Ataxia, Dementia, and Hypogonadotropism Caused by Disordered Ubiquitination

David H. Margolin, M.D., Ph.D., Maria Kousi, Ph.D., Yee-Ming Chan, M.D., Ph.D., Elaine T. Lim, M.S., Jeremy D. Schmahmann, M.D., Marios Hadjivassiliou, M.D., Janet E. Hall, M.D., Ibrahim Adam, M.D., Andrew Dwyer, N.P., Lacey Plummer, B.S., Stephanie V. Aldrin, B.A., Julia O’Rourke, Ph.D., Andrew Kirby, B.S., Kasper Lage, Ph.D., Aubrey Milunsky, M.B., B.Ch., D.Sc., Jeff M. Milunsky, M.D., Jennifer Chan, M.D., E. Tessa Hedley-Whyte, M.D., Mark J. Daly, Ph.D., Nicholas Katsanis, Ph.D., and Stephanie B. Seminara, M.D.

From the Department of Neurology (D.H.M., J.D.S.), Harvard Reproductive Sciences Center and Reproductive Endocrine Unit (Y.-M.C., J.E.H., A.D., L.P., S.V.A., J.O., S.B.S.), Analytic and Translational Genetics Unit (E.T.L., A.K., K.L., M.J.D.), Department of Medicine, Pediatric Surgical Research Laboratories (K.L.), and Department of Neuropathology (E.T.H.-W.), Massachusetts General Hospital, Division of Endocrinology, Department of Medicine, Boston Children’s Hospital (Y.-M.C.), and Department of Pathology, Brigham and Women’s Hospital (J.C.) — all in Boston; Center for Human Genetics, Cambridge, MA (A.M., J.M.M.); Center for Human Disease Modeling, Department of Cell Biology (M.K., N.K.), and Department of Pediatrics (N.K.), Duke University Medical Center, Durham, NC; Department of Neurology, Royal Hallamshire Hospital, Sheffield, United Kingdom (M.H.); Specialty Hospital, Amman, Jordan (J.A.); and Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, and Center for Protein Research, University of Copenhagen, Copenhagen (K.L.). Address reprint requests to Dr. Seminara at the Reproductive Endocrine Unit, Massachusetts General Hospital, Boston, MA 02115, or at seminara.stephanie@mgh.harvard.edu; or to Dr. Katsanis at the Center for Human Disease Modeling, Duke University, Durham NC 27710, or at katsanis@cellbio.duke.edu.

Drs. Margolin, Kousi, and Y.-M. Chan and Drs. Katsanis and Seminara contributed equally to this article.

This article was published on May 8, 2013, at NEJM.org.


Copyright © 2013 Massachusetts Medical Society.

ABSTRACT

BACKGROUND

The combination of ataxia and hypogonadism was first described more than a century ago, but its genetic basis has remained elusive.

METHODS

We performed whole-exome sequencing in a patient with ataxia and hypogonadotropic hypogonadism, followed by targeted sequencing of candidate genes in similarly affected patients. Neurologic and reproductive endocrine phenotypes were characterized in detail. The effects of sequence variants and the presence of an epistatic interaction were tested in a zebrafish model.

RESULTS

Digenic homozygous mutations in RNF216 and OTUD4, which encode a ubiquitin E3 ligase and a deubiquitinase, respectively, were found in three affected siblings in a consanguineous family. Additional screening identified compound heterozygous truncating mutations in RNF216 in an unrelated patient and single heterozygous deleterious mutations in four other patients. Knockdown of rnf216 or otud4 in zebrafish embryos induced defects in the eye, optic tectum, and cerebellum; combinatorial suppression of both genes exacerbated these phenotypes, which were rescued by nonmutant, but not mutant, human RNF216 or OTUD4 messenger RNA. All patients had progressive ataxia and dementia. Neuronal loss was observed in cerebellar pathways and the hippocampus; surviving hippocampal neurons contained ubiquitin-immunoreactive intranuclear inclusions. Defects were detected at the hypothalamic and pituitary levels of the reproductive endocrine axis.

