Received: 11 January 2023 | Revised: 3 March 2023 | Accepted: 14 March 2023

DOI: 10.1002/ccr3.7165

CASE REPORT

Clinical Case Reports Open Access WILEY

RNA analysis of intronic variants in the LAMA2 gene detected by whole genome sequencing confirms a rare dual diagnosis of incontinentia pigmenti with limb-girdle muscular dystrophy

Camerun Washington[1] | **Elliot S. Stolerman**[1] | **Jessica A. Cooley-Coleman**[1] | **Julie R. Jones**[1] | **Xiangwen Chen-Deutsch**[2]

[1]Greenwood Genetic Center, Greenwood, South Carolina, USA

[2]PerkinElmer Genomics, Pittsburgh, Pennsylvania, USA

Correspondence
Camerun Washington, 14 Edgewood Dr, Greenville, SC 29605, USA.
Email: cwashington@ggc.org

Abstract

We see that a multiple methods approach to diagnosis remains necessary in the era of whole genome sequencing. We also observe that reproductive risk genetic counseling can be a motivating factor for further testing along the diagnostic odyssey.

KEYWORDS

dual diagnosis, genome sequencing, incontinentia pigmenti, *LAMA2*, muscular dystrophy, RNA analysis

1 | INTRODUCTION

A 27-year-old female with incontinentia pigmenti, *LAMA2*-related muscular dystrophy and *WNT10A*-related tooth agenesis was diagnosed using a multi-omics approach. This report adds RNA evidence of splicing alterations in previously reported genomic *LAMA2* variants and insights into reproductive genetic counseling as a motivator for further testing.

With the increasing clinical use of whole genome sequencing (WGS), clinicians and scientists will continue to uncover and face challenges interpreting variants in the noncoding regions of the genome. In the absence of experimentally confirmed functional data, these variants may be dismissed, remain classified as variants of uncertain significance or not reported at all.[1,2] Few genetic diagnostic laboratories routinely offer RNA analysis as part of their oncology test menus (Ambry Genetics, Invitae). However, not all laboratories will have a method or time to experimentally confirm these variants, posing problems for interpretation.

Whole genome-based approaches may reveal more than one molecular diagnosis for patients. This potential presents a considerable challenge for tailored management recommendations and predicting disease prognosis by the clinician. This also requires advanced skills in risk communication and genetic counseling to obtain informed consent and make clear the recurrence risk of each condition, which may have different modes of inheritance. The art of clinical genetics evaluation is in identifying recognizable and sometimes subtle phenotypic deviations from normal. When the clinician suspects they are dealing with a new clinical feature or symptom that cannot be explained by an existing diagnosis, further investigation, including laboratory exploration and confirmation, should be pursued.

2 | METHODS

2.1 | Biomedical ethics

Written informed consent for treatment, specimen collection, and publication of relevant findings, including photographs, was obtained from the patient and family. This report was deemed exempt from formal review by our institutional ethics committee.

2.2 | Whole exome sequencing

The Agilent SureSelect Clinical Research Exome kit was used to target known coding sequences and splice junctions of known protein-coding genes associated with disease using genomic DNA from the submitted sample(s). The DNA was sequenced using an Illumina NextSeq 500® Sequencing System (Illumina Inc.) per the manufacturer's protocol. Using NextGENe software (SoftGenetics, LLC) and an in-house bioinformatics pipeline, the DNA sequence was aligned and compared to the human genome build 19 (hg19/NCBI build 37). The average depth of coverage across all targeted regions was 169×. The Cartagenia Bench Lab next generation sequencing (NGS) software (Agilent Technologies) was used to filter and analyze sequence variants identified in the patient and compared them to the sequences of family members. All reported variants were confirmed by Sanger sequencing for the patient and any submitted family member samples.

2.3 | Whole genome sequencing

WGS was performed on genomic DNA using 2×150 bp reads on Illumina NGS systems at a mean coverage of 40× in the target region. A base is considered to have sufficient coverage at 20× and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20× or more. PerkinElmer Genomics has curated deep intronic pathogenic variants in public databases and these are tagged for identification during analysis. Alignment to the human reference genome (hg19) was performed, and annotated variants were identified in the targeted region. Primary data analysis was performed using Illumina DRAGEN Bio-IT Platform v.2.03. Secondary and tertiary data analysis was performed using PerkinElmer's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v6.1 for CNVs.

