

Long-read sequencing is required for precision diagnosis of incontinentia pigmenti

Monica H. Wojcik,^{1,14} Robin D. Clark,² Abdallah F. Elias,^{3,4,5} Casie A. Genetti,^{1,14} Jill A. Madden,^{1,14} Dana Simpson,⁶ Linda Golkar,⁷ Miranda P.G. Zalusky,⁸ Angela L. Miller,⁸ Araceli Rodriguez,² Joy Goffena,⁸ Camille A. Dash,^{1,14} Nikhita Damaraju,⁸ Sophia B. Gibson,^{8,9} Sophie H.R. Storz,⁸ Zachary B. Anderson,⁸ Jonas A. Gustafson,^{8,10} Isabelle Thiffault,¹¹ Emily G. Farrow,¹¹ Tomi Pastinen,¹¹ Jasmine Lin,^{1,14} Jennifer T. Huang,¹ Alan H. Beggs,^{1,14} Pankaj B. Agrawal,^{1,14} Genomics Research to Elucidate the Genetics of Rare Diseases (GREGoR) Consortium, David T. Miller,¹ and Danny E. Miller^{8,9,12,13,15,*}

Summary

Incontinentia pigmenti (IP) is caused by loss-of-function variants in *IKBK*, with molecular genetic diagnosis complicated by a pseudogene. We describe seven individuals from three families with IP but negative clinical genetic testing in whom long-read sequencing identified causal variants, including one family with the common exon 4–10 deletion not identified by conventional clinical genetic testing. Concurrent methylation analysis explained disease severity in one individual who died from neurologic complications, identified a mosaic variant in an individual with an atypical presentation, and confirmed skewed X chromosome inactivation in an XXY individual.

Incontinentia pigmenti (IP; MIM: 308310) is an X-linked disorder caused by loss-of-function variants in *IKBK* that is typically male (XY) lethal, with phenotypic heterogeneity in females (XX) caused by variable X chromosome inactivation.¹ Genetic diagnosis of IP is complicated by the presence of a pseudogene, *IKBKGP1*, making it difficult for currently available clinical tests to accurately sequence the region and identify disease-causing variants.^{2,3} Long-read sequencing (LRS) is an emerging technique that is better able to evaluate these complex genomic regions due to the length of the reads generated compared to short-read approaches.⁴ Using LRS, we evaluated seven individuals from three families with a clinical diagnosis of IP but negative clinical genetic testing, including an individual with Klinefelter syndrome. We identified one common variant missed by clinical genetic testing, provided insight into disease severity in one individual, and, in all cases, demonstrated how LRS can be used for comprehensive clinical evaluation of complex genomic regions.

Individuals from family 1 were enrolled in the Manton Center for Orphan Disease Research under a protocol

approved by the Boston Children's Hospital institutional review board (IRB). Individuals from family 2 were recruited using protocol 7064 (University of Washington, Repository for Mendelian Disorders). Individuals from family 3 were recruited using protocol 20161 (University of Washington, Genomic Discovery Initiative). All participants or their legal guardian provided written consent.

In family 1, a female neonate (V:1) who presented with seizures, strokes, and a Blaschkolinear rash characteristic of IP (Figure 1A) died in the setting of severe neurological complications at 13 days of age (supplemental information). Although the neonate's mother (IV:1), maternal aunt (IV:3), and maternal grandmother (III:1) had been clinically diagnosed with IP (Figure 1B), prior clinical genetic testing, including both targeted evaluation of *IKBK* and exome sequencing, was negative. We performed research LRS using blood-derived DNA from the proband and her mother, aunt, and grandmother and detected a complex structural variant (SV) in *IKBK* composed of a ~2,350-bp deletion that included exons 8–10 flanked by an ~80-kbp inversion (Figures 1C and 1D). Reads spanning this region indicated that the causal

¹Boston Children's Hospital and Harvard Medical School, Boston, MA, USA; ²Division of Clinical Genetics, Loma Linda University Children's Hospital and Loma Linda University School of Medicine, Loma Linda, CA, USA; ³Department of Medical Genetics, Shodair Children's Hospital, Helena, MT, USA; ⁴Division of Biological Sciences, University of Montana, Missoula, MT, USA; ⁵Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT, USA; ⁶Randall Children's Hospital, Portland, OR, USA; ⁷Department of Dermatology, Loma Linda University School of Medicine, Loma Linda, CA, USA; ⁸Division of Genetic Medicine, Department of Pediatrics, University of Washington and Seattle Children's Hospital, Seattle, WA, USA; ⁹Department of Genome Sciences, University of Washington, Seattle, WA, USA; ¹⁰Molecular and Cellular Biology Program, University of Washington, Seattle, WA, USA; ¹¹Children's Mercy Hospital, Kansas City, MO, USA; ¹²Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA; ¹³Brotman Baty Institute for Precision Medicine, University of Washington, Seattle, WA, USA; ¹⁴Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA

¹⁵Lead contact

*Correspondence: dm1@uw.edu

<https://doi.org/10.1016/j.xhgg.2025.100468>.