CONCLUSIONS

The syndrome of hypogonadotropic hypogonadism, ataxia, and dementia can be caused by inactivating mutations in RNF216 or by the combination of mutations in RNF216 and OTUD4. These findings link disordered ubiquitination to neurodegeneration and reproductive dysfunction and highlight the power of whole-exome sequencing in combination with functional studies to unveil genetic interactions that cause disease. (Funded by the National Institutes of Health and others.)
I
n recent years, we have seen great advances in the elucidation of genetic causes of cerebellar ataxia, with newly identified genes regulating a wide spectrum of cellular functions, including intracellular signaling, tau regulation, and mitochondrial function. However, a genetic defect cannot be found in approximately 40% of patients with ataxia, including those in whom ataxia is associated with reproductive endocrine failure, a syndrome first reported by Gordon Holmes in 1908. Most patients with this syndrome have a hypogonadotropic condition, with defective secretion of gonadotropins by the pituitary gland. Strikingly, genes associated with ataxia have little functional overlap with genes associated with hypogonadotropic hypogonadism, which encode proteins involved in the biologic function of the neurons that secrete gonadotropin-releasing hormone (GnRH).

A decade ago, we described a consanguineous family with a syndrome of cerebellar ataxia, dementia, and hypogonadotropic hypogonadism. Here we report the results of whole-exome and targeted sequencing performed to identify mutations that underlie the syndrome in this kindred and in unrelated patients.

METHODS

STUDY PATIENTS
Our study included 12 patients with ataxia and hypogonadotropic hypogonadism from eight families. The pedigrees of the index family and four of the other seven families are shown in Figure 1. The patients were referred to the Massachusetts General Hospital for clinical or genetic evaluation between 2000 and 2010. The study was approved by the hospital’s human research committee, and written informed consent for all participants was provided by the participant or an authorized representative.

GENETIC ANALYSIS
We performed exome sequencing with DNA from Patient 3 in the index family. The data sets used for exome analysis in this study were obtained from dbGaP at www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000475.v1.p1. Candidate genes were sequenced in family members and in unrelated affected persons. Computer algorithms were used to predict the pathogenicity of variants and to identify interactions between candidate genes and genes known to be associated with ataxia or hypogonadotropic hypogonadism. Allele-specific reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays were performed with RNA from Patients 5, 6, and 7 (see the Methods section in the Supplementary Appendix, available with the full text of this article at NEJM.org).

NEUROPATHOLOGICAL AND ENDOCRINE EVALUATION
The brain of Patient 2 was obtained within 6 hours after death. Immunohistochemical analysis was performed with the use of antibodies against ubiquitin, tau, and α-synuclein. Electron microscopy was performed according to standard procedures. Detailed reproductive endocrine phenotyping was performed in 5 patients, as described in our previous report and in the Methods section in the Supplementary Appendix.

ZEBRAFISH INVESTIGATIONS
Morpholino oligonucleotides (MO) for the silencing of zebrafish rnf216 and otud4 were injected either alone or with nonmutant or mutant human messenger RNA (mRNA) encoding RNF216, mRNA encoding OTUD4, or both (see the Methods section in the Supplementary Appendix).

