

X-MAID disease caused by a novel synonymous *MSN* variant that disrupts mRNA splicing

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INTRODUCTION

- MSN* encodes moesin, a cytoskeletal adaptor protein that is critical in maintaining cell rigidity and is primarily expressed in lymphocytes and endothelial cells.

- Missense and premature stop variants in the *MSN* gene are known to cause X-linked moesin-associated immunodeficiency (X-MAID), a rare, X-linked disease.

RESULTS

1 Potential moesin splicing variant in brothers with a similar phenotype

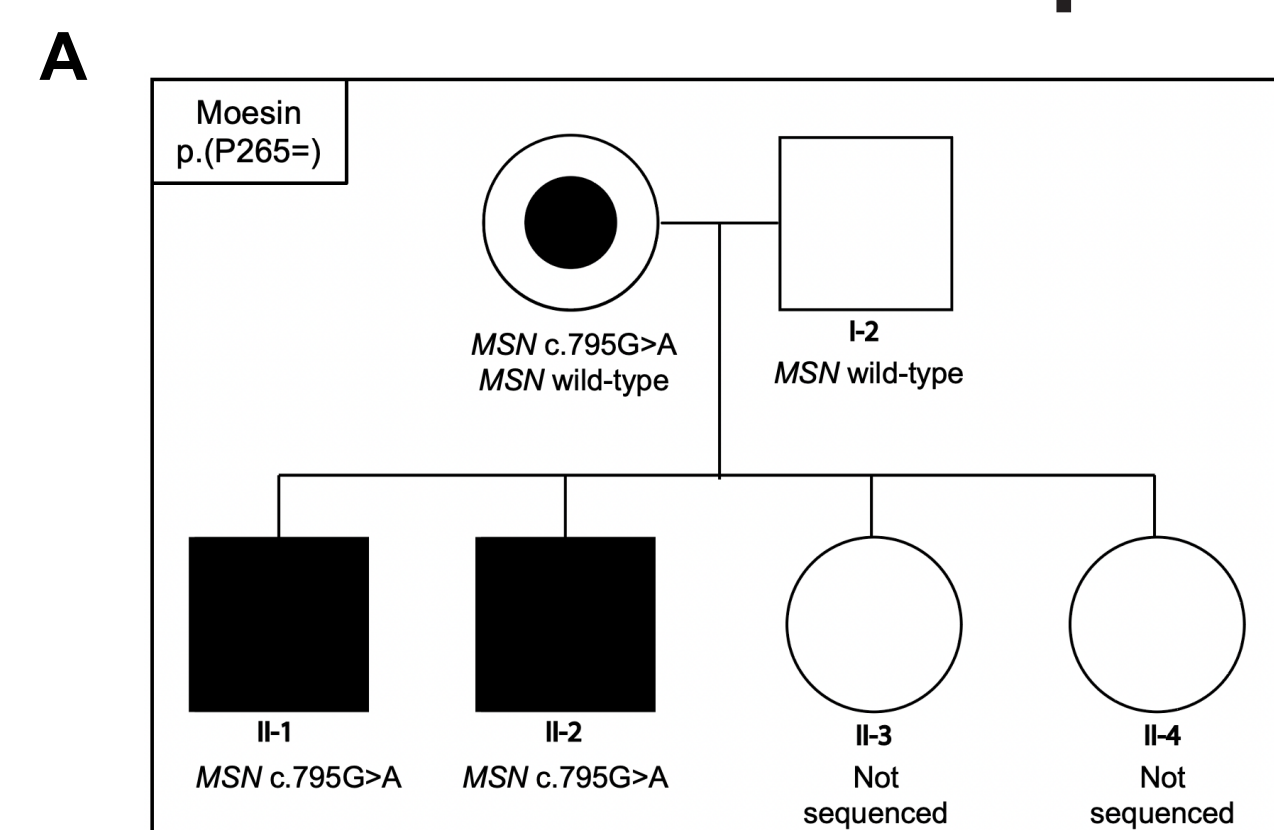


FIGURE 1: A Family pedigree. The *MSN* c.795G>A variant was discovered by an IEL gene panel and segregated with disease in the family. The brothers inherited the variant from their healthy, carrier mother (*MSN* is on the X chromosome). The variant is not reported in gnomAD and has a CADD score of 25 (damaging score). **B** The brothers presented with a very similar phenotype of severe lower leg dermatitis, chronic non-healing ulcers, clinical features of venous insufficiency, hypogammaglobulinemia, and mild lymphopenia. Images used with the consent of the family.

