

# Gordon Holmes syndrome and Huntington-like disease: Two types of *RNF216*-related disorders

Chujun Wu (✉ [wuchujun612@163.com](mailto:wuchujun612@163.com))

Beijing Tiantan Hospital <https://orcid.org/0000-0003-1810-2331>

Zaiqiang Zhang

Beijing Tiantan Hospital <https://orcid.org/0000-0002-0053-3451>

---

## Research Article

**Keywords:** RNF216, Gordon Holmes syndrome, ataxia, chorea, hypogonadotropic hypogonadism

**Posted Date:** February 7th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1310364/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background and purpose:** Cases of *RNF216*-related disorders have been reported sporadically. The systemic clinical classification and phenotype-genotype correlations of these disorders have not been fully studied.

**Methods:** We report the case of a patient with a novel homozygous mutation in *RNF216* and review the cases of all patients with *RNF216* mutations reported in the literature. These patients were classified by clinical phenotypes into those with Gordon Holmes syndrome (GHS) and those with Huntington-like disease (HLD). Clinical and genetic features were compared between the groups.

**Results:** The cases of twenty-one patients from 14 families with *RNF216* mutations were identified and collected. Eleven (52.4%) patients presented with GHS, and eight (38.1%) presented with HLD. GHS patients had a younger age of onset (24.2 vs. 36.9 years,  $P < 0.001$ ) and a higher percentage of male patients (81.8% vs. 25.0%,  $P = 0.024$ ) than HLD patients. Five (62.5%) HLD patients had ataxia, but only one (9.1%) GHS patient developed chorea. Male patients had a higher percentage of poor pubertal development than female patients (69.2% vs. 12.5%,  $P = 0.024$ ). White matter lesions and cerebellar atrophy were the most common imaging findings in both groups. The mutations in nine (64.3%) families were inherited in a monogenic recessive pattern, whereas the mutations in five (35.7%) were inherited in an oligogenic pattern by acting with mutations in *OTUD4*, *SRA1* or other unknown genes. All eight mutations found in GHS patients and 57.1% (4/7) of mutations found in HLD patients resulted in amino acid changes or the truncation of the “RING-between-RING” (RBR) domain, which plays a key role in the ubiquitin E3 ligase activity of *RNF216*.

**Conclusion:** Patients with *RNF216* mutations mainly presented with GHS or HLD in a monogenic autosomal-recessive pattern or an oligogenic pattern. The mutations in GHS patients affected the RBR domain and are thought to abrogate ubiquitin E3 ligase activity. The pathogenic mechanism underlying *RNF216*-related HLD is still unknown.

## Introduction

The gene *RNF216* is an E3 ubiquitin ligase of the “RING-between-RING” (RBR) class that attaches ubiquitin to protein substrates, designating them for proteasome-mediated degradation or signal transduction.(1) Gordon Holmes syndrome (GHS) is an autosomal-recessive cerebellar ataxia characterized by ataxia, dementia and hypogonadotropic hypogonadism. In 2013, David et al reported *RNF216* mutations in patients with GHS for the first time.(2) Subsequently, more studies on *RNF216*-related disorders have been published.(3–10) In addition to GHS, some of those patients presented with Huntington-like disease (HLD), 4H syndrome (hypodontia, hypomyelination, ataxia and hypogonadotropic hypogonadism) and congenital hypogonadotropic hypogonadism (CHH), confirming the clinical heterogeneity of *RNF216*-related disorders.(3, 4, 8–10) The inheritance pattern of *RNF216*-related disorders includes a monogenic recessive pattern and an oligogenic pattern by acting with other genes.(2,

4, 10) Several different types of mutations have been observed, comprising missense, nonsense, splice site, and frameshift mutations and large deletions.(2, 5, 9) Although the number of case reports on *RNF216*-related disorders has been gradually increasing, a comprehensive review that covers all patients thus far is missing. Moreover, group comparisons and phenotype-genotype correlations have not been studied before.

In this study, we report the case of a patient with a novel homozygous mutation in *RNF216* and summarize the clinical and genetic features of all described patients. Furthermore, we aim to explore the phenotype-genotype correlations by comparing the clinical and genetic characteristics between different groups.

## Methods

### Patients

We performed whole-exome sequencing (WES) in a patient with the GHS phenotype using the Illumina HiSeq sequencing platform. The variants identified in WES were validated by Sanger sequencing. The disease-causing mutations were identified by bioinformatics analysis and pedigree analysis. The clinical and imaging information of the patient was collected and analysed.