2.4 | RNA analysis

For RT-PCR, RNA was first isolated from peripheral blood using the PAXgene Blood RNA kit (Qiagen) and then reverse transcribed into cDNA using the SuperScript™ III First-Strand Synthesis System Kit (Thermo Fisher Scientific). PCR was performed following standard procedures using combinations of exon-specific and exon-spanning primers. PCR products were Sanger sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit and then run on an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific).

3 | CASE REPORT

Our patient was born full term via a spontaneous vaginal delivery to nonconsanguineous parents at 2.86 kg weight, 49.53 cm length, and with a head circumference of 33 cm. The Apgar scores were 9 and 9 with early temperature control problems. She was discharged with her mother at 2 days of age. She returned to medical attention at 5 days of age with concerns of a rash with the appearance of brown, "marbled" lesions across her trunk, abdomen, and thighs and verrucous lichenoid lesions along the right knee, ankle, and foot. Genetics was consulted and the characteristic dermatologic findings plus a family history of incontinentia pigmenti (IP) for her mother, full sister, and two maternal half sisters were sufficient for a clinical diagnosis of IP for our patient.

Our patient sat unassisted at 6 months, crawled at 12 months, and walked between 18 and 19 months. She had noticeable balance issues with frequent falls, which prompted a neurologic evaluation between the age of 5 and 6 years. Her brain MRI at this time was nonspecific but compatible with dysmyelination of the supratentorial white matter predominating in a subcortical location. Electromyogram (EMG) results showed a mildly abnormal tracing possibly suggestive of a myopathic process, and a muscle biopsy of the right anterior leg showed benign myopathic changes with variation in fiber size, stained for dystrophin. Her nerve conduction study was normal. At a follow-up clinical genetics visit at age 9, we noticed a wide-based gait and elicited a history of persistent balance issues, difficulty ascending and descending stairs without assistance, and trouble getting in and out of cars. On physical exam, she had proximal weakness of her upper extremities and distal weakness of the lower extremities. Her cognitive, speech and language, and fine motor skills were all within the normal range. We recommended a chromosome analysis and SNP microarray for

her developmental delays and referral to cardiology and creatine kinase (CK) analysis for her muscle weakness. Cytogenetic studies returned within the normal range. Her CK analysis was slightly elevated at 765 units/L (normal range: 45–198 units/L). At age 11, she had two generalized tonic–clonic seizures. The EEG results were abnormal, showing frequent paroxysms of brief multispike wave discharges with a low seizure threshold. Following additional breakthrough seizures, she was diagnosed with a primary seizure disorder at age 12 and started on levetiracetam. When we met with her again at 13 years of age, she had been diagnosed with a dilated cardiomyopathy from her cardiac evaluation and was started on lisinopril. We noted reduced flexion in her elbows and tightening of her heel cords. She also had appreciable scoliosis, which would be surgically corrected, and still had gait difficulties and weakness requiring motorized wheelchair use during school. By this time, most physicians presumed her symptoms were a phenotypic expansion of IP, likely citing the Huttner et al.[3] paper of a woman with IP and unexplained neuromuscular symptoms.

We then recommended an exome duo analysis and X-inactivation studies at age 23 years. The X-inactivation studies showed a highly skewed pattern of 99:1. Her affected mother had an identical pattern. The exome duo analysis with a maternal sample showed a pathogenic variant in *WNT10A* (NM_025216.3) c.321C>A, p.Cys107* inherited from her mother and a variant in *LAMA2* (NM_000426.3) c.5562+5G>C that was not maternally inherited. This single nucleotide variant in the *LAMA2* gene involves the consensus splice site sequence of intron 38. The second alteration in *LAMA2* was not identified. Targeted Sanger sequencing later confirmed the paternal inheritance of this variant. The rest of the