© 2025 The Authors. Published by Elsevier Inc. on behalf of American Society of Human Genetics.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



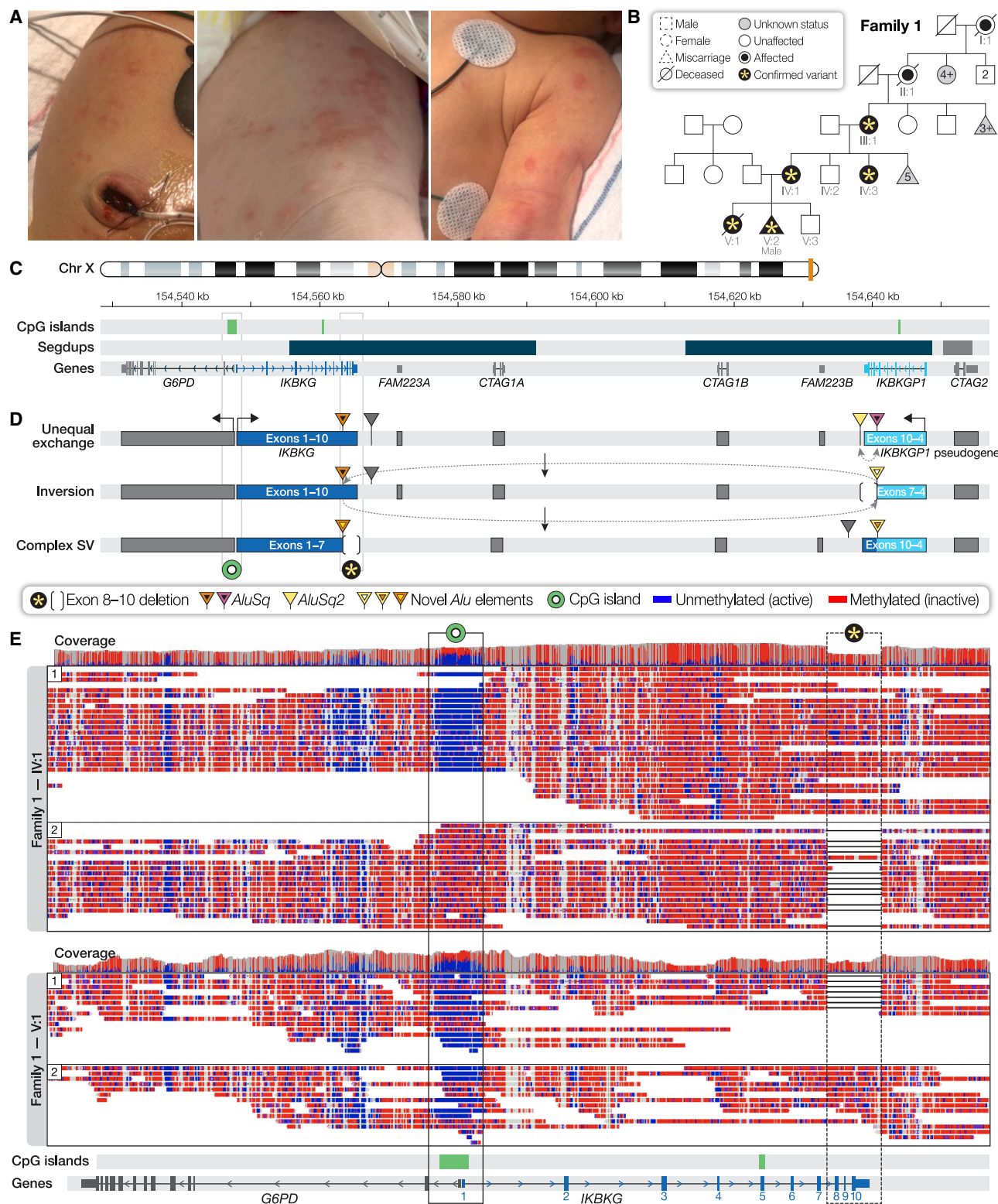


Figure 1. A deletion of *IKBKG* exons 8–10 likely resulted from an inversion

(A) The neonate displayed erythematous papules and vesicles in a Blaschkoid pattern on the abdomen, legs, and arms, consistent with the early findings of IP.

