CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Late-Onset Ataxia-Telangiectasia Presenting With Dystonia and Tremor

The Use of Nanopore Long-Read Sequencing Solving the Variant Phase

Bora Jin, MD,* Jihoon G. Yoon, MD, PhD,* Aryun Kim, MD, Jangsup Moon, MD, PhD,† and Han-Joon Kim, MD, PhD†

Correspondence
Dr. Moon
jangsup.moon@gmail.com
or Dr. Kim
movement@snu.ac.kr

Neurol Genet 2024;10:e200141. doi:10.1212/NXG.0000000000200141

MORE ONLINE

Video

Abstract

Objectives

This study investigates atypical late-onset ataxia-telangiectasia (AT) cases in a Korean family, diagnosed via Nanopore long-read sequencing, diverging from the typical early childhood onset caused by biallelic pathogenic ATM variants.

Methods

A 52-year-old Korean woman exhibiting dystonia and tremor, with a family history of similar symptoms in her older sister, underwent comprehensive tests including routine laboratory tests, neuropsychological assessments, and neuroimaging. Genetic analysis was conducted through targeted sequencing of 29 dystonia-associated genes and Nanopore long-read sequencing to assess the configuration of 2 *ATM* gene variants.

Results

Routine blood tests and brain imaging studies returned normal results, except for elevated α-fetoprotein levels. Neurologic examination revealed dystonia in the face, hand, and trunk, along with cervical dystonia in the proband. Her sister exhibited similar symptoms without evident telangiectasia. Genetic testing revealed 2 heterozygous pathogenic *ATM* gene variants (p.Glu2014Ter and p.Glu2052Lys). Nanopore long-read sequencing confirmed these variants were in *trans* configuration, establishing a definite molecular diagnosis in the proband.

Discussion

This report expands the known clinical spectrum of AT, highlighting a familial case of atypical AT. Moreover, it underscores the clinical utility of Nanopore long-read sequencing in phasing variant haplotypes, essential for diagnosing autosomal recessive disorders, especially beneficial for cases without parental samples.

Introduction

Ataxia-telangiectasia (AT; OMIM #607585) is a rare autosomal recessive disorder, characterized by early childhood–onset ataxia, multisystem involvement, neurodegeneration, and immunodeficiency.[1] This condition is caused by biallelic pathogenic variants in the *ATM* gene, which encodes a serine/threonine protein kinase. To date, more than 500 distinct pathogenic variants have been reported,[2] and short-read sequencing techniques have been widely used in

*These authors contributed equally as cofirst authors.