RESULTS

GENETIC STUDIES
The consanguineous pedigree of the index family (a self-reported Palestinian family) includes three siblings (Patients 1, 2, and 3) with ataxia and hypogonadotropic hypogonadism. Exome sequencing performed with DNA from Patient 3 identified 13 homozygous variants that were rare and predicted to be deleterious (Table S1 in the Supplementary Appendix), 2 of which were also homozygous in the two other affected siblings: RNF216 (NM_207111.3) c.2251C→T, p.R751C and OTUD4 (NM_001102653.1) c.998G→T, p.G333V; these variants were not identified or were heterozygous in the unaffected family members (Fig. 1). RNF216 encodes an E3 ubiquitin-protein ligase. The R751 residue of RNF216 resides within the second of two domains called “really interesting new gene” (RING) finger domains and is conserved across vertebrates (Fig. S1 in the Supplementary Appendix). The R751C variant is predicted to be deleterious by four prediction tools.
and in 672 chromosomes from Middle Eastern persons (including 36 chromosomes from Palestinian persons). OTUD4 encodes a deubiquitinase...
Supplementary Appendix). Patient 4 had com-
in four probands (Fig. 1, and Fig. S1 in the
considered to be overtly deleterious and nine con-
in the ESP, which did identify five other variants
OTUD4, families. No rare variants were identified in
nine affected persons from seven unrelated
chance (P<1×10
sidered likely to be deleterious. Given this back-
prediction programs.
c.721C→T, p.Q241X in Patient 7; and c.2149C→T,
zygous mutations were identified: c.414delG,
[c.1791T→A; p.C597X]). Three additional hetero-
mutations ([c.615_616delGA; p.E205DfsX15] and
pound heterozygous frameshift and nonsense
Eastern persons.
2 of the 13,006 chromosomes from the ESP and
were used to identify potential
by Fisher’s exact test). Grail,
were found. Allele-specific RT-PCR assays failed
mRNA stability in the nonmutant alleles
of Patients 5, 6, and 7 (Fig. S2 in the Supplementary
Appendix).

Figure 1 (facing page). Segregation of RNF216 and
OTUD4 Mutations in the Index Pedigree and Identifi-
cation of Additional RNF216 Mutations in Unrelated
Probands.
The seven-generation pedigree shown in Panel A includes
Patients 1, 2, and 3, all of whom presented with ataxia,
dementia, and hypogonadotropic hypogonadism and
were homozygous for both RNF216 p.R751C and OTUD4
p.G333V. Double lines indicate consanguineous unions.
Genotyped, unaffected family members are shown to
be either homozygous for the nonmutated alleles (de-
noted with a + symbol) or heterozygous for one or both
changes. The pedigrees shown in Panel B are for the
families of additional RNF216 mutation-positive patients
(Patients 4 through 8), all of whom presented with
ataxia and hypogonadotropic hypogonadism. Squares
denote male family members, circles female family
members, solid symbols affected family members,
slashes deceased family members, diamonds siblings
of either sex, the triangle miscarriages, and Arabic
numbers the number of siblings or miscarriages.

(Fig. S1 in the Supplementary Appendix). The
G333V variant is predicted to be deleterious by
three of four prediction programs and is found in
2 of the 13,006 chromosomes from the ESP and
in none of the 672 chromosomes from Middle
Eastern persons.

Both OTUD4 and RNF216 were sequenced in
nine affected persons from seven unrelated
families. No rare variants were identified in
OTUD4, but mutations in RNF216 were identified
in four probands (Fig. 1, and Fig. S1 in the
Supplementary Appendix). Patient 4 had com-
pound heterozygous frameshift and nonsense
mutations (c.615_616delGA; p.E205DfsX15) and
c.1791T→A; p.C597X)). Three additional hetero-
zygous mutations were identified: c.414delG,
p.G333V. Double lines indicate consanguineous unions.
Genotyped, unaffected family members are shown to
be either homozygous for the nonmutated alleles (de-
noted with a + symbol) or heterozygous for one or both
changes. The pedigrees shown in Panel B are for the
families of additional RNF216 mutation-positive patients
(Patients 4 through 8), all of whom presented with
ataxia and hypogonadotropic hypogonadism. Squares
denote male family members, circles female family
members, solid symbols affected family members,
slashes deceased family members, diamonds siblings
of either sex, the triangle miscarriages, and Arabic
numbers the number of siblings or miscarriages.
nadotropic hypogonadism; no such connections
were found. Allele-specific RT-PCR assays failed
to identify occult mutations affecting transcrip-
tion or mRNA stability in the nonmutant alleles
of Patients 5, 6, and 7 (Fig. S2 in the Supplementary
Appendix).

