle who died of acute heart failure at 20 years of age. Clinical features include severe speech and language delay, intellectual disability, autism, dysmorphic features, and a VSD. He has macrocephaly and an OFC of 57.3 cm (>99th percentile) at 6 years, 10 months of age. However, this might be familial given that his mother's OFC is 59.7 cm (+ 2.5 SD).

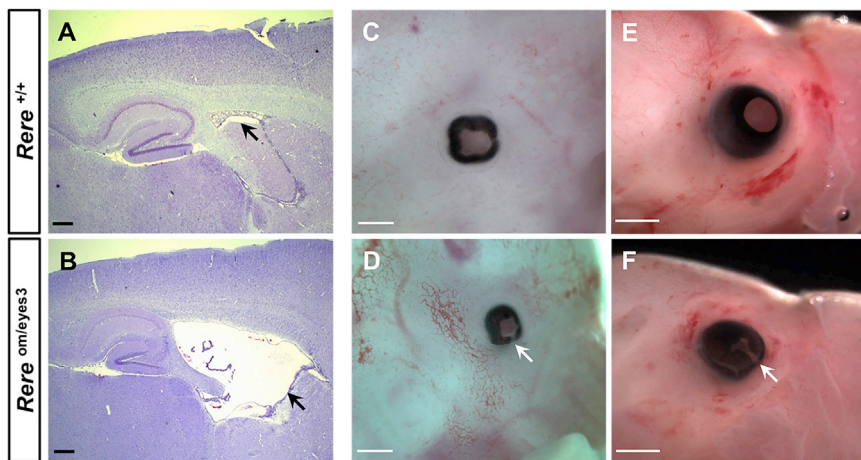
While investigating the genetic causes of autism spectrum disorder in a large cohort, Krumm et al. identified a 14-year-old non-Hispanic female (subject 11654.p1) with autism spectrum disorder who carried a de novo c.2278C>T, p.Gln760\* change in *REER*.<sup>18</sup> This mutation was estimated to be disease causing by MutationTaster. Here, we refer to her as subject 10. Her clinical features include autism spectrum disorder, sleep apnea, and nocturnal enuresis.

All of the subjects presented here have neurodevelopmental disorders—intellectual disability, developmental delay, and/or autism (Table 2, Table S2). Recurrent neurological abnormalities reported in these subjects include seizures, hypotonia, behavioral problems, ADHD, and problems with feeding and swallowing. Brain anomalies were documented in 88% (7/8) of subjects who had an MRI and included abnormalities and/or thinning of the corpus callosum (6/8, or 75%), diminished white matter volume (6/8, or 75%), abnormal cerebellar vermis (4/8, or 50%), and ventriculomegaly (3/8, or 38%). Structural eye defects were seen in 40% (4/10) of subjects and included coloboma, optic atrophy and/or hypoplasia, microphthalmia, Peter anomaly, iris anomalies, and blepharophimosis.

Congenital heart defects were seen in 40% (4/10) of subjects, and VSDs were the most common defect. Genitourinary defects were seen in 50% (5/10), and vesicoureteral reflux was documented in 30% (3/10) of subjects.

In addition to these examples of extra-neuronal organ involvement, a variety of dysmorphic features were also observed in our subjects (Table S2), including some features previously associated with deletions of the proximal 1p36 critical region, such as frontal bossing, low-set, posteriorly rotated ears, epicanthic folds, anteverted nares, and broad eyebrows (Figure 1).<sup>9</sup> Our cohort also demonstrates a variety of growth patterns—some subjects have short stature (3/9, or 33%) or microcephaly (2/9, or 22%), whereas others have tall stature (2/9, or 22%) or macrocephaly (2/9, or 22%). This suggests that physicians should not rely heavily on the identification of a characteristic constellation of physical features but rather on a grouping of medical conditions, such as brain, eye, and cardiac developmental defects, when considering the possible diagnosis of an *REER*-related disorder.