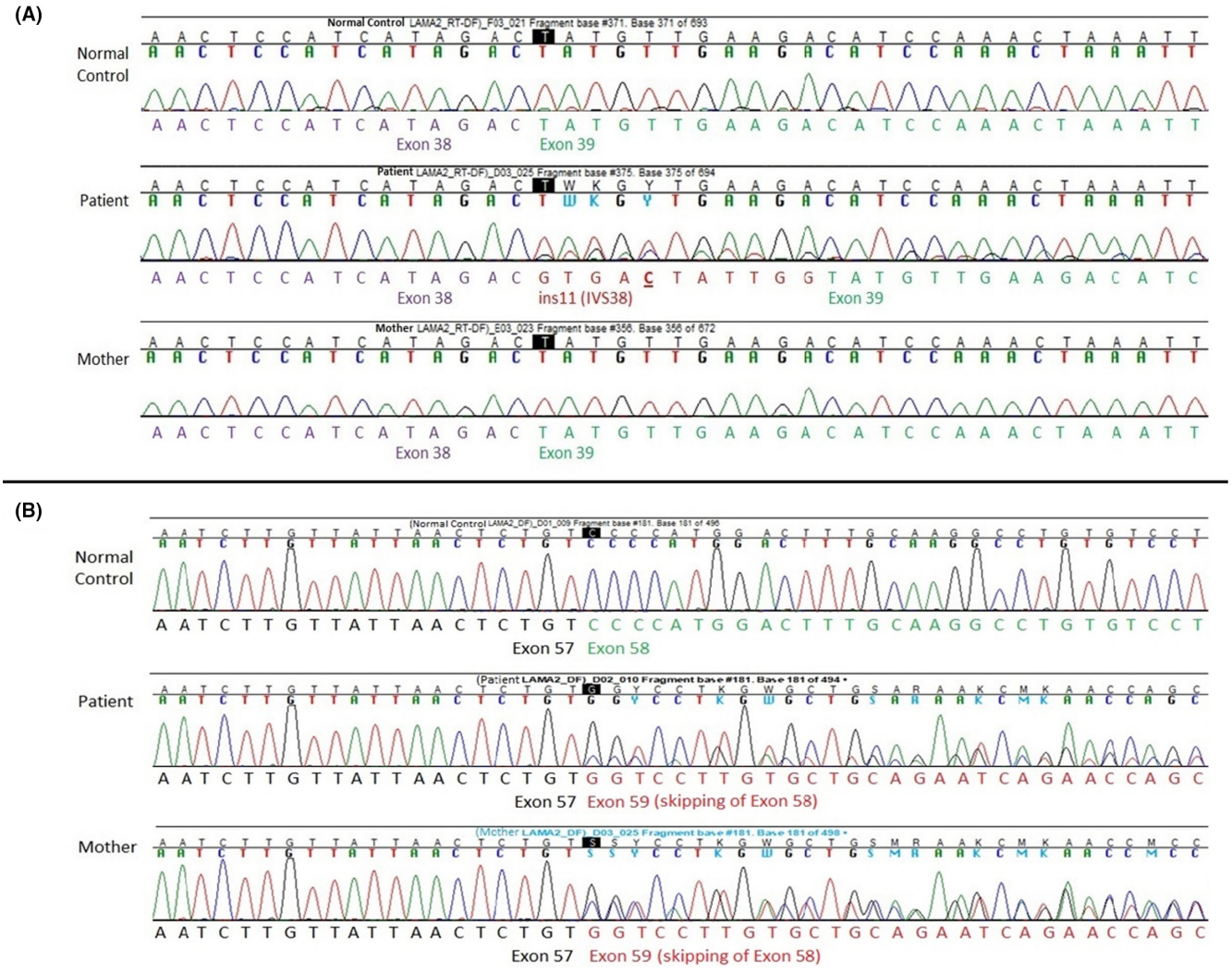

FIGURE 1 RT-PCR Sanger sequence results. (A) The *LAMA2* c.5562+5G>C variant results in the insertion of 11 nucleotides of intron 38 within the transcript, generating a premature stop codon (p.Tyr1855Leufs*5) in the patient but not her mother. This variant was confirmed to be paternally inherited by DNA testing. The father was not available for RNA testing. (B) The *LAMA2* c.8244+3_8244+6del variant resulted in the skipping of exon 58 in both the patient and her mother.

analysis was normal. The original exome sequence reporting classified the paternal *LAMA2* variant as a variant of uncertain significance. By request, our laboratory undertook an investigational RNA analysis for our patient which proved a splicing defect with the introduction of 11 nucleotides of intronic sequence generating a premature stop codon (p.Tyr1855Leufs*5) (Figure 1) for our patient. This evidence was sufficient to reclassify the variant as likely pathogenic (Table 1). We did not perform RNA analysis for her father. From these results, we strongly considered that she was a possible manifesting heterozygote of *LAMA2*, since *LAMA2*-related muscular dystrophies are autosomal recessive traits (MIM# 156225). However, we struggled to find evidence in the medical literature to support this. We discussed Emery–Dreifuss muscular dystrophy, given her dilated cardiomyopathy and elbow contractures, although absence of a sequence or copy number variant in *EMD*, *FHL1*, and *LMNA* precluded this diagnosis.