FUNCTIONAL TESTING
Zebrafish were used to interrogate the functional
consequences and suggestive epistatic interac-
tions of the RNF216 and OTUD4 mutations.19,20
The injection of a MO that disrupted the splicing
of zebrafish rnf216 (Fig. S3 in the Supplementary
Appendix) caused a reduction in the size of the
eye cup and optic tecta and disorganization of the
cerebellum, as well as a slight reduction in
overall head size (Fig. 2 and 3, and Fig. S4 in the
Supplementary Appendix). The tectal phenotype
was rescued by the coinjection of human RNF216
mRNA, but the injection of human RNF216
mRNA encoding R751C failed to rescue the phen-
type (Fig. 2), suggesting that the mutation af-
fected the function of the encoded protein. The
injection of a splice-blocking MO against zebra-
fish otud4 (Fig. S3 in the Supplementary Appen-
dix) also induced a reduction in size of the optic
tecta and cerebellum (Fig. 3).

Coinjection of both rnf216 and otud4 MOs
induced a significant reduction in the size of the
optic tecta as compared with the injection of the
rnf216 MO alone (P<0.001) (Fig. 3). Double-MO
injection also caused marked disorganization of
the cerebellum in more than 60% of embryos
(Fig. 3) and the development of a severe cerebel-
lar phenotype with complete loss of structural
integrity in approximately 30% of embryos, as
compared with only 5 to 10% of embryos injected
with either the rnf216 or the otud4 MO alone
(data not shown). Furthermore, double-MO in-
jection resulted in marked microphthalmia as
compared with modest microphthalmia when
either MO injection was used alone (Fig. S4 in the
Supplementary Appendix). These phenotypes
were specific, since coinjection of nonmutant hu-
man RNF216 or OTUD4 mRNA rescued all phen-
otypes (Fig. 3, and Fig. S4 in the Supplementary
Appendix). RNF216 mRNA encoding R751C
and OTUD4 mRNA encoding G333V were less
effective in rescuing the phenotypes induced by
double-MO injection (Fig. 3, and Fig. S4 in the
Supplementary Appendix), suggesting not only
that these mutant alleles encode functionally
deficient proteins but also that epistatic interac-

n engl j med 368;21 nejm.org may 23, 2013
1995
The New England Journal of Medicine
Downloaded from nejm.org at PARTNERS HEALTHCARE on July 13, 2015. For personal use only. No other uses without permission.
Copyright © 2013 Massachusetts Medical Society. All rights reserved.
tions between these mutations contribute to the disease phenotype in the index pedigree.

CLINICAL CHARACTERISTICS OF THE STUDY PATIENTS

Patients 1 through 8, who carried variants in RNF216, had similar clinical histories (Table 1). They presented in adolescence or early adulthood with hypogonadotropic hypogonadism but no other pituitary abnormalities. Dysarthria was the initial neurologic symptom in some patients, but ataxia developed in all patients, leading to wheelchair dependency and to bed confinement for some patients. Dementia was also prominent, with personality changes and memory loss occurring at the onset of the disease and mutism and uncoordinated, purposeless movements during the end stages. Nystagmus was absent. The presentation of Patients 9 through 12, who did not have variants in RNF216, was quite different from that of Patients 1 through 8 (Table 1). Extensive evaluation did not reveal any known causes of ataxia in any of the patients; mitochondrial abnormalities were identified in Patients 7 and 8 (Table S2 in the Supplementary Appendix).

Neuroimaging performed in Patients 1 through 8 revealed striking similarities, with cerebellar and cortical atrophy but no abnormalities of the pituitary gland. The subcortical white matter contained patchy areas of hyperintensity on T2-weighted imaging and fluid-attenuated inversion recovery (FLAIR) imaging (Table 1 and Fig. 4). In Patient 7, these areas of hyperintensity were present approximately 9 years before the onset of neurologic symptoms; the cerebellum appeared normal at that earlier point in time.