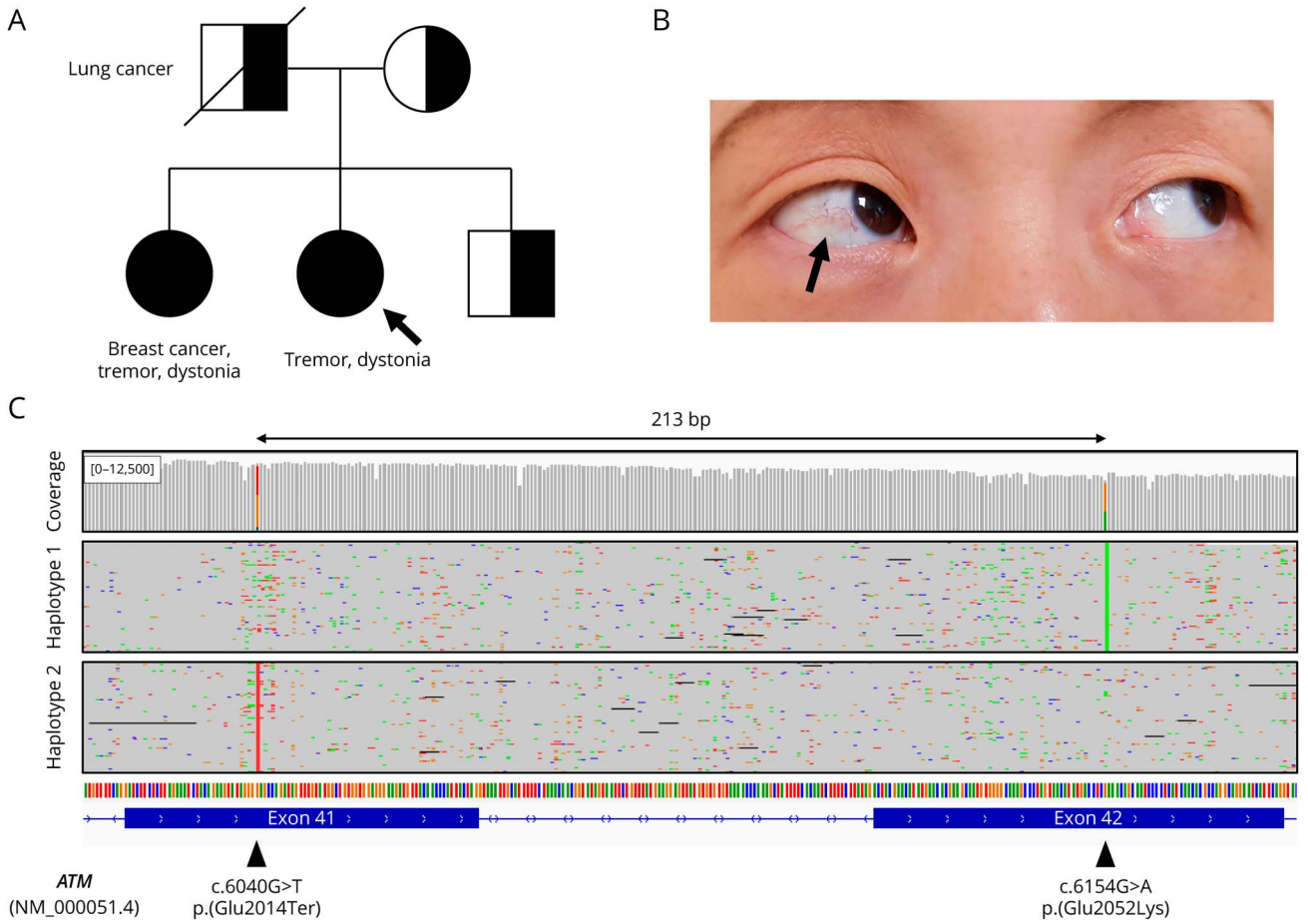
†These authors contributed equally as cosenior authors.

From the Department of Neurology (B.J., J.M., H.-J.K.), Seoul National University Hospital and Seoul National University College of Medicine; Department of Genomic Medicine (J.G.Y., J.M.), Seoul National University Hospital; Department of Laboratory Medicine (J.G.Y), Gangnam Severance Hospital and Yonsei University College of Medicine; and Department of Neurology (A.K.), Chungbuk National University Hospital and Chungbuk National University College of Medicine, Cheongju-si, Republic of Korea.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Figure Clinical and Genomic Findings of the Patient



(A) Family pedigree. The patient's father succumbed to lung cancer, and the elder sister exhibited breast cancer and neurologic symptoms similar to the patients, while the younger brother exhibited no relevant presentations. Subsequent Sanger sequencing confirmed that the elder sister had the identical 2 pathogenic variants (p.Glu2014Ter and p.Glu2052Lys) in the *ATM* gene, while the younger brother harbored 1 (p.Glu2052Lys). (B) Ocular telangiectasia (indicated by a black arrow) observed in our patient. (C) Integrative Genomic Viewer (IGV) findings of the amplicon sequenced with Nanopore long-read sequencing. Nanopore long-read sequencing resolved haplotype configurations of 2 pathogenic *ATM* variants (p.Glu2014Ter and p.Glu2052Lys) located in exon 41 (red) and 42 (green), respectively, thus confirming the *trans* configuration.

identifying these variants. However, this method often requires additional parental sampling to conclusively determine the allelic origins of variants. Recently, long-read sequencing technologies have shown promise in detecting tandem repeats and complex structural variations and resolving haplotypes in Mendelian disorders and various cancers.[3-5] This advancement is highly beneficial in autosomal recessive conditions such as AT, where determining the configuration of compound heterozygotes is essential for accurate diagnosis. In this study, we present a patient with late-onset AT in whom the configuration of 2 pathogenic variants was successfully determined using Nanopore long-read sequencing.