At her most recent evaluation with genetics at age 26, she returned as an adult to review her case and discuss reproductive recurrence risk. At this visit, we noted generalized muscle weakness involving upper and lower extremities, abnormal gait, scoliosis repair, and easy fatiguability. Dermatologic signs of IP had resolved, with faint hyperpigmented areas remaining on her flank and axillar regions. She reported sparse eyebrows and eyelashes, patchy hair loss, brittle nails, hypotonia, and conoid teeth (reportedly more significantly affected than her sisters with IP), without ophthalmologic findings. She was diagnosed with hypothyroidism and prescribed synthroid 3 years prior. Given her complex phenotype, we recommended confirmatory genetic testing for IP with an *IKBKG* analysis through GeneDx Laboratories and requested targeted deletion/duplication analysis for *LAMA2*. Targeted DNA analysis for *IKBKG* detected the recurrent 11.7 kb microdeletion involving exons 4–10 (Table 1), and deletion/duplication studies for *LAMA2* were normal. Several months later, once clinically available, we offered clinical proband-only WGS through PerkinElmer Genomics Laboratories, which revealed a second heterozygous likely pathogenic intronic variant c.8244+3_8244+6del in the *LAMA2* gene (NM_000426.3). This variant is a four nucleotide deletion involving the consensus splice donor sequence of intron 58 of the *LAMA2* gene. She was also a carrier of *GJB2*-related hearing loss by detection of a heterozygous pathogenic variant c.101T>C, p.Met34Thr (NM_004004.6) in the *GJB2* gene uncovered from requesting a carrier status analysis from WGS. Given that phase was unclear for the newly found likely pathogenic splicing variant in *LAMA2*, we investigated our patient's and mother's samples with targeted RNA analyses. This experiment showed that both patient and maternal *LAMA2* gene transcripts demonstrated skipping of exon 58 (Figure 1), providing inheritance data and evidence of pathogenicity (Table 1). This in-house investigation was sufficient to call a molecular diagnosis of *LAMA2*-related muscular dystrophy for our patient. We banked DNA for future diagnostic studies.

At present, our patient (Figure 2) maintains eligibility for disability services and does not work due to her easy fatigue and muscle weakness. She was advised to avoid statins and succinylcholine due to the risk of muscle damage, anesthetic sensitivity, and cardiac rhythm disorders, respectively. She has normal intelligence, a kind disposition and is currently married.

4 | DISCUSSION

IP, or Bloch-Sulzberger syndrome, is a rare genetic predominately female condition that presents with characteristic skin lesions early in life and can increase the lifetime risk for seizures, intellectual disability, and developmental delays. The major clinical diagnostic features are the characteristic skin lesions in one of four stages with diagnostic support from minor criteria involving the retina, hair, nails, teeth, and family history.[4,5] *LAMA2* muscular dystrophy is a multisystem autosomal recessive disorder with a disease spectrum including a severe congenital form, clinically presenting at birth with hypotonia, muscle wasting and weakness, respiratory difficulty, joint contractures, and feeding difficulties, and a later-onset form with ambulation and a limb-girdle pattern of muscle weakness. Individuals with reduced expression of laminin α-2 protein present with a milder form of disease, while those with the severe form tend to have virtually absent laminin α-2 expression. There are no published clinical diagnostic criteria for *LAMA2* muscular dystrophy. The diagnosis is suspected from suggestive clinical, molecular, brain MRI findings, and family history.[6,7]

In hindsight, there were a number of clues suggesting a second or alternative diagnosis for our patient. Neurologic manifestations, such as seizures, strokes, and abnormalities on brain imaging, are present in 30% of individuals with IP.[8,9] Given this report, an observer may not be impressed by some degree of motor developmental delay or muscle weakness in a child with IP. For our case, delayed motor milestones and complaints of clumsiness and frequent falls in the first decade of life were the first clue of a deviation from the expected natural history. In clinically ambiguous instances such as this, confirmatory molecular genetic testing of IP could be pursued. The combination of dilated cardiomyopathy, marked scoliosis, and joint contractures at the elbows and ankles in the second decade of life were the next

TABLE 1 Molecularly confirmed results.