NEUROPATHOLOGICAL STUDIES

The formalin-fixed brain of Patient 2 weighed 940 g (normal weight, 1300 g). The cerebellum and inferior olives were atrophic. Histopathological analysis revealed gliosis and virtually complete loss of inferior olivary neurons, cerebellar Purkinje's cells, and neurons in hippocampal regions CA3 and CA4, whereas neurons were well preserved in regions CA1 and CA2. Ubiquitin-immunoreactive nuclear inclusions were present in 1 to 5% of the pyramidal neurons in hippocampal regions CA1 and CA2 (Fig. 4). In Patient 7, these areas of hyperintensity were present approximately 9 years before the onset of neurologic symptoms; the cerebellum appeared normal at that earlier point in time.
On electron microscopy, the intranuclear inclusions appeared as aggregates of fine filaments and granular material (Fig. 4).

**REPRODUCTIVE ENDOCRINE STUDIES**

When Patient 6 reached 32 years of age, 1 year after the development of neurologic symptoms, low-amplitude pulses of luteinizing hormone were detected, indicating that GnRH secretion, although present, was diminished (Fig. 5). The administration of pulsatile GnRH for 7 days induced robust increases in levels of gonadotropins and estradiol (Fig. 5) as well as the growth of a dominant ovarian follicle, observed on ultrasonography (not shown). Although the secretion of luteinizing hormone increased in response to the administration of GnRH, the typical peaked pattern of luteinizing hormone pulses was not seen, which suggested a degree of pituitary dysfunction. Indeed, the patient’s pituitary responsiveness waned over time, with a diminished response to GnRH on day 1 of treatment 15 months after the initial endocrine study (Fig. 5).

