

# Ataxia and hypogonadotropic hypogonadism with intrafamilial variability caused by *RNF216* mutation

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## Abstract

Gordon Holmes syndrome (GHS) is a distinct phenotype of autosomal recessive cerebellar ataxia, characterized by ataxia, dementia, reproductive defects and hypogonadism; it has been recently found to be associated with *RNF216* mutation. We performed whole-exome sequencing and filtered the resulting novel variants by the coordinates of the shared autozygome. We identified a novel splicing variant in *RNF216* that is likely to abolish the canonical splice site at the junction of exon/intron 13 (NM\_207111.3:c.2061G>A). We herein report two patients with GHS caused by a novel *RNF216* mutation as the first follow up report on *RNF216*-related GHS, and show inter-familial variability of phenotype supporting the previously reported *RNF216*-related cases.

## Introduction

Autosomal recessive cerebellar ataxias are a clinically and genetically heterogeneous group of neurological disorders. A distinct phenotype within this group is cerebellar ataxia associated with hypogonadotropic hypogonadism; this association has been found in two diseases, Gordon Holmes (GHS) and Boucher Neuhauser syndromes.<sup>1</sup> GHS (MIM 212840) is a rare phenotype, first described in 1907 by Holmes,<sup>2</sup> and comprises adolescence or early adulthood age of onset, isolated idiopathic hypogonadotropic hypogonadism and progressive neurodegenerative manifestations including cognitive impairment, dysarthria, cerebellar ataxia. Recently, mutations in genes involved in ubiquitination (*RNF216*, *OTUD4*, *STUB1*) were identified in families with GHS.<sup>3,4</sup> Margolin *et al.* identified mutations in the *RNF216* gene either alone or in combination (digenic mutations) with mutations in *OTUD4* as a cause of GHS.<sup>3</sup> We report two patients with GHS caused by a novel *RNF216* mutation as the first follow up report on *RNF216*-related GHS, and show inter-familial variability of phenotype supporting the previ-

ously reported *RNF216*-related cases. Our study includes 2 out of 9 brothers of a consanguineous Middle Eastern parents (Figure 1), presented with poor development of secondary sexual characteristics and ataxia.

## Case Report #1

A 26-year-old male, presented at the age of 20 with poor development of secondary sexual characteristics, independent for his activities of daily living (ADLs). His examination revealed poor facial hair growth, gynecomastia hypogonadism, and mild cerebellar ataxia. No nystagmus or cognitive impairment were noted. His examinations showed low serum testosterone level of 0.12 nmol/L (reference range for his age is 9.9-27.8 nmol), free androgen index 1.4% (14.8-94.8) and low luteinizing hormone level. Evaluation for bone growth using hand X-ray showed that the metacarpal heads and growth plates of the phalanges are not fused yet. The carpal bones are well ossified. The bone age is about 14 years to 15 years, chronological age at time of the study was 20 years (Figure 2). Brain magnetic resonance imaging (MRI) revealed mild cerebellar atrophy, significant subcortical white matter confluent patchy areas of hyperintensity on T2-weighted imaging and fluid-attenuated inversion recovery (FLAIR) imaging, and partial empty sella with no mass lesions identified (Figure 3).

## Case Report #2

A 31 year old male, developed ataxic gait at the age of 24 with features of poor development of secondary sexual characteristics. His disease progressively worsened with clear cognitive deterioration and need for support in daily activities to the degree that he became totally dependent. His examination revealed no facial hair, hypogonadism, gynecomastia, cognitive impairment, dysarthria, broken saccadic eye

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movement, appendicular and truncal cerebellar ataxia, and exaggerated deep tendon reflexes. No nystagmus was noted. His workup showed low luteinizing hormone level, low testosterone level of 0.44 nmol/L (9.9-27.8 nmol), free androgen index 1.5% (14.8-94.8). X-ray study of his hands at the age of 25 years showed that the growth plate of the distal radius and ulna are not fused yet where it normally starts at the age of 17 to 19 and maximally should be fused by the age of 20 (Figure 2). Brain MRI revealed significant cerebellar atrophy, subcortical white matter patchy areas of hyperintensity on T2-weighted imaging and fluid-attenuated inversion recovery (FLAIR) imaging, and normal pituitary gland (Figure 3).