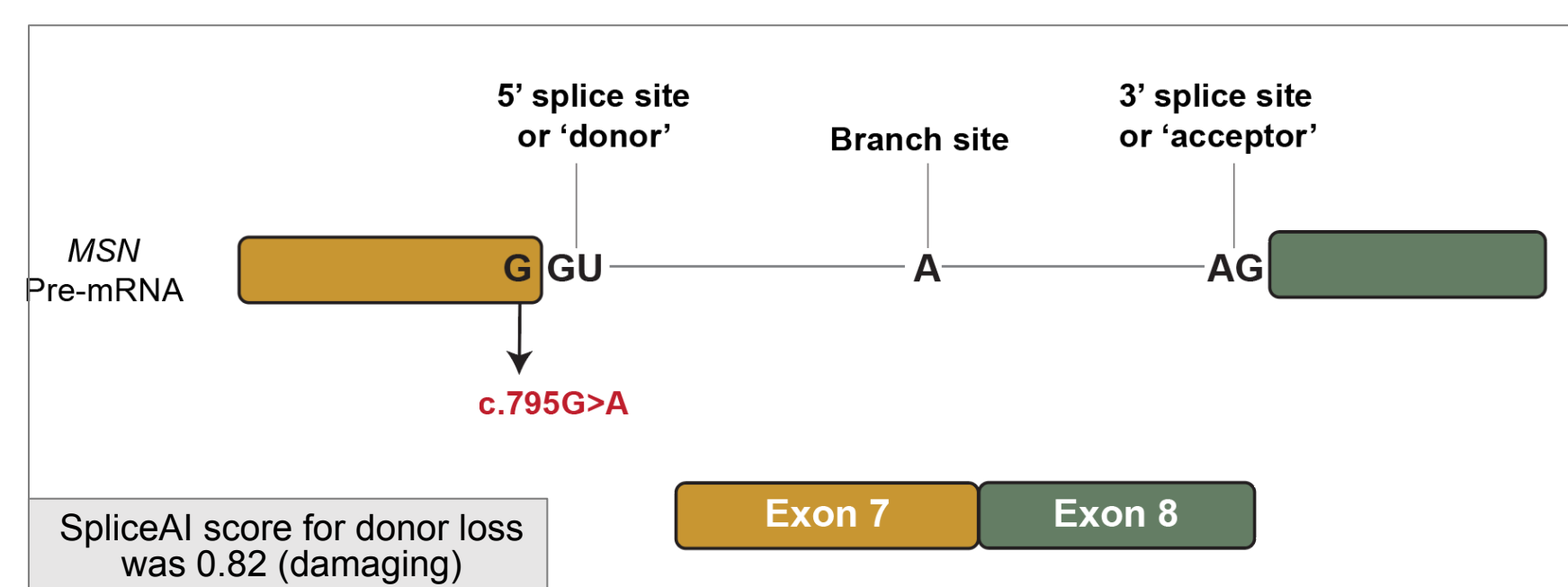


FIGURE 2: The variant is located beside the canonical splice donor site and is predicted to impact splicing based on in silico prediction tools, such as SpliceAI.