In Patient 8, in whom endocrine function was initially assessed before the onset of neurologic symptoms, there was an absence of endogenous pulsatile luteinizing hormone secretion (Fig. 5).
<table>
<thead>
<tr>
<th>Patient and Race or Ethnic Group</th>
<th>Sex</th>
<th>Clinical Features</th>
<th>Imaging Findings</th>
<th>RNF216 Genotype</th>
<th>OTUD4 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1, Palestinian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>Male</td>
<td>No spontaneous puberty; at 22 yr, dysarthria, followed by progressive ataxia and dementia; at 43 yr, death (aspiration pneumonia)</td>
<td>At 30 yr, CT revealed prominent cerebellar and mild cortical atrophy, with hypodensities in cerebral white matter</td>
<td>R751C + R751C</td>
<td>G333V + G333V</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Female</td>
<td>At 16 yr, menarche, followed by secondary amenorrhea; at 20 yr, personality change; at 30 yr, dysarthria, followed by progressive ataxia and dementia; at 41 yr, death (aspiration pneumonia)</td>
<td>At 30 yr, CT revealed prominent cerebellar and mild cortical atrophy, with hypodensities in cerebral white matter</td>
<td>R751C + R751C</td>
<td>G333V + G333V</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Male</td>
<td>Normal puberty; at 20 yr, erectile dysfunction; at 29 yr, dysarthria, followed by progressive ataxia and dementia; at 47 yr, death (possibly from pulmonary embolism)</td>
<td>At 35 yr, MRI revealed diffuse parenchymal volume loss in cerebellum and cerebral cortex, with multiple punctate and confluent areas of hyperintensity on T2-weighted and FLAIR imaging</td>
<td>R751C + R751C</td>
<td>G333V + G333V</td>
</tr>
<tr>
<td>Family 2, white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 4</td>
<td>Male</td>
<td>No spontaneous puberty; at 22 yr, dysarthria, ataxia, and dementia; at 30 yr, prominent chorea; at 36 yr, death</td>
<td>At 23 yr, MRI revealed cerebellar atrophy and widespread foci of hyperintensity in cerebral white matter and thalami</td>
<td>CS97X + E205DfsX15</td>
<td>Nonmutant + Nonmutant</td>
</tr>
<tr>
<td>Patient 5</td>
<td>Male</td>
<td>Normal puberty; at 36 yr, hypogonadotropism and chorea, followed by progressive ataxia and dementia</td>
<td>At 42 yr, MRI revealed global atrophy, with diffusely scattered periventricular foci of hyperintensity on T2-weighted and FLAIR imaging</td>
<td>G138GfsX74 + Nonmutant</td>
<td>Nonmutant + Nonmutant</td>
</tr>
<tr>
<td>Patient 6</td>
<td>Female</td>
<td>Normal puberty; at 27 yr, oligomenorrhea, followed by amenorrhea; memory problems; at 31 yr, chorea, followed by progressive ataxia and dementia</td>
<td>At 31 yr, MRI revealed mild-to-moderate cerebellar atrophy and mild prominence of ventricles and sulci, with multiple small foci of hyperintensity on T2-weighted imaging</td>
<td>G138GfsX74 + Nonmutant</td>
<td>Nonmutant + Nonmutant</td>
</tr>
<tr>
<td>Family 3, white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 7</td>
<td>Female</td>
<td>Primary amenorrhea; at 27 yr, ataxia and dysarthria, followed by progressive ataxia and dementia</td>
<td>At 18 yr, MRI revealed a normal cerebellum and multiple foci of hyperintensity in subcortical white matter on T2-weighted imaging; at 35 yr, MRI revealed marked cerebellar atrophy, with an increased number of foci of T2-weighted hyperintensity</td>
<td>Q241X + Nonmutant</td>
<td>Nonmutant + Nonmutant</td>
</tr>
<tr>
<td>Family 4, white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 8</td>
<td>Male</td>
<td>No spontaneous puberty; at 19–21 yr, partial response to treatment with exogenous pulsatile GnRH; at 21 yr, slurred speech and imbalance, followed by progressive ataxia, mood changes, and memory impairment</td>
<td>At 17 yr, MRI revealed slight prominence of fissures in cerebellum; at 23 yr, MRI revealed severe cerebellar and mild cerebral atrophy, multiple foci of T2-weighted and FLAIR hyperintensity</td>
<td>R717C + Nonmutant</td>
<td>Nonmutant + Nonmutant</td>
</tr>
</tbody>
</table>
### Family 6, white

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Puberty</th>
<th>Symptoms</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Male</td>
<td>No spont.</td>
<td>at 5 yr, ataxia, nystagmus; at 34 yr, still able to walk</td>
<td>Nonmutant +</td>
<td>Nonmutant</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>No spont.</td>
<td>at 5 yr, ataxia, nystagmus; at 32 yr, still able to walk</td>
<td>Nonmutant +</td>
<td>Nonmutant</td>
</tr>
</tbody>
</table>

### Family 7, white

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Puberty</th>
<th>Symptoms</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Male</td>
<td>No spont.</td>
<td>at 4 yr, ataxia; at 28 yr, ataxia stable</td>
<td>Nonmutant +</td>
<td>Nonmutant</td>
</tr>
</tbody>
</table>

### Family 8, Asian

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Puberty</th>
<th>Symptoms</th>
<th>Genotype 1</th>
<th>Genotype 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Female</td>
<td>Normal</td>
<td>at 17 yr, behavior problems, followed by tremor, dysarthria, and ataxia; at 19 yr, internuclear ophthalmoplegia; at 24 yr, hypogonadotropism</td>
<td>Nonmutant +</td>
<td>Nonmutant</td>
</tr>
</tbody>
</table>

* CT denotes computed tomography, FLAIR fluid-attenuated inversion recovery, GnRH gonadotropin-releasing hormone, and MRI magnetic resonance imaging.