A systematic literature review was performed via PubMed using the keyword "*RNF216*" in November 2021. Twenty patients with *RNF216* mutations from 13 families were identified. The clinical and genetic information of these patients was collected from the literature.

### Study design

GHS was defined as an autosomal-recessive cerebellar ataxia characterized by ataxia, dementia and hypogonadotropic hypogonadism. HLD was defined as characteristic clinical features of chorea, behavioural disturbances, dementia, and a lack of CAG repeat expansion in the HTT gene. According to the clinical manifestations, all of the patients were classified into a GHS group or a HLD group except for two, who were reported to have 4H syndrome or CHH. The clinical and imaging characteristics, as well as the mutation distribution, were compared between the groups.

## Data analysis

Student's t test was used for continuous data, and Fisher's exact test (two-tailed) was used for categorical data.  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 26.

## Results

### Case report

A 27-year-old male born to consanguineous Chinese parents (Figure 1A) presented with progressive slurred speech, balance difficulties and memory decline since the age of 26. He had poor development of secondary sexual characteristics due to hypogonadotropic hypogonadism (Additional file 1: Table S1). There was no relevant family history. Neurological examination showed dysarthria, lower limb and gait ataxia, as well as brisk tendon reflexes. No nystagmus was noted. The Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) scores were 30 and 24, respectively. A brain magnetic resonance imaging (MRI) series demonstrated diffuse white matter lesions and cerebellar atrophy years before the onset of neurological symptoms (Figure 1B-M). Fluid attenuated inversion recovery (FLAIR) imaging revealed patchy and confluent white matter lesions affecting the brainstem and the periventricular and subcortical areas. Hyperintensity in the thalami was found on FLAIR images (Figure 1C, 1G, 1K). Neither gadolinium-enhanced lesions nor diffusion-restricted lesions were detected. The patient was clinically diagnosed with GHS. Whole-exome sequencing identified a novel homozygous nonsense mutation c.1549C>T (p.R517X) in the *RNF216* gene (NM\_207111.3). This mutation was predicted to create a premature stop codon, thus producing a truncated protein without a key RBR domain and C-terminal extension (Figure 2). Both parents were unaffected carriers of this mutation. This mutation was classified as pathogenic according to the American College of Medical Genetics (ACMG) guidelines.

## Literature review

## Clinical data

A total of 21 patients from 14 families carrying *RNF216* mutations were identified (Additional file 2: Table S2). Thirteen patients were male, and 8 were female. Eleven (52.4%) patients presented with GHS, eight (38.1%) presented with HLD and the remaining two (9.5%) presented with 4H syndrome or CHH. Patients from the same family shared similar symptoms and were classified into the same group. The mean age at the onset of neurological symptoms was  $29.5 \pm 8.6$  years (mean  $\pm$  standard deviation (SD), range 20-49 years). Five patients had died, including 4 patients diagnosed with GHS and 1 patient with HLD. The mean age at death was  $42.6 \pm 4.4$  years (mean  $\pm$  SD, range 36-47 years), and the disease duration was  $17.6 \pm 3.5$  (mean  $\pm$  SD, range 14-21 years). Compared with the HLD group, patients in the GHS group had a younger age of onset (24.2 vs. 36.9 years,  $P < 0.001$ ) and a higher percentage of male patients (81.8% vs. 25.0%,  $P = 0.024$ ) (Table 1). The neurological symptoms included cognitive decline (90.5%, 19/21), ataxia (81.0%, 17/21), dysarthria (66.7%, 14/21), chorea (42.9%, 9/21), psychological and behavioural abnormalities (33.3%, 7/21), pyramidal signs (23.8%, 5/21), broken saccadic eye movements (14.3%, 3/21) and gaze-evoked nystagmus (4.8%, 1/21). It should be noted that 62.5% (5/8) of HLD patients had ataxia, but only 9.1% (1/11) of GHS patients presented with chorea. Dysarthria was more common in GHS patients (90.9% in GHS vs. 50.0% in HLD,  $P = 0.111$ ), whereas psychological and behavioural abnormalities were more common in HLD patients (62.5% in HLD vs. 18.2% in GHS,  $P = 0.074$ ). Almost all patients suffered from hypogonadotropic hypogonadism to different degrees. Interestingly, male patients were more vulnerable to hypogonadotropic hypogonadism than female patients. No spontaneous or partial puberty occurred in 69.2% (9/13) of male patients but only 12.5% (1/8) of female patients

(P=0.024). GHS patients had a higher percentage of patients with poor pubertal development than HLD patients (81.8% vs. 0%, P=0.001), which might be associated with the sex differences between the two groups.