Phenotype	Prevalence	Phenotype MIM number	Zygosity/ inheritance	Gene/locus	DNA change	RNA change	Protein change	Classification
Allele-specific PCR results								
Incontentia pigmenti	1–9/100,000 or 900 to 1200 known affected individuals	303800	XLD/unknown	IKBKG/Xq28	c.400_1260del861		Deletion of exons 4–10	Pathogenic
Whole exome sequencing results								
Autosomal recessive limb-girdle muscular dystrophy 23	1–9/100,000 or 1 in 50,000 to 400,000	607855	Heterozygous/ AR/paternal	*LAMA2*/6q22.33	c.5562+5G>C	Frameshift due to an insertion of 11 nucleotides	p.Tyr1855Leufs*5	Likely pathogenic
Selective tooth agenesis 4	Unknown	150400	Heterozygous/ AD/maternal	*WNT10A*/2q35	c.321C>A		p.Cys107*	Pathogenic
WGS results								
Autosomal recessive limb-girdle muscular dystrophy 23	1–9/100,000 or 1 in 50,000 to 400,000	607855	Heterozygous/ AR/maternal	*LAMA2*/6q22.33	c.8244+3_8244+6del	Skipping of exon 58 disrupts the reading frame	p. –	Pathogenic

Note: The asterisk is standard molecular genetic nomenclature for a "premature stop signal." It refers to a genetic sequence variant that results in a stop codon, which typically truncates/alters the mRNA product.

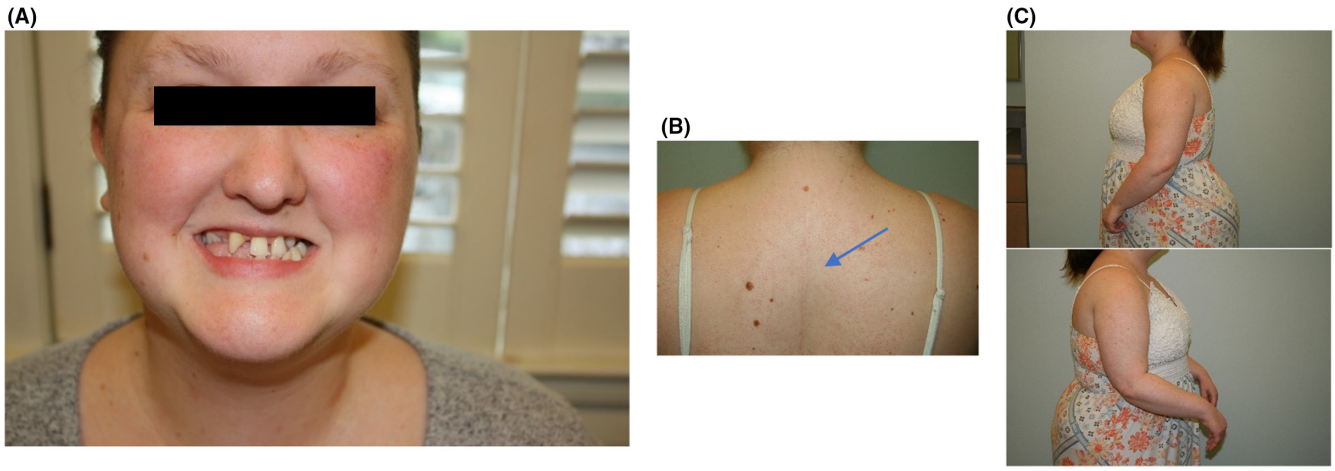

FIGURE 2 Our patient. (A) Note the sparse eyebrows and eyelashes and marked dental anomalies (conoid teeth and hypodontia) likely due to pathogenic *IKBKG* and *WNT10A* alterations. (B) Scar from growing rod scoliosis repair. (C) Our patient has bilateral elbow contractures photographed at resting position and explained by biallelic *LAMA2* alterations.

clues for an alternative neuromuscular diagnosis. These clinical features should be investigated with a thorough neuromuscular workup, including a serum CK level and evaluation by a neuromuscular specialist. The report by Huttner et al.[3] is the most similar to our patient, with a woman with IP affected by proximal muscle weakness and dilated cardiomyopathy. While their patient did not have joint contractures, scoliosis, or delayed motor milestones, she presented with scapular winging and esotropia, two alternative signs of a neuromuscular disorder. Our patients similarly had modestly elevated serum CK levels, significant white matter changes on brain MRI, and myopathic patterns on EMG. While their patient had a muscle MRI and was tested for myotonic dystrophy, ours was not. Indeed, the initial lack of support from the neuromuscular and molecular evaluations can lead one to consider the possibility of an unrevealed molecular explanation or expansion of a known phenotype. Our case directly answers and adds to the call from Huttner et al. to provide a molecular characterization of neuromuscular symptoms in a patient with IP.