## DISCUSSION

The underpinnings for the association of ataxia with hypogonadotropic hypogonadism have eluded investigators for more than a century. This report shows that ataxia with hypogonadotropic hypogonadotropism can be caused by mutations in RNF216 or OTUD4 in zebrafish, resulting in cerebellar hypoplasia, microcephaly, and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease. Indeed, the phenotype in the index pedigree appears to have been caused by the interaction of hypomorphic mutations in RNF216 and OTUD4 in zebrafish, resulting in cerebellar hypoplasia and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease.

Heterozygous RNF216 mutations were found in Patients 5 through 8 but did not cause disease in their parents. Oligogenic inheritance for these observed patterns of hypogonadotropic hypogonadism can act with mutations at other genetic loci to cause disease. Indeed, the phenotype in the index pedigree appears to have been caused by the interaction of hypomorphic mutations in RNF216 and OTUD4 in zebrafish, resulting in cerebellar hypoplasia and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease.

Heterozygous RNF216 mutations were found in Patients 5 through 8 but did not cause disease in their parents. Oligogenic inheritance for these observed patterns of hypogonadotropic hypogonadism can act with mutations at other genetic loci to cause disease. Indeed, the phenotype in the index pedigree appears to have been caused by the interaction of hypomorphic mutations in RNF216 and OTUD4 in zebrafish, resulting in cerebellar hypoplasia and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease.

Inhibition of either RNF216 or OTUD4 in zebrafish resulted in cerebellar hypoplasia, microcephaly, and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease. Indeed, the phenotype in the index pedigree appears to have been caused by the interaction of hypomorphic mutations in RNF216 and OTUD4 in zebrafish, resulting in cerebellar hypoplasia and isolated hypogonadotropic hypogonadism that can act with mutations at other genetic loci to cause disease.

Although pituitary responsiveness was assessed, there was no detectable luteinizing hormone secretion and no measurable change in the gonadotropin level in response to the administration of pulsatile exogenous GnRH (Fig. 5).
Thalmia, and small optic tecta. The concordance of these phenotypes suggests that RNF216 and OTUD4 operate in the same pathway. This possibility is bolstered by the observation of epistatic interactions between RNF216 and OTUD4, with simultaneous knockdown of both genes resulting in more severe phenotypes. Taken together, these findings support a digenic model in which the OTUD4 mutation, in conjunction with the RNF216 mutation, played an essential role in causing disease in the index pedigree. By extension, in Patients 5 through 8, it is possible that mutations in other, currently unidentified loci may have acted in conjunction with the heterozygous mutations in RNF216 to cause disease. Oligogenicity is likely to be increasingly recognized as methods for detecting this genetic architecture, such as exome sequencing, are more widely adopted.
RNF216 encodes an E3 ubiquitin ligase that attaches ubiquitin to protein substrates, marking them for proteasome-mediated degradation. Known targets of RNF216 include upstream activators of nuclear factor κB signaling, which regulates diverse cellular processes.\textsuperscript{28-31} RNF216
is structurally similar to parkin, an E3 ubiquitin ligase that is mutated in a recessive form of Parkinson’s disease. The finding of neuronal intranuclear inclusions in Patient 2 may indicate that RNF216-associated neurodegeneration has similarities not only with Parkinson’s disease but also with other neurodegenerative disorders in which protein aggregates are found, such as Huntington’s disease and Alzheimer’s disease.

OTUD4 encodes a deubiquitinating enzyme. Deubiquitinases allow target proteins and ubiquitin itself to be recycled and often function in partnership with specific E3 ligases. For example, the deubiquitinase ataxin-3 counteracts the ability of parkin to ubiquitinate itself. On the basis of this and other examples, OTUD4 and RNF216 may be similarly linked in a coregulatory partnership.

The progressive and debilitating dementia observed in the patients with RNF216 mutations (Patients 1 through 8) distinguishes them from the other patients with ataxia and hypogonadotropic hypogonadism. Furthermore, we observed changes in cerebral white matter in all the patients with RNF216-associated neurodegeneration, suggesting that such changes may constitute a consistent feature of this syndrome. None of these patients had oculomotor abnormalities such as the nystagmus and ophthalmoplegia seen in Patients 9, 10, and 12, who did not have RNF216 mutations.