To determine the subset of 1p36 deletion phenotypes that could possibly be explained by *REER* haploinsufficiency, we compared the spectrum of structural birth defects seen in our subjects to a cohort of 31 individuals with isolated 1p36 deletions involving *REER* (Table 2, Table S1, Table S3, and Figure S1). These individuals were either previously described in the literature or provided informed consent and were enrolled in an institutional-review-board-approved research study.<sup>9,16,28–33</sup> In all cases, the procedures followed were in accordance with the ethical standards of the institution's committee on human research and were in keeping with national standards. Within this cohort, 24 individuals carried larger terminal or interstitial deletions that included all or part of both the distal and proximal 1p36 deletion critical regions, and seven individuals carried interstitial 1p36 deletions that did not include the distal critical region (Figure S1). We found that the majority of recurrent neurologic, ophthalmologic, cardiac, and gastrointestinal phenotypes seen in our subjects were also seen in individuals in the 1p36 deletion cohort. Overlap was also seen for



**Figure 2. RERE-Deficient *Rere*<sup>om/eyes3</sup> Mice and Embryos Have Ventriculomegaly, Microphthalmia, and Coloboma** (A and B) Representative cresyl-violet-stained sagittal brain sections from a wild-type mouse (A) and its *Rere*<sup>om/eyes3</sup> littermate (B) show enlargement of the lateral ventricles (black arrows). Ventriculomegaly was seen in 3/3 *Rere*<sup>om/eyes3</sup> adult mice harvested between 5 and 10 months of age. Scale bar represents 200  $\mu$ m. (C–F) Representative eyes from wild-type embryos harvested at E13.5 (C) and E17.5 (E) and their *Rere*<sup>om/eyes3</sup> littermates (D and F) demonstrate microphthalmia and incomplete closure of the optic fissure (white arrows in D and F). Failure of closure was seen in 8/10 (80%) of *Rere*<sup>om/eyes3</sup> eyes examined at E13.5 and in 6/6 (100%) of *Rere*<sup>om/eyes3</sup> eyes examined at E17.5. Scale bar represents 0.5 mm (C and D) or 1 mm (E and F).

sensorineural hearing loss, cleft lip, duodenal atresia, cryptorchidism, hip dysplasia, and scoliosis, all of which were identified in only one of our subjects. This overlap suggests that haploinsufficiency of *RERE* might also be sufficient to cause these additional phenotypes.

The phenotypic overlap between *Rere*-deficient zebrafish and RERE-deficient mice and our subjects provides further evidence that the deleterious sequence changes observed in *RERE* are the cause of many of the phenotypes observed in our subjects. This overlap also suggests that these animals represent useful models in which to study RERE's role in the development of many organ systems (Table 2). Specifically, we note that zebrafish carrying homozygous mutations in *reera*—the zebrafish homolog of *RERE*—have microphthalmia, inconsistent startle response, and decreased microphonic potentials.<sup>13,17</sup> This provides further evidence that changes in *RERE* are likely to be responsible for the eye anomalies and sensorineural hearing loss seen in our subjects.

Mice that are compound heterozygous for an *Rere*-null allele (*om*) and a hypomorphic allele (*eyes3*) have an even greater phenotypic overlap with our subjects. We have previously shown that these mice have microphthalmia, optic nerve hypoplasia, sensorineural hearing loss, congenital heart defects, and post-natal growth deficiency.<sup>14</sup> They also have abnormal brain development including a reduced brain size and weight, decreased numbers of NeuN-positive hippocampal neurons, cerebellar foliation defects, and delayed Purkinje cell maturation and migration in the cerebellum.<sup>14,15</sup>

Ventriculomegaly is seen in 38% (3/8) of our subjects. To determine whether *Rere*<sup>om/eyes3</sup> mice also have ventriculomegaly, we compared cresyl-violet-stained sagittal brain sections from adult *Rere*<sup>om/eyes3</sup> mice and their wild-type littermates. Obtaining large numbers of *Rere*<sup>om/eyes3</sup> mice for study is difficult because only a small fraction live into adulthood.<sup>14</sup> However, in the three adult *Rere*<sup>om/eyes3</sup> brains examined, all had enlarged lateral ventricles in com-

parison to those of their littermate controls (Figures 2A and 2B). Given that 20% (2/10) of our subjects have colobomas, we also looked for evidence of incomplete closure of the optic fissure—a precursor to the development of coloboma—in *Rere*<sup>om/eyes3</sup> embryos. Although the optic fissures of wild-type mice close between embryonic days 11 (E11) and E13, we found incomplete closure of the optic fissure in 80% (8/10) of *Rere*<sup>om/eyes3</sup> eyes examined at E13.5 and 100% (6/6) of the *Rere*<sup>om/eyes3</sup> eyes examined at E17.5 (Figures 2C–2F).<sup>34</sup> In contrast, the optic fissures of all wild-type control littermates were closed at these time points.