Both of our patient's *LAMA2* splicing variants have been reported in affected individuals as homozygotes or compound heterozygotes.[10,11] In silico modeling almost unanimously predicted a deleterious effect on RNA splicing. We directly tested these predictions using a custom cDNA analysis for the *LAMA2* gene on a research basis. The Greenwood Genetic Center Molecular Diagnostic Laboratory is a CLIA- and CAP-accredited diagnostic laboratory in Greenwood, South Carolina, with in-house capability and experience in RNA analysis and diagnostics for rare and undiagnosed diseases. Our laboratory scientists developed and optimized protocols for reverse transcriptase-PCR followed by direct sequencing in analysis of *LAMA2* RNA transcripts altered by genomic variants identified in our patient. In our case, the ability to design a custom functional assay to determine the true outcome of the genomic variants and phase was critical for a diagnosis. When our patient underwent her first exome in 2018, it was performed as a duo analysis. Four years later, after WGS was clinically available, her mother was deceased and her father was incarcerated. Thus, without further molecular characterization, the diagnosis of muscular dystrophy would have remained speculative. There is no established regulatory framework for functional transcript analysis in the clinical setting, which is a barrier to its widespread use (particularly for biobanking and the ethics of informed consent). The testing center's expertise in RNA diagnostics, quality standards for RNA diagnostics, alignment with ACMG-AMP interpretation guidelines, and cost of custom assays are also key factors.[12] Despite a low GTEX value for *LAMA2* in blood but we were still able to get amplification and use it as a proxy for skeletal muscle tissue. Our protocol may assist future molecular analyses of suspected *LAMA2*-related disorders.

Previous research suggests that when exome analysis results are negative, families are less likely to request further genetic testing.[13] We report genetic counseling for recurrence risk and family planning as a primary motivating factor for our patient for further testing. We initially provided genetic counseling for IP as 1 in 3 (33%) reproductive risk for an affected female, unaffected female, or unaffected male, and *WNT10A* as 50%, regardless of gender. We did not provide specific recurrence risk numbers for her muscular dystrophy as it was not yet diagnosed but cautioned of the potential for heritability. With definitive molecular diagnoses for each of her conditions, we can now provide accurate recurrence risk genetic counseling for X-linked, autosomal

dominant, and autosomal recessive inheritance patterns. We offered carrier testing for *LAMA2* and *GJB2* for all at-risk family members. Her current partner is negative for variants in *LAMA2*. He has not yet been tested for variants in *GJB2*.

Ongoing communication and follow-up with the patient, her family, and a team of interdisciplinary specialists were critical to the outcome of this case. This group remained curious about her unusual clinical presentation and persisted in asking questions. We must emphasize that the diagnostic journey is fraught with limitations in medical testing. Although next-generation sequencing by exome analysis identified the first hit in *LAMA2*, and targeted CNV analysis was normal, advanced whole genomic analysis identified the second hit. Next, the original muscle biopsies for our patient lacked specific protein staining for *LAMA2*, providing nonspecific results early in her neuromuscular workup. Lastly, the common *IKBKG* exon 4–10 deletion was acknowledged but not reported from the genome analysis due to a lack of validation for this gene/region (in part due to the *IKBKG2* pseudogene). Single gene testing through a laboratory validated to report changes in this gene was necessary for a confirmatory molecular diagnosis of IP in this patient, which was necessary given her complicated phenotype. With such a rare combination of pathogenic genomic variants, this case demonstrates many of the challenges and rewards of health care in the genomic era.

AUTHOR CONTRIBUTIONS

Camerun Washington: Conceptualization; data curation; project administration; writing – original draft; writing – review and editing. **Elliot S. Stolerman:** Supervision; writing – review and editing. **Jessica A. Cooley Coleman:** Formal analysis; investigation; methodology; writing – review and editing. **Julie R. Jones:** Formal analysis; methodology; writing – review and editing. **Xiangwen Chen-Deutsch:** Formal analysis; methodology; writing – review and editing.