The patients with RNF216-associated neurodegeneration had dysfunction at multiple levels of the reproductive endocrine axis. In Patients 6 and 8, reproductive function was restored with extended GnRH treatment, which suggests that hypothalamic GnRH deficiency was the primary cause of their reproductive endocrine dysfunction. However, these two patients also appeared to have an element of pituitary dysfunction, given the diminishing responses to GnRH over time in Patient 6 and the observation of a right-shifted dose–response curve in Patient 8. In Patients 1 and 2, who were evaluated late in the course of their disease, the complete absence of response after 7 days of treatment with GnRH may represent progression of this pituitary dysfunction. The basis for the selective vulnerability of particular neuronal and pituitary cell types is currently unexplained.

In conclusion, we have identified loss-of-function mutations in RNF216 that cause a syndrome of ataxia, dementia, and hypogonadotropic hypogonadism. Genetic and in vivo evidence suggests that mutations affecting RNF216, an E3 ubiquitin ligase, and OTUD4, a deubiquitinase, can synergize to cause this syndrome, reinforcing the notion that the mutational load within biologic pathways can drive disease manifestation. Taken together, these data highlight a hitherto unknown role of the ubiquitination system in disorders of combined neurodegeneration and reproductive dysfunction. More broadly, our findings show the value of combining individual whole-exome sequencing with in vivo functional studies to identify disease-causing gene mutations and epistatic interactions.

The views expressed in this article are those of the authors and do not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health.

Supported by grants from the Eunice K. Shriver National Institute for Child Health and Human Development (K24 HD067388, R01 HD043341, R01 HD042601, and U54 HD028138); from the National Institute of Diabetes and Digestive and Kidney Diseases (P50 DK096415); by Harvard Catalyst and the Harvard Clinical and Translational Science Center (funded by the National Center for Research Resources and the National Center for Advancing Translational Sciences [UL1TR025758 and M01RR01066]) and Harvard University and its affiliated academic health care centers); by a grant from the National Human Genome Research Institute to the Broad Institute (U54 HG009367); and by grants from the National Heart, Lung, and Blood Institute for the Exome Sequencing Project (HL102923, HL102924, HL102925, HL102926, and HL103010). Dr. Chan is the recipient of a Charles A. King Trust postdoctoral fellowship and a Career Development Award from Boston Children’s Hospital.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients and their families for their cooperation in these studies; Dr. Fawzi al-Hammouri and the physicians and staff of the Specialty Hospital, Amman, Jordan, for assisting with clinical care for Patients 1, 2, and 3; Dr. Momen al-Hadidi and the staff of the Forensic Medicine Department at Al-Basheer Government Hospital, Amman, Jordan, for performing the autopsy and obtaining the brain after the death of Patient 2; Drs. Daniel Metzger, Susan Ratzan, Meriel McIntagart, and Sandra Sirrs for patient referrals; Dr. J. Michael Andresen for performing the initial linkage analysis; Drs. Christopher Walsh and Timothy Yu at Boston Children’s Hospital for sharing data from the Middle Eastern control patients; Carlotta Fitch and Dr. Kathy Newell for assisting with the neuropathological analyses; Dr. Omar Abu Hijleh for providing the early endocrine history of Patients 1 and 2; the staff of the Massachusetts General Hospital (MGH) Clinical Research Center for assisting with clinical protocols; members of the MGH Reproductive Endocrine Unit; and Eric Lander and the Broad Institute for generating high-quality sequence data.

The New England Journal of Medicine
Downloaded from nejm.org at PARTNERS HEALTHCARE on July 13, 2015. For personal use only. No other uses without permission. Copyright © 2013 Massachusetts Medical Society. All rights reserved.
References


Copyright © 2013 Massachusetts Medical Society.