(B) Pedigree consistent with X-linked inheritance of an XY male-lethal disorder. The neonate's mother, maternal aunt, and grandmother had received a clinical diagnosis of IP, which was also suspected in the maternal great-grandmother and great-great-grandmother given their clinical history, recurrent miscarriages, and predominantly XX female offspring.

(C) Structure of the *IKBKG* locus showing the position of *IKBKG*, two large segmental duplications, and the pseudogene *IKBKG1*, which contains nearly identical copies of *IKBKG* exons 3–10.

(legend continued on next page)

variant likely originated in a distant relative after recombination between *Alu* transposable elements in *IKBKG* and *IKBKGP1*. Custom long-range PCR clinically confirmed the variant in the neonate's mother and grandmother (supplemental information) and in a subsequent male miscarriage (V:2) (Figure 1B). Because LRS also captures methylation status, which can be used to evaluate X-inactivation patterns, we determined that the mildly affected female family members had complete inactivation of the haplotype with the deletion, while the affected neonate had variable inactivation (Figure 1E; supplemental information). While it is unclear why the affected neonate did not have skewed X-inactivation, follow-up studies could look for a disease-causing variant on the paternally inherited X, which may be selected against. The family had a healthy male infant who was conceived spontaneously and tested negative for the *IKBKG* variant postnatally and is now using this information for preimplantation genetic testing.

The proband (II:1) from family 2 was a 2-year-old female diagnosed with IP in infancy based on physical findings of linear papules with hyperpigmentation and necrotic keratinocytes by punch biopsy at 14 days of age (Figure 2A). Because the proband was the only affected member of the family, a *de novo* variant in *IKBKG* was suspected, but targeted clinical genetic testing was negative. We performed research LRS of blood-derived DNA from the proband and her unaffected mother (I:1) and identified an atypical inversion bisecting *IKBKG* in the proband only (Figure 2B). Evaluation of phased reads from the proband revealed that the 27 reads spanning the inversion breakpoint and assigned to haplotype 1 were from the maternally derived X chromosome and showed no evidence of an inversion. In contrast, 6 of 21 reads assigned to haplotype 2 and 7 of 7 unphased reads showed evidence of an inversion, suggesting that 13 of 55 reads (24%) were likely from a chromosome carrying an inversion (Figure 2C). Reads that were split from those mapping to the left side of the inversion breakpoint within *IKBKG* also mapped to an intron of *GAB3*, suggesting an approximately 150-kb inversion event that duplicated the proximal part of *GAB3* (Figure 2D). Evaluation of methylation across both haplotypes revealed a mixed methylation pattern at the CpG island shared by *G6PD* and *IKBKG* for both haplotypes, but all 7 reads from haplotype 2 that were from the chromosome with the inversion were methylated, suggesting selection against chromosomes carrying this inversion during development (Figure 2C). Analysis of local methylation patterns in both the proband and mother revealed no

skewed X-inactivation in either individual (Figure S5). Identification of an atypical mosaic inversion on the paternally derived chromosome allowed for more accurate counseling regarding recurrence risk for this family.