ACKNOWLEDGMENTS

We are grateful to our patient for participating in and approving the study. This study did not receive grant funding from any agency in the public, commercial, or not-for-profit sectors. Camerun Washington gathered the data, wrote and conceptualized the manuscript, and contributed to clinical care. Elliot S. Stolerman contributed to clinical care and revised the article for intellectual content. Jessica Cooley-Coleman generated data and described RT-PCR methods. Julie Jones and Xiangwen Chen-Deutsch analyzed and interpreted molecular data. All authors approved the final version of the article.

CONFLICT OF INTEREST STATEMENT

The Greenwood Genetic Center receives revenue from diagnostic testing performed in the GGC Molecular Diagnostic Laboratory. Otherwise, the authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study were obtained from clinical laboratories and are openly available in ClinVar at https://www.ncbi.nlm.nih.gov/clinvar/.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Camerun Washington https://orcid.org/0000-0001-6166-6613
Elliot S. Stolerman https://orcid.org/0000-0001-9536-4621

REFERENCES

1. Truty R, Ouyang K, Rojahn S, et al. Spectrum of splicing variants in disease genes and the ability of RNA analysis to reduce uncertainty in clinical interpretation. *Am J Hum Genet*. 2021;108(4):696-708.
2. Gonorazky HD, Naumenko S, Ramani AK, et al. Expanding the boundaries of RNA sequencing as a diagnostic tool for rare Mendelian disease. *Am J Hum Genet*. 2019;104(3):466-483.
3. Huttner HB, Richter G, Jünemann A, et al. Incontinetia pigmenti-related myopathy or unsolved "double trouble"? *Neuromuscul Disord*. 2010;20(2):139-141.
4. Minić S, Trpinac D, Obradović M. Incontinentia pigmenti diagnostic criteria update. *Clin Genet*. 2014;85(6):536-542.
5. Scheuerle AE, Ursini MV. Incontinentia pigmenti. In: Adam MP, Mirzaa GM, Pagon RA, et al. eds. *GeneReviews® [Internet]*. University of Washington; 1999-2023. https://www.ncbi.nlm.nih.gov/books/NBK1472/
6. Sarkozy A, Foley AR, Zambon AA, Bönnemann CG, Muntoni F. LAMA2-related dystrophies: clinical phenotypes, disease biomarkers, and clinical trial readiness. *Front Mol Neurosci*. 2020;13:123.
7. Oliveira J, Parente Freixo J, Santos M, Coelho T. *LAMA2* muscular dystrophy. In: Adam MP, Mirzaa GM, Pagon RA, et al. eds. *GeneReviews® [Internet]*. University of Washington; 2012-2020. https://www.ncbi.nlm.nih.gov/books/NBK97333/
8. Meuwissen ME, Mancini GM. Neurological findings in incontinentia pigmenti; a review. *Eur J Med Genet*. 2012;55(5):323-331.
9. Minić S, Trpinac D, Obradović M. Systematic review of central nervous system anomalies in incontinentia pigmenti. *Orphanet J Rare Dis*. 2013;8(1):1-10.
10. Oliveira J, Gruber A, Cardoso M, et al. LAMA2 gene mutation update: toward a more comprehensive picture of the laminin-α2 variome and its related phenotypes. *Hum Mutat*. 2018;39(10):1314-1337.

11. Xiong H, Tan D, Wang S, et al. Genotype/phenotype analysis in Chinese laminin-α2 deficient congenital muscular dystrophy patients. *Clin Genet*. 2015;87(3):233-243.
12. Bournazos AM, Riley LG, Bommireddipalli S, et al. Standardized practices for RNA diagnostics using clinically accessible specimens reclassifies 75% of putative splicing variants. *Genet Med*. 2022;24(1):130-145.
13. Mollison L, O'Daniel JM, Henderson GE, Berg JS, Skinner D. Parents' perceptions of personal utility of exome sequencing results. *Genet Med*. 2020;22(4):752-757.

How to cite this article: Washington C, Stolerman ES, Cooley-Coleman JA, Jones JR, Chen-Deutsch X. RNA analysis of intronic variants in the LAMA2 gene detected by whole genome sequencing confirms a rare dual diagnosis of incontinentia pigmenti with limb-girdle muscular dystrophy. *Clin Case Rep*. 2023;11:e7165. doi:10.1002/ccr3.7165