Case Report

A 52-year-old woman presented with worsening tremors that had begun to worsen 5 years ago. She was the second of 3 children in the family, having 1 elder sister and 1 younger brother (Figure, A). She noticed slower handwriting compared with her peers at the age of 7 years. Between the ages of 9 and 10 years, she intermittently had trouble controlling her left hand when washing her face. Intermittent bilateral action tremors started at the age of 13 years. Her symptoms remained stable until her late teens. Intermittent gait disturbances began in her early twenties, while the action tremors in her hands remained stable. At that time, they did not significantly impede her daily life. However, in her late 40s, hand and jerky head tremors worsened to the point of disrupting daily activities.

During her first visit at 52 years of age, a neurologic examination revealed dystonia in the face, hand, and trunk, in addition to cervical dystonia (Video 1). Dystonic postural tremors in both hands were also observed. A saccadic pursuit was suspected during the extraocular movement examination, and ocular telangiectasia was noted (Figure, B). In the cerebellar function test, limb dysmetria was not observed in the finger-to-nose and heel-to-shin tests. She demonstrated a normal base with no difficulty in walking but exhibited instability during turning. In the tandem gait test, she was able

to perform 4–5 steps. Despite mild gait ataxia, she was able to walk independently. Cognitive function was normal (the Korean Mini-Mental State Examination score: 28/30). A routine blood test and a brain imaging study yielded normal results, while an increased level of α-fetoprotein was noted (107 ng/mL; reference range: <11.90 ng/mL). Informed consent was appropriately obtained from the patient, and the Institutional Review Board of Seoul National University Hospital, Seoul, Korea, approved the study (2006-083-1132).

In addition, the proband had a family history of similar symptoms in her older sister, aged 54 years, who began experiencing a tremor in her right hand during her early 20s. This tremor progressively worsened with age but remained considerably less severe than that of the proband. In addition to the tremor, she reported involuntary elevation of her right shoulder and difficulties with handwriting due to the involuntary flexion of her right wrist since adolescence. On neurologic examination, mild dysarthria, a head tremor, cervical dystonia, and kinetic, intention, and dystonic postural tremors in both hands, notably worse on the right side, were observed. In addition, cerebellar function tests, including the finger-to-nose, heel-to-shin, and tandem gait tests, revealed normal performance. Furthermore, no definite evidence of telangiectasia was noted. An elevated α-fetoprotein level of 108 ng/mL was detected, while other blood tests returned normal results. Notably, she had been diagnosed with breast cancer 5 years earlier.

Because a genetic cause was strongly suspected in this family, targeted panel sequencing including 29 hereditary dystonia–related genes was performed in the proband. As a result, we identified 2 heterozygous variants in the *ATM* gene (NM_000051.4): c.6040G>T, p.Glu2014Ter, and c.6154G>A, p.Glu2052Lys. While these variants have been reported as pathogenic variants,[6-8] it was difficult to determine their allelic origin without parental sampling. However, sampling was unavailable for her deceased father. Therefore, we conducted PCR amplification using the primers (forward: 5′-CAATAATGCTTACCACAGTAGC-3′, reverse: 5′- GAGCTCAAAGGGCTCTAATA-3′) containing these 2 variants (amplicon size: 1.4 kb) and sequenced them using Nanopore long-read sequencing (Figure, C). Long reads were aligned to the human reference genome hg38 using minimap2[9] and provided sufficient coverage of both variants, which were distanced by 213 base pairs. Haplotype analysis using WhatsHap[10] and visual inspection through the Integrative Genomic Viewer confirmed their allele positions in *trans*, concluding the molecular diagnosis. Further familial evaluations using Sanger sequencing in her siblings revealed the same pathogenic variants in her older sister and carrier status of the p.Glu2052Lys variant in her younger brother. Similar to the reports of the efficacy of levodopa for cervical dystonia in a patient with AT,[5] our patient also revealed a mild benefit in dystonic tremor amplitude.