Family 3 was identified during the preconception evaluation of a 25-year-old female with personal and maternal family histories of IP. IP had been clinically diagnosed over three generations, affecting the proband (III:1), her mother (II:1), maternal grandmother (I:1), and a brother (III:3), who also had Klinefelter syndrome (47,XXY) (Figure 2E). The proband showed classic IP skin findings of blistering at birth that progressed to hyperkeratotic lesions and swirling hyperpigmented patterns along Blaschko's lines. In adulthood, she had sparse eyelashes/eyebrows, nail ridges (Figure 2F), hypodontia, and linear hypopigmented atrophic patches but normal growth and development without neurologic or significant ophthalmic issues. Clinical genetic testing performed on blood-derived DNA from the proband, including next-generation sequencing of *IKBKG* and an oligonucleotide array, was negative. X chromosome inactivation was extremely skewed. Her younger brother (III:3) also presented with a characteristic IP rash from birth. A skin biopsy confirmed IP, and the karyotype showed 47,XXY. Both the mother and maternal grandmother exhibited typical IP skin changes and hair/nail abnormalities, with multiple miscarriages reported. Targeted LRS of the proband and affected brother revealed an ~11.7-kbp deletion that included exons 4–10 of *IKBKG* (Figure 2G), and both individuals had skewed X-inactivation (Figure S5B). The deletion breakpoints overlapped precisely with the breakpoints reported for the common exon 4–10 deletion, which arises by unequal exchange between two ERV1 elements (MER67B; Figure S6), suggesting that the deletion was missed by prior clinical genetic testing.^{5–7} Evaluation of primer sequences used to evaluate the common exon 4–10 deletion did not reveal polymorphisms in the family members that might interfere with primer binding and PCR; thus, the reason that clinical genetic testing did not identify the deletion remains unclear. While approximately 5% of individuals with a clinical diagnosis of IP have negative clinical and research testing, we do not know whether these individuals have a deletion missed by standard testing or have a novel variant that is unlikely to be detected by standard clinical genetic testing, similar to families 1 and 2.⁸

In summary, we describe three families with X-linked IP, all of whom were clinically diagnosed with this condition but lacked molecular confirmation due to

(D) The deletion identified in the family likely arose via a two-step mechanism. First, an unequal exchange event between *Alu* transposable elements (triangles) at *IKBKGP1* removed exons 8–10 of the pseudogene. Second, an exchange event between the *Alu* in intron 7 of *IKBKG* and the remaining *Alu* in *IKBKGP1* resulted in an inversion of the locus, which moved exons 8–10 of *IKBKG* to *IKBKGP1*. (E) Phased LRS data shows skewed X-inactivation over a CpG island within *IKBKG* (box) in the mildly affected mother (IV:1) but random X-inactivation in the more severely affected proband (V:1). Methylation status at CpG islands directs the expression of the gene at that site; methylated (inactive) CpG sites are shown in red and unmethylated (active) CpG sites in blue. The familial exon 8–10 deletion is also indicated (dashed box). Numbers indicate *IKBKG* exons.



Figure 2. Disease-causing variation missed by prior clinical genetic testing in two families with IP

(A–D) Family 2.

(A) The proband (II:1) displayed a rash consistent with IP at birth.

(B) The proband was the only affected individual in the family; thus, a *de novo* variant was suspected.

(C) Phased IGV view of LRS data with relevant gene regions shown. An atypical inversion bisects *IKBKG* and *GAB3* (dashed boxes) on the paternal haplotype (2). Arrows indicate reads that span from the promoter region of *IKBKG* to the inversion breakpoint within *IKBKG* and show that these reads are methylated. The inversion breakpoint within an intron of *GAB3* creates a small duplication, leaving one intact copy of *GAB3* on the affected haplotype.

(D) Subway plot demonstrates the structure of the complex SV, which includes the inversion and duplication.

(E–G) Family 3.

(E) Multiple females from family 3 with clinical diagnoses of IP also had miscarriages. The proband (III:1) had a mildly affected male sibling who was found to be 47,XXY.

(F) A photo of the proband's left hand shows abnormal nail beds.

(G) Analysis of LRS data from individual III:3 (shown) as well as III:1 (not shown) revealed the common exon 4–10 11.7-kbp deletion and skewed X-inactivation. Some reads in individual III:3 carrying the deletion are assigned to haplotype 1 as a result of poor phasing of shorter fragments generated using DNA isolated from saliva.

the limitations of commonly available clinical short-read technologies, such as exome, single-gene, or panel sequencing. Despite high suspicion for IP based on clinical features, a molecular diagnosis was desired for reproductive planning and counseling in all three families due to the high recurrence risk for affected mothers. Using LRS, we identified the causal variants for all three families and provided a likely mechanism underlying the formation of the variant in family 1 and an explanation for why the proband in family 1 was more severely affected than other females in the family. This informa-

tion enabled families 1 and 3 to pursue *in vitro* fertilization with preimplantation genetic testing to avoid transmission in future pregnancies, while family 2 received more accurate counseling regarding recurrence risk for their future offspring. These cases demonstrate the utility of LRS as a single test to simultaneously resolve complex SVs and phase variants and evaluate methylation patterns in complex regions of the genome. In addition, our results show that clinical genetic testing may not fully capture all variant types, especially within complex regions, suggesting that LRS as a single test is able to

reduce diagnostic complexity. Finally, our results demonstrate the utility of LRS for evaluating X-linked conditions where phenotypic manifestations may depend on the degree of X-inactivation. Overall, our findings support the broader development of LRS-based methods for conditions with limited or complex clinical genetic testing options.