Table 1  
Summarized clinical and imaging features of RNF216-related GHS and HLD

	GHS(n=11)	HLD(n=8)	P value
<b>Clinical features</b>			
Age at onset, y, mean (range)	24.2(20-29)	36.9(27-49)	<0.001
Male, n(%)	9(81.8%)	2(25.0%)	0.024
Cognitive decline, n(%)	10(90.9%)	8(100%)	1.000
Ataxia, n(%)	11(100%)	5(62.5%)	0.058
Chorea, n(%)	1(9.1%)	8(100%)	<0.001
Dysarthria, n(%)	10(90.9%)	4(50.0%)	0.111
Psychological and behavioural abnormalities, n(%)	2(18.2%)	5(62.5%)	0.074
Poor pubertal development, n(%)	9(81.8%)	0(0%)	0.001
<b>Imaging features</b>			
White matter lesions, n(%)	11(100%)	8(100%)	
Cerebellar atrophy, n(%)	11(100%)	7(87.5%)	0.421
Cerebral atrophy, n(%)	7(63.6%)	5(62.5%)	1.000
Brainstem, n(%)	2(18.2%)	5(62.5%)	0.074
Basal ganglia, n(%)	1(9.1%)	3(37.5%)	0.262
Thalami, n(%)	2(18.2%)	2(25.0%)	1.000

#### Imaging data

White matter lesions (95.2%, 20/21) and cerebellar atrophy (90.5%, 19/21) were the most common imaging findings. Cerebral atrophy (57.1%, 12/21) and abnormal signalling in the brainstem (38.1%, 8/21), basal ganglia (23.8%, 5/21) and thalami (23.8%, 5/21) were also observed. However, gadolinium-enhanced lesions or diffusion-restricted lesions were not reported in any patient. Although there was no significant difference in imaging manifestations between the GHS group and the HLD group, we found that bilateral confluent white matter lesions surrounding the basal ganglia were associated with chorea (44.4% in patients with chorea vs. 0% in patients without chorea, P=0.033).

#### Genetic data

Seventeen mutations were detected in 21 patients from 13 families, including 7 missense mutations, 4 nonsense mutations, 3 splice site mutations, 2 frameshift mutations and 1 deletion of exon 2 (Figure 2). The mutations of nine (64.3%) families were inherited in monogenic recessive mode, and the mutations of 5 (35.7%) families were inherited in oligogenic mode by acting with mutations in *OTUD4* (c.998G>T, NM\_001102653.1), *SRA1* (c.2374G>A, NM\_001035235.3) or other unknown genes. All the mutations in GHS patients and 4 (57.1%) mutations in HLD patients resulted in amino acid changes or the truncation of the RBR domain. Another three mutations (c.1367G>A, c.1616A>G and a deletion of exon 2) in HLD patients were outside the RBR domain. The remaining two mutations in patients with 4H syndrome or CHH were located in the C-terminal extension of the RBR domain. GHS patients tended to have more mutations influencing RBR domain than HLD patients, although the difference did not reach statistical significance (100% vs. 57.1%,  $P=0.077$ ).

## Discussion

In this article, we report the case of a Chinese patient with a novel homozygous mutation, c.1549C>T, in *RNF216* and review the clinical, neuroimaging and genetic data of all patients with *RNF216* mutations published in the literature thus far.