## Results

Reproductive endocrine evaluation in both patients revealed only low levels of LH and

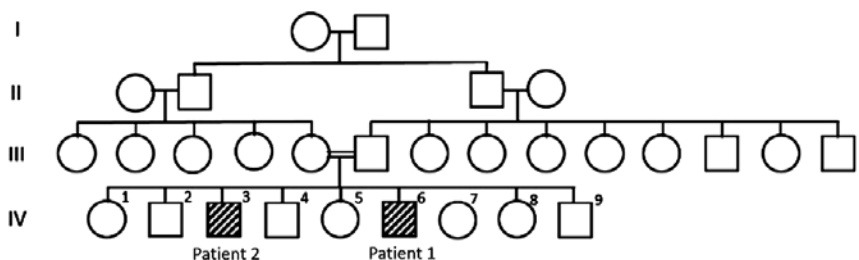


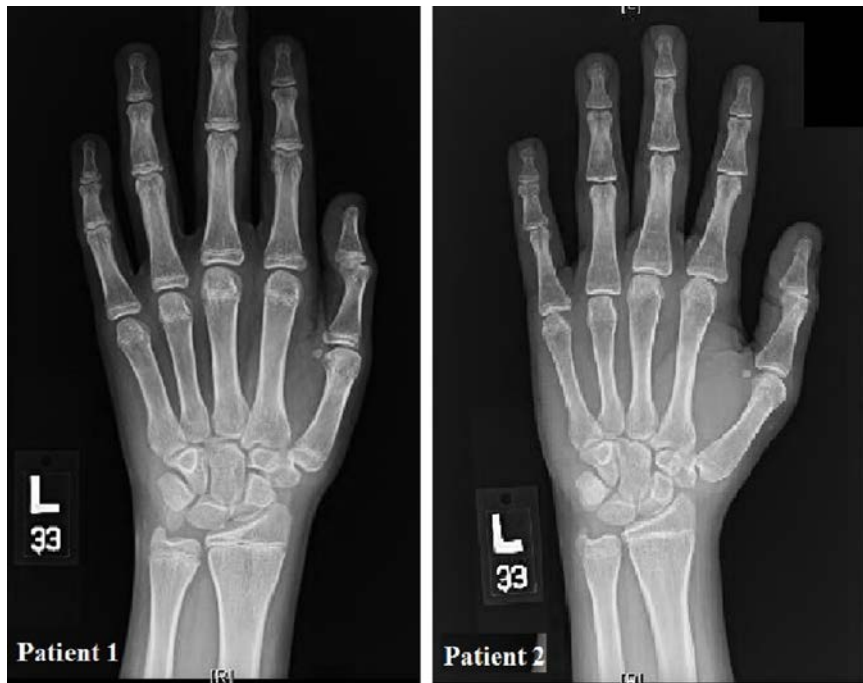
Figure 1. Family pedigree.

testosterone, no other pituitary hormones were affected. Cerebrospinal fluid (CSF) analysis, evoked potential and metabolic workup were normal.

Both patients were started on testosterone injection 250 mcg IM every 3 weeks. Both showed good response to treatment regarding secondary sexual characteristics, in the form of facial, axillary and pubic hair growth, and deepening of voice. There was no change in their neurological symptoms with treatment.