Data and code availability

The accession number for the sequence data presented in this paper is AnVIL Portal: phs003047.

Acknowledgments

We thank the families for their participation in this study. M.H.W. is supported by NIH/NICHD grant K23HD102589. The GREGoR Consortium is funded by the National Human Genome Research Institute of the NIH through the following grants: U01HG011758, U01HG011755, U01HG011745, U01HG011762, U01HG011744, and U24HG011746. D.E.M. is supported by NIH grant DP5OD033357. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Declaration of interests

M.H.W. has consulted for Illumina and Sanofi and received speaking honoraria from Illumina, Sanofi, and GeneDx. D.E.M. is engaged in a research agreement with Oxford Nanopore Technologies (ONT), is on a scientific advisory board at ONT and Basis Genetics, has received travel support from ONT and Pacific Biosciences, and holds stock options in MyOme and Basis Genetics.

Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.xhgg.2025.100468>.

Web resources

OMIM, <https://www.omim.org>

Received: February 25, 2025

Accepted: June 6, 2025

References

1. Aradhya, S., Woffendin, H., Jakins, T., Bardaro, T., Esposito, T., Smahi, A., Shaw, C., Levy, M., Munnich, A., D'Urso, M., et al. (2001). A recurrent deletion in the ubiquitously expressed NEMO (IKK- γ) gene accounts for the vast majority of incontinentia pigmenti mutations. *Hum. Mol. Genet.* 10, 2171–2179. <https://doi.org/10.1093/hmg/10.19.2171>.
2. Frans, G., Meert, W., Van der Werff Ten Bosch, J., Meyts, I., Bossuyt, X., Vermeesch, J.R., and Hestand, M.S. (2018). Conventional and Single-Molecule Targeted Sequencing Method for Specific Variant Detection in IKBKG while Bypassing the IKBKGPI Pseudogene. *J. Mol. Diagn.* 20, 195–202. <https://doi.org/10.1016/j.jmoldx.2017.10.005>.
3. Pipko, N., Oh, R.Y., Kaplan, A., Shugar, A., Szuto, A., Weinstein, M., Yoon, G., Mendoza-Londono, R., Pope, E., Young, T., et al. (2025). Genome sequencing reveals novel IKBKG structural variants associated with incontinentia pigmenti. *Br. J. Dermatol.* 192, 933–935. <https://doi.org/10.1093/bjd/ljae462>.
4. Logsdon, G.A., Vollger, M.R., and Eichler, E.E. (2020). Long-read human genome sequencing and its applications. *Nat. Rev. Genet.* 21, 597–614. <https://doi.org/10.1038/s41576-020-0236-x>.
5. Bardaro, T., Falco, G., Sparago, A., Mercadante, V., Gean Molins, E., Tarantino, E., Ursini, M.V., and D'Urso, M. (2003). Two cases of misinterpretation of molecular results in incontinentia pigmenti, and a PCR-based method to discriminate NEMO/IKK γ gene deletion. *Hum. Mutat.* 21, 8–11. <https://doi.org/10.1002/humu.10150>.
6. Song, M.-J., Chae, J.-H., Park, E.-A., and Ki, C.-S. (2010). The Common NF- κ B Essential Modulator (NEMO) Gene Rearrangement in Korean Patients with Incontinentia Pigmenti. *J. Korean Med. Sci.* 25, 1513–1517. <https://doi.org/10.3346/jkms.2010.25.10.1513>.
7. Zhou, W., Zhang, F., Chen, X., Shen, Y., Lupski, J.R., and Jin, L. (2013). Increased genome instability in human DNA segments with self-chains: homology-induced structural variations via replicative mechanisms. *Hum. Mol. Genet.* 22, 2642–2651. <https://doi.org/10.1093/hmg/ddt113>.
8. Fusco, F., Paciolla, M., Conte, M.I., Pescatore, A., Esposito, E., Mirabelli, P., Lioi, M.B., and Ursini, M.V. (2014). Incontinentia pigmenti: report on data from 2000 to 2013. *Orphanet J. Rare Dis.* 9, 93. <https://doi.org/10.1186/1750-1172-9-93>.