*RNF216* disorders span a phenotypic continuum characterized by variable combinations of cerebellar ataxia, chorea, cognitive decline and hypogonadotropic hypogonadism. According to the clinical features, patients with *RNF216* mutations were categorized as having GHS or HLD, except for two with 4H syndrome or CHH. Four genes related to GHS include *RNF216*, either alone or in association with *OTUD4*, *STUB1* and *PNPLA6*.(2, 11, 12) *RNF216*-related GHS is characterized by dementia and confluent white matter lesions, which are rare in GHS patients with other gene mutations.(12, 13) In our review, GHS patients with *RNF216* mutations developed neurological symptoms in their 20s. Over 90% of those patients typically presented with ataxia, cognitive decline and dysarthria. HLD was another common clinical phenotype of *RNF216*-related disorders and characterized by chorea, cognitive decline, and psychological and behavioural abnormalities. Interestingly, we found that the average age at the onset of neurological symptoms in HLD patients was approximately 12 years older than that in GHS patients. The HLD group was predominantly female, whereas the GHS group was predominantly male. Despite the clinical heterogeneity, there was also an overlap between different phenotypes. For instance, cognitive decline was very common in both groups. More than half of HLD patients developed ataxia during the course of disease, and one GHS patient had accompanying chorea. Apart from GHS and HLD, a male patient with the homozygous mutation c.2453-2A>G in *RNF216* was reported to present with 4H syndrome.(3) However, it is still controversial to classify the patient as having 4H syndrome on the basis of atypical dental abnormalities and imaging manifestations. Nicole et al considered that this patient should be diagnosed with GHS rather than 4H syndrome.(14) Furthermore, a 19-year-old male patient, in whom digenic mutations in *RNF216* (c.2374G>A) and *SRA1* (c.2374G>A, NM\_001035235.3) were observed, presented with CHH without obvious neurological symptoms or MRI abnormalities.(10) Considering the age at the onset of neurological symptoms in other patients with *RNF216* mutations, we thought this patient might be too young to show neurological symptoms.

Another prominent feature in patients with *RNF216* mutations is hypogonadotropic hypogonadism due to diminished GnRH secretion and pituitary dysfunction.(2) Interestingly, male patients seemed to be more vulnerable than female patients. Most male patients suffered from poor pubertal development. In contrast, the majority of female patients had normal puberty, and three of them delivered successfully.(4, 8) This phenomenon was consistent with the results of previous animal experiments. The targeted deletion of the *RNF216* gene in mice resulted in disruption in spermatogenesis and male infertility, but *RNF216* was not required for female fertility.(15)

White matter lesions and cerebellar atrophy were the most common imaging findings and were present in over 90% of patients. Cerebral atrophy and the involvement of the brainstem, basal ganglia and thalami were also observed in some patients. However, gadolinium-enhanced lesions or diffusion-restricted lesions were not detected in any patient. Characteristic bilateral white matter lesions surrounding the basal ganglia, which have been reported to be related to Huntington's disease,(16) were exclusively found in patients with chorea.(4, 6, 9)

*RNF216* is a ubiquitin ligase of the RBR class that attaches ubiquitin chains to substrates. For *RNF216*, various substrates have been reported, including TOLL-like receptors, tumour necrosis factor-receptor associated factor 3 (TRAF3), the autophagy regulator Beclin and the synaptic regulator activity regulated cytoskeleton associated protein (ARC).(1, 17–19) The ubiquitin ligase activity of *RNF216* requires not only the RBR domain but also an additional C-terminal extension of the RBR domain.(19, 20) All mutations in patients with GHS, 4H or CHH resulted in amino acid changes or the truncation of the RBR domain/C-terminal extension of the RBR domain, which was presumed to abrogate ubiquitin E3 ligase activity. However, three of seven mutations (c.1367G>A, c.1616A>G and a deletion in exon 2) in HLD patients did not affect the RBR region/C-terminal extension of the RBR domain. Further functional studies confirmed that the two missense mutations (c.1367G>A and c.1616A>G) in HLD patients reversed the E3 ubiquitin ligase activity of *RNF216*, implying that these mutations caused disease by mechanisms other than by influencing ubiquitin E3 ligase activity.(19) These findings indicated that *RNF216*-related GHS was strongly associated with mutations affecting the ubiquitin E3 ligase activity of *RNF216*. However, the pathogenic mechanism underlying *RNF216*-related HLD is more complicated and remains to be explored in future studies.

In summary, patients with *RNF216*-related disorders typically presented with GHS or HLD in a monogenic autosomal-recessive pattern or an oligogenic pattern. Mutations in GHS patients were closely related to the RBR domain and ubiquitin E3 ligase activity of *RNF216*. However, the pathogenic mechanism underlying *RNF216*-related HLD remains to be explored in future studies.

## Abbreviations

Gordon Holmes syndrome  
GHS  
Huntington-like disorders

HLD

hypodontia, hypomyelination, ataxia and hypogonadotropic hypogonadism

4H syndrome

congenital hypogonadotropic hypogonadism

CHH

RING-between-RING

RBR

## Declarations

### Ethics approval and consent to participate

The institutional ethics committee of Beijing Tian Tan Hospital, Capital Medical University approved this study. Written, informed consent was obtained from the patient.

### Consent for publication

Consent to publish has been obtained from participants to report individual patient data.