### Genetic analysis

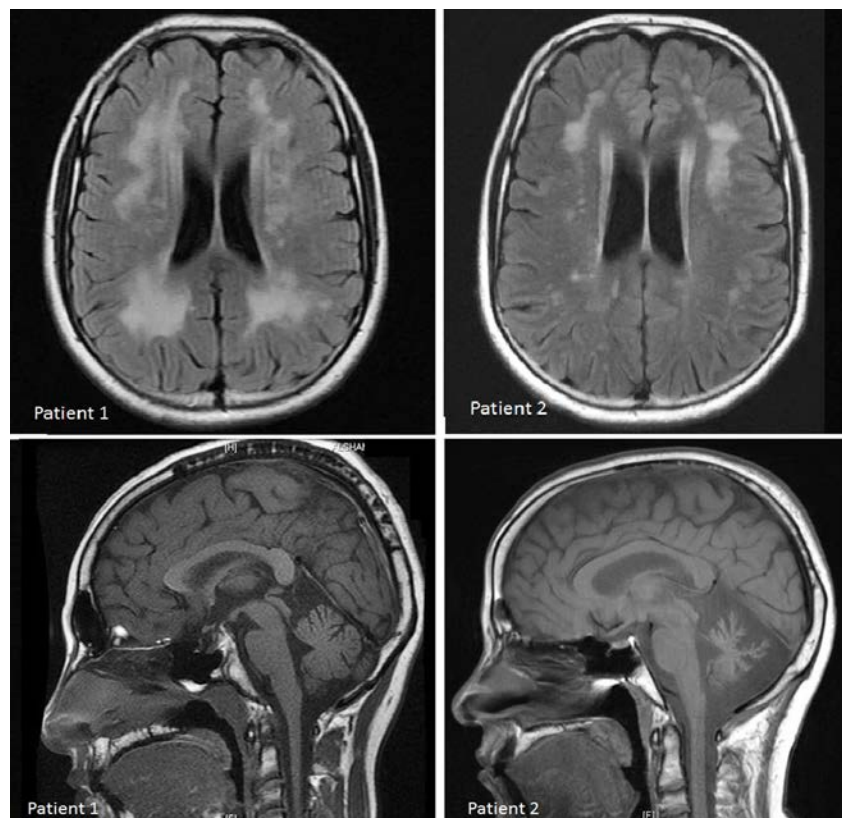
The shared autozygome of the two individuals was checked against known disease genes but no good candidate was identified (*RNF216* had not been identified). Therefore, we proceeded with whole-exome sequencing and filtered the resulting novel variants by the coordinates of the shared autozygome. This resulted in the identified of a novel splicing variant in *RNF216* that is likely to abolish the canonical splice site at the junction of exon/intron 13 (NM\_207111.3:c.2061G>A). This variant was absent in ~600 ethnically matched exomes, in the 1000 genomes and ExAC browser. Homozygosity for this mutation was confirmed by Sanger sequencing in both patients.<sup>5</sup>



**Figure 2.** Hand X-ray showed delayed bone growth. Patient 1: The chronological age of this patient is 20 years. The metacarpal heads and growth plates of the phalanges are not fused yet. The carpal bones are well ossified. The bone age of this patient is about 14 years to 15 years. Patient 2: The chronological age for the patient is 25 years. The growth plate of the distal radius and ulna are not fused yet where it normally starts at the age of 17 to 19 and maximally should be fused by the age of 20.

### Discussion

*RNF216* (ring finger protein 216) is an E3 ubiquitin ligase involved in regulation of autophagy which is a cellular process concerned with cellular homeostasis via the degradation of the cell own cytosolic components or protein aggregates.<sup>6</sup> Margolin *et al.* identified digenic homozygous mutations in *RNF216* and *OTUD4* in three affected siblings of a consanguineous family originally from the Middle East, as well as a compound heterozygous truncating and missense mutations in *RNF216* and single mutations in another family.<sup>3</sup> Homozygous *STUB1* mutation have recently been reported in a consanguineous family with GHS thus expanding the genetic heterogeneity of this condition.<sup>7</sup> The phenotype of *RNF216*-mediated neurodegeneration have been expanded recently to include Huntington-like disorder after identifying of *RNF216* mutation in Belgian families presented initially with prominent chorea, behavioral problems, severe dementia and Low gonadotropin serum levels.<sup>8</sup> The novel mutation we identified in this report further expands the allelic heterogeneity of this neurodegenerative disease. Our patients presented in their early adulthood with idiopathic hypogonadotropic hypogonadism and no other pituitary abnormalities. We observed in patient 1, normal cognitive functions and only mild cerebellar ataxia consistent with the radiological evidence of mild cerebellar atrophy,



**Figure 3.** Brain magnetic resonance imaging. Patient 1: mild cerebellar atrophy, mild cortical atrophy, significant bilateral confluent white matter abnormalities and partial empty sella with no mass lesions identified. Patient 2: significant cerebellum atrophy, mild cortical atrophy, mild white matter changes and normal pituitary gland.

although the white matter involvement was significant. In contrast, patient 2 suffered from a more progressive phenotype manifesting as dysarthria, cerebellar ataxia and cognitive impairment with radiological evidence of prominent cerebellar atrophy but paradoxically milder picture of leukodystrophy in comparison with patient 1 (Figure 3). We compared the clinical features of our patients to other patients with GHS caused by *RNF216* mutation reported by Margolin *et al.* and we noticed that they share same age of onset, cerebellar ataxia, lack of nystagmus, cerebellar atrophy, white matter changes and normal pituitary appearance, of note that our first patient who had partial empty sella which had not been reported previously.

## Conclusions

In summary, we report two patients with

GHS caused by a novel *RNF216* mutation and show Interfamilial variability of phenotype supporting the previously reported *RNF216*-related cases.

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