Discussion

Typical AT is characterized by childhood-onset cerebellar ataxia, ocular telangiectasia, elevated α-fetoprotein levels, and the typical progression to wheelchair dependency at an average age of 10 years.[11,12] By contrast, late-onset AT exhibited a generally milder course of the disease, usually with a later onset in adolescent or adulthood.[6] In cases of late-onset AT, extrapyramidal symptoms such as rest tremor, dystonia, and choreoathetosis often appear to predominate the clinical presentation instead of the cardinal ataxia symptoms.[11] Immunodeficiency and pulmonary manifestations are less common, while there is a higher risk of malignancy in later stages. These clinical features are delineated by residual expression of the *ATM* gene with low but sufficient kinase activity, which plays a role in DNA repair processes.[13] Our case was notable due to the patient exhibiting dystonia and dystonic tremor with slow progression as the primary symptoms rather than ataxia. In addition, the patient was able to maintain an independent gait even in her early 50s. These relatively mild clinical features might result from the residual activity in the hypomorphic missense variant (p.Glu2052Lys), as previously reported.[6]

Nanopore long-read sequencing is emerging as a powerful tool in the diagnosis and understanding of many recessive neurologic diseases,[14] particularly those requiring detection of structural variations and variant phasing for haplotype confirmation.[3,4] Although the detection of a single heterozygous allele in siblings would have been a robust indicator of the *trans* effect of the 2 variants, our approach using Nanopore sequencing provided direct and clear haplotype-resolved information for the 2 *ATM* gene variants. In addition, while we conducted PCR amplification before sequencing, a noteworthy development is the potential use of amplification-free sequencing. This method, which involves CRISPR/Cas9 enrichment, could offer a more direct and less biased approach.[15] It is particularly effective for assessing haplotype-resolved single-nucleotide variants, structural variations, and CpG methylation. The exploration of Cas9-mediated Nanopore sequencing in future research may expand its applicability and provide deeper insights into diverse neurologic disorders.

In summary, we report a patient with late-onset AT, characterized by extrapyramidal symptoms with a very slow progression, whose molecular diagnosis was confirmed using Nanopore long-read sequencing. This study underscores the utility of Nanopore long-read sequencing in phasing variant haplotypes, crucial for diagnosing autosomal recessive disorders, especially beneficial for cases without parental samples.

Study Funding

This research was supported by funds from the Research of Korea Centers for Disease Control and Prevention (2020-ER6904-01) and the Seoul National University Hospital Research Fund (0320210170). J. Moon was supported by grant (KSMG-AR-202203) from the Korean Society of Medical Genetics and Genomics.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* December 21, 2023. Accepted in final form January 24, 2024. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

Appendix Authors

Name	Location	Contribution
Bora Jin, MD	Department of Neurology, Seoul National University Hospital and Seoul National University College of Medicine, Republic of Korea	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data
Jihoon G. Yoon, MD, PhD	Department of Genomic Medicine, Seoul National University Hospital; Department of Laboratory Medicine, Gangnam Severance Hospital and Yonsei University College of Medicine, Republic of Korea	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Aryun Kim, MD	Department of Neurology, Chungbuk National University Hospital and Chungbuk National University College of Medicine, Cheongju-si, Republic of Korea	Major role in the acquisition of data
Jangsup Moon, MD, PhD	Department of Neurology, Seoul National University Hospital and Seoul National University College of Medicine; Department of Genomic Medicine, Seoul National University Hospital, Republic of Korea	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and study concept or design
Han-Joon Kim, MD, PhD	Department of Neurology, Seoul National University Hospital and Seoul National University College of Medicine, Republic of Korea	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and study concept or design