### Availability of data and materials

Supporting information is available from the corresponding author upon request.

### Competing interest

The authors declare that they have no competing interests.

### Funding

This project did not receive any financial support and was self-funded.

### Authors' contributions

CW collected the information of the patient, performed the systematic literature search and drafted the manuscript. ZZ substantively revised the work. Both authors read and approved the final manuscript.

### Acknowledgement

The authors thank the patient and his family for their cooperation in this study.

## References

1. Chuang TH, Ulevitch RJ. Triad3A, an E3 ubiquitin-protein ligase regulating Toll-like receptors. *Nature immunology*. 2004;5(5):495–502.

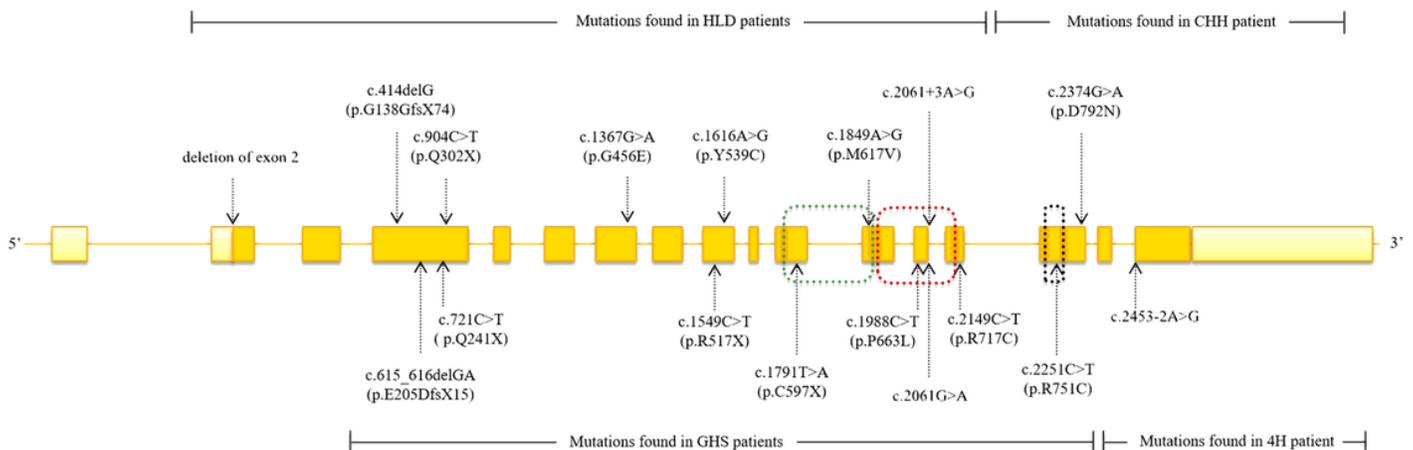
2. Margolin DH, Kousi M, Chan YM, Lim ET, Schmahmann JD, Hadjivassiliou M, et al. Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N Engl J Med*. 2013;368(21):1992–2003.
3. Ganos C, Hersheson J, Adams M, Bhatia KP, Houlden H. The 4H syndrome due to RNF216 mutation. *Parkinsonism Relat Disord*. 2015;21(9):1122–3.
4. Santens P, Van Damme T, Steyaert W, Willaert A, Sablonniere B, De Paepe A, et al. RNF216 mutations as a novel cause of autosomal recessive Huntington-like disorder. *Neurology*. 2015;84(17):1760–6.
5. Alqwaify M, Bohlega S. Ataxia and Hypogonadotropic Hypogonadism with Intrafamilial Variability Caused by RNF216 Mutation. *Neurology international*. 2016;8(2):6444.
6. Mehmood S, Hoggard N, Hadjivassiliou M. Gordon Holmes syndrome: finally genotype meets phenotype. *Pract Neurol*. 2017;17(6):476–8.
7. Calandra CR, Mocarbel Y, Vishnopolska SA, Toneguzzo V, Oliveri J, Cazado EC, et al. Gordon Holmes Syndrome Caused by RNF216 Novel Mutation in 2 Argentinean Siblings. *Movement disorders clinical practice*. 2019;6(3):259–62.
8. Lieto M, Galatolo D, Roca A, Cocozza S, Pontillo G, Fico T, et al. Overt Hypogonadism May Not Be a Sentinel Sign of RING Finger Protein 216: Two Novel Mutations Associated with Ataxia, Chorea, and Fertility. *Movement disorders clinical practice*. 2019;6(8):724–6.
9. Chen KL, Zhao GX, Wang H, Wei L, Huang YY, Chen SD, et al. A novel de novo RNF216 mutation associated with autosomal recessive Huntington-like disorder. *Ann Clin Transl Neurol*. 2020;7(5):860–4.
10. Neocleous V, Fanis P, Toumba M, Tanteles GA, Schiza M, Cinarli F, et al. GnRH Deficient Patients With Congenital Hypogonadotropic Hypogonadism: Novel Genetic Findings in ANOS1, RNF216, WDR11, FGFR1, CHD7, and POLR3A Genes in a Case Series and Review of the Literature. *Front Endocrinol*. 2020;11:626.
11. Synofzik M, Gonzalez MA, Lourenco CM, Coutelier M, Haack TB, Rebelo A, et al. PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. *Brain*. 2014;137(Pt 1):69–77.
12. Shi CH, Schisler JC, Rubel CE, Tan S, Song B, McDonough H, et al. Ataxia and hypogonadism caused by the loss of ubiquitin ligase activity of the U box protein CHIP. *Human molecular genetics*. 2014;23(4):1013–24.
13. Locci S, Bianchi S, Tessa A, Santorelli FM, Mignarri A. Gordon Holmes syndrome caused by two novel mutations in the PNPLA6 gene. *Clinical neurology neurosurgery*. 2021;207:106763.
14. Wolf NI, Bernard G. Mutations in RNF216 do not cause 4H syndrome. *Parkinsonism Relat Disord*. 2015;21(11):1387–8.
15. Melnick AF, Gao Y, Liu J, Ding D, Predom A, Kelly C, et al. RNF216 is essential for spermatogenesis and male fertility. *Biol Reprod*. 2019;100(5):1132–4.
16. Zhang J, Gregory S, Scahill RI, Durr A, Thomas DL, Lehericy S, et al. In vivo characterization of white matter pathology in premanifest huntington's disease. *Ann Neurol*. 2018;84(4):497–504.