Phenotype-genotype correlations are often not possible to discern with a cohort of ten individuals, given that genetic and non-genetic influences outside of the gene can also affect the development of the final phenotype. However, we have observed a partial association between missense mutations in the atrophin 1 domain and clinical severity. Thus, subjects 1–3 and subject 5, all of whom have missense mutations in this domain, have anatomic abnormalities of both the eye and brain. Subjects 1–3 all have VSDs. Subjects 3–5 all have vesicoureteral reflux, and subjects 1 and 2 present with other genitourinary malformations (Table S1). However, it is also possible that the phenotypic differences seen between subjects are due to the effects of other genetic, epigenetic, environmental, and/or stochastic factors. This hypothesis is supported by subjects 4 and 5 whose missense mutations in *RERE* result in the same amino acid change. Although they share many phenotypes in common—intellectual disability, developmental delay, seizures, abnormal and/or thin corpora callosa, abnormal cerebellar vermis, vesicoureteral reflux, deep-set eyes, and abnormal ears—they have other features that are discordant. This hypothesis is also consistent with the phenotypic variation seen in RERE-deficient mice on different genetic backgrounds.<sup>14</sup>

RERE has previously been shown to positively regulate retinoic acid signaling during somite development.<sup>11</sup> Perturbations of retinoic acid signaling could also be the

underlying cause of RERE deficiency's effects on eye and heart development. We note that autosomal-recessive mutations in stimulated by retinoic acid 6 (*STRA6* [MIM: 610745])—which encodes a transmembrane protein that transports retinol into cells—have been shown to cause microphthalmia, isolated with colobomas 8 (MIM: 601186), and microphthalmia, syndromic 9 (MIM: 601186), also known as Matthew-Wood syndrome. This syndrome is characterized by congenital heart defects and eye anomalies—microphthalmia and coloboma—that are similar to those seen in subjects 1 and 2.<sup>35–37</sup> Similarly, recessive loss-of-function mutations in aldehyde dehydrogenase 1 family, members A3 (*ALDH1A3* [MIM: 600463]), which encodes an enzyme that converts retinaldehyde to retinoic acid, cause microphthalmia, isolated 8 (MIM: 615113).<sup>38</sup>

In conclusion, we have shown that mutations in *RERE* cause an autosomal-dominant genetic syndrome characterized by neurodevelopmental defects, hypotonia, seizures, behavioral problems, structural CNS anomalies—abnormalities and/or thinning of the corpus callosum, diminished white matter volume, abnormal cerebellar vermis, and ventriculomegaly—ophthalmologic anomalies, congenital heart defects, and genitourinary abnormalities. Many of these phenotypes are seen in individuals with proximal 1p36 deletions that include *RERE*. This suggests that haploinsufficiency of *RERE* might be sufficient to cause many of the phenotypes associated with proximal 1p36 deletions. These phenotypes are also recapitulated in *RERE*-deficient zebrafish and mice, which might serve as effective models for elucidating the molecular mechanisms by which *RERE* acts in various organ systems.

### Supplemental Data

Supplemental Data include a Supplemental Note, one figure, and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.03.002>.

### Acknowledgments

The authors thank the family members for participating in this research. This work was supported by the National Institute of Neurological Disorders and Stroke (grant R01 NS058721 to E.H.S.), the Netherlands Organization for Health Research and Development (grant 912-12-109 to B.B.A.d.V), and ODAS Stichting (to D.G.M.B. and B.B.A.d.V). E.H.S is a member of the clinical advisory board of InVitaie and consults for Personalis. M.T.C, R.S., K.G.M, and J.J. are all employees of GeneDx, which provides exome sequencing on a clinical basis. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from clinical laboratory testing conducted at Baylor Miraca Genetics Laboratories, which provides exome sequencing on a clinical basis.

Received: December 14, 2015

Accepted: March 2, 2016

Published: April 14, 2016

### Web Resources

dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/>  
 ExAC Browser, <http://exac.broadinstitute.org/>  
 GenBank, <http://www.ncbi.nlm.nih.gov/genbank/>  
 MutationTaster, <http://www.mutationtaster.org/>  
 NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>  
 OMIM, <http://www.omim.org/>  
 PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>  
 SFARI-base, <http://sfari.org/resources/sfari-base>.  
 SIFT, <http://sift.bii.a-star.edu.sg/>  
 UniProtKB, <http://www.uniprot.org/help/uniprotkb>

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