References

1. Levy A, Lang AE. Ataxia-telangiectasia: a review of movement disorders, clinical features, and genotype correlations. *Mov Disord.* 2018;33(8):1238-1247. doi: 10.1002/mds.27319
2. Hall MJ, Bernhisel R, Hughes E, et al. Germline pathogenic variants in the ataxia telangiectasia mutated (ATM) gene are associated with High and moderate risks for multiple cancers. *Cancer Prev Res.* 2021;14(4):433-440. doi:10.1158/1940-6207.CAPR-20-0448
3. Mastrorosa FK, Miller DE, Eichler EE. Applications of long-read sequencing to Mendelian genetics. *Genome Med.* 2023;15(1):42. doi:10.1186/s13073-023-01194-3
4. Miller DE, Sulovari A, Wang T, et al. Targeted long-read sequencing identifies missing disease-causing variation. *Am J Hum Genet.* 2021;108(8):1436-1449. doi:10.1016/j.ajhg.2021.06.006
5. Sakamoto Y, Miyake S, Oka M, et al. Phasing analysis of lung cancer genomes using a long read sequencer. *Nat Commun.* 2022;13(1):3464. doi:10.1038/s41467-022-31133-6
6. Necpál J, Zech M, Škorvánek M, et al. Ataxia telangiectasia gene mutation in isolated segmental dystonia without ataxia and telangiectasia. *Mov Disord Clin Pract.* 2018; 5(1):89-91. doi:10.1002/mdc3.12564
7. Charlesworth G, Mohire MD, Schneider SA, Stamelou M, Wood NW, Bhatia KP. Ataxia telangiectasia presenting as dopa-responsive cervical dystonia. *Neurology*. 2013; 81(13):1148-1151. doi:10.1212/WNL.0b013e3182a55fa2
8. Sandoval N, Platzer M, Rosenthal A, et al. Characterization of ATM gene mutations in 66 ataxia telangiectasia families. *Hum Mol Genet.* 1999;8(1):69-79. doi:10.1093/hmg/8.1.69
9. Li H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*. 2018; 34(18):3094-3100. doi:10.1093/bioinformatics/bty191
10. Martin M, Ebert P, Marschall T. Read-Based Phasing and Analysis of Phased Variants with WhatsHap. *Methods Mol Biol*. 2023;2590:127-138. doi: 10.1007/978-1-0716-2819-5_8
11. Verhagen MMM, Abdo WF, Willemsen MaaP, et al. Clinical spectrum of ataxia-telangiectasia in adulthood. *Neurology*. 2009;73(6):430-437. doi:10.1212/WNL.0b013e3181af33bd
12. Rothblum-Oviatt C, Wright J, Lefton-Greif MA, McGrath-Morrow SA, Crawford TO, Lederman HM. Ataxia telangiectasia: a review. *Orphanet J Rare Dis.* 2016;11(1):159. doi:10.1186/s13023-016-0543-7
13. Verhagen MMM, Last JI, Hogervorst FBL, et al. Presence of ATM protein and residual kinase activity correlates with the phenotype in ataxia-telangiectasia: a genotype–phenotype study. *Hum Mutat.* 2012;33(3):561-571. doi:10.1002/humu.22016
14. Cho J, Yoon JG, Lee S, et al. Expansion of clinico-genetic spectrum of PRDX3 disease: a literature review with two additional cases. *Brain Comm.* 2023;5:fcad233. doi: 10.1093/braincomms/fcad233
15. Gilpatrick T, Lee I, Graham JE, et al. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat Biotechnol.* 2020;38(4):433-438. doi:10.1038/s41587-020-0407-5