17. Nakhaei P, Mesplede T, Solis M, Sun Q, Zhao T, Yang L, et al. The E3 ubiquitin ligase Triad3A negatively regulates the RIG-I/MAVS signaling pathway by targeting TRAF3 for degradation. *PLoS pathogens*. 2009;5(11):e1000650.
18. Xu C, Feng K, Zhao X, Huang S, Cheng Y, Qian L, et al. Regulation of autophagy by E3 ubiquitin ligase RNF216 through BECN1 ubiquitination. *Autophagy*. 2014;10(12):2239–50.
19. Schwintzer L, Aguado Roca E, Broemer M. TRIAD3/RNF216 E3 ligase specifically synthesises K63-linked ubiquitin chains and is inactivated by mutations associated with Gordon Holmes syndrome. *Cell death discovery*. 2019;5:75.
20. Seenivasan R, Hermanns T, Blyszcz T, Lammers M, Praefcke GJK, Hofmann K. Mechanism and chain specificity of RNF216/TRIAD3, the ubiquitin ligase mutated in Gordon Holmes syndrome. *Human molecular genetics*. 2019;28(17):2862–73.

## Figures

**Figure 1**

Pedigree and brain MRI scans of the patient. A, a novel homozygous nonsense mutation, c.1549C>T (p.R517X), in the *RNF216* gene (NM\_207111.3) was found in the patient. Both of his parents were unaffected carriers of this mutation. B-M, a series of brain MRI scans of the patient from 17 years old to 26 years old. B-E, MRI scans of the patient at 17 years old showing patchy white matter lesions (WMLs) affecting the brainstem and periventricular and subcortical areas on FLAIR. F-I, MRI scans at 22 years old showing confluent WMLs on FLAIR and cerebellar atrophy on T1-weighted coronal images. J-M, MRI scans at 26 years old showing the progression of WMLs and cerebellar atrophy.



**Figure 2**

Position of reported and novel mutations in the gene *RNF216*. *RNF216* is located on chromosome 7p22 and contains 17 exons. *RNF216* is an E3 ubiquitin ligase of the “RING-between-RING” (RBR) class, including two RING domains and an “in-between-RING” (IBR) domain. The green box represents the RING1 domain, the red box represents the IBR (in-between-RING) domain and the black box represents the RING2 domain.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1TableS1.docx](#)
- [Additionalfile2TableS2.docx](#)