2 RNA-seq revealed aberrant splicing of the *MSN* transcript in the brothers

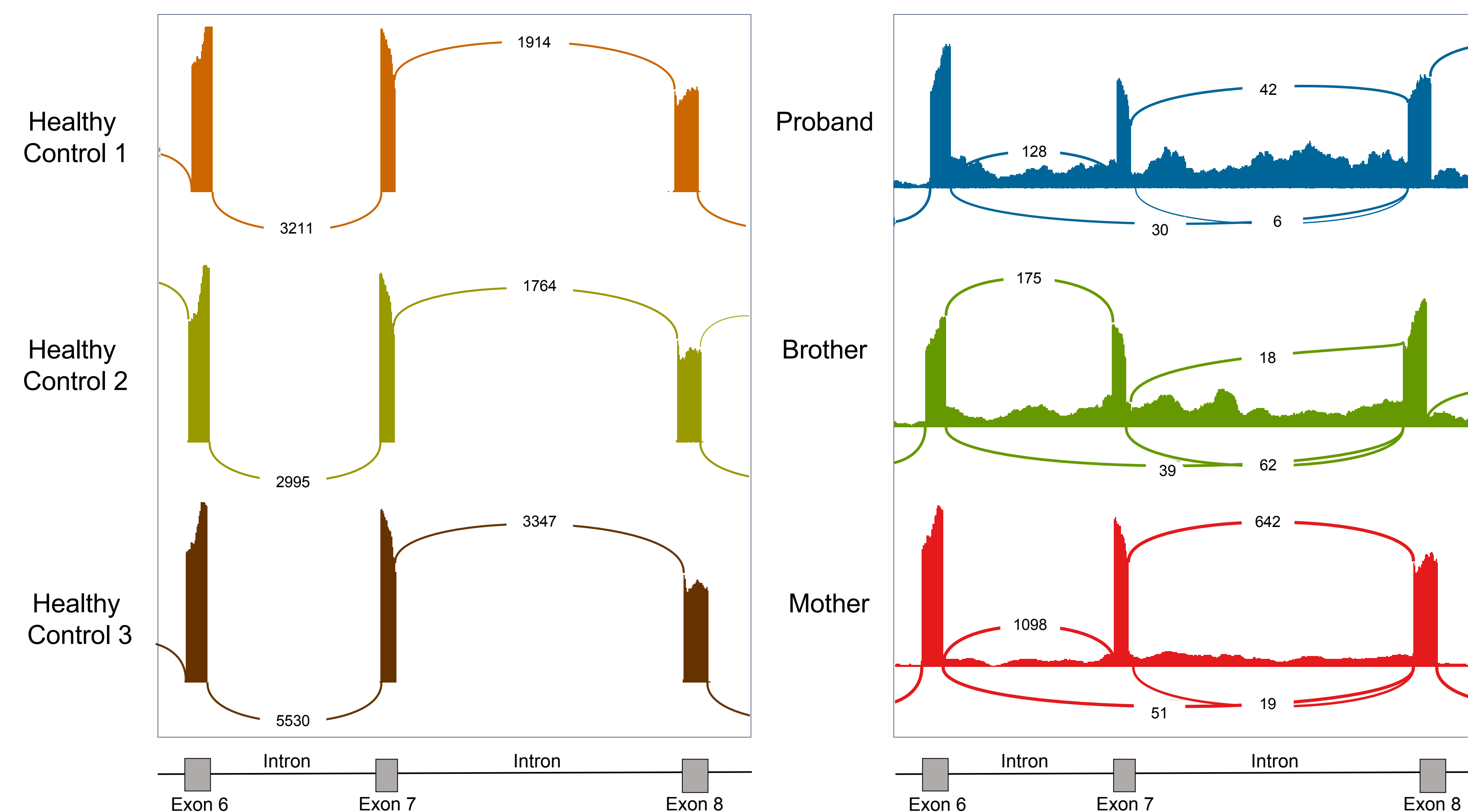


FIGURE 3: RNA sequencing revealed aberrant splicing of the *MSN* transcript in the brothers, consisting of either a complete skip of exon 7 or the retention of intronic sequences that result in a premature stop codon. These aberrant splicing events were observed at low levels in the carrier mother and were absent in the healthy controls.

3 qPCR analysis validated RNA sequencing results

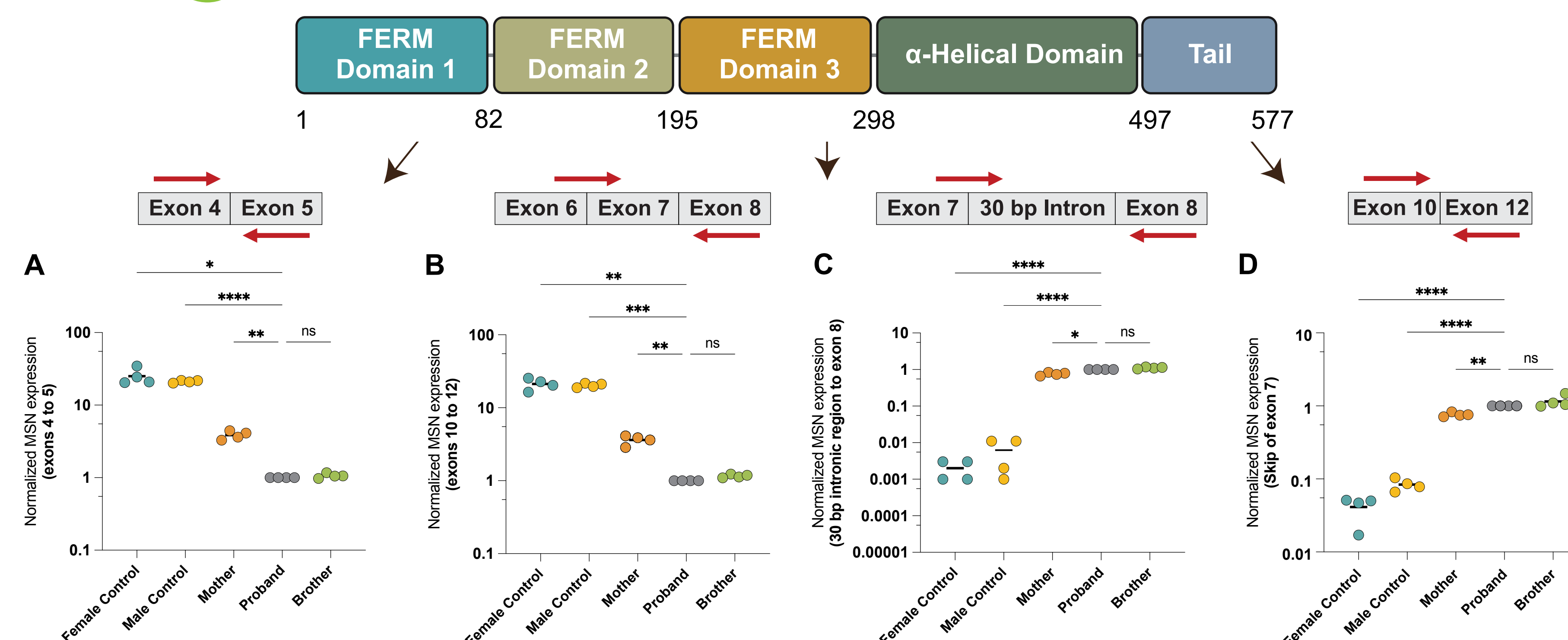


FIGURE 4: Quantitative PCR validated the splicing events, revealing minimal *MSN* transcript levels in the brothers, consistent with transcript degradation.

4 Moesin expression is absent in the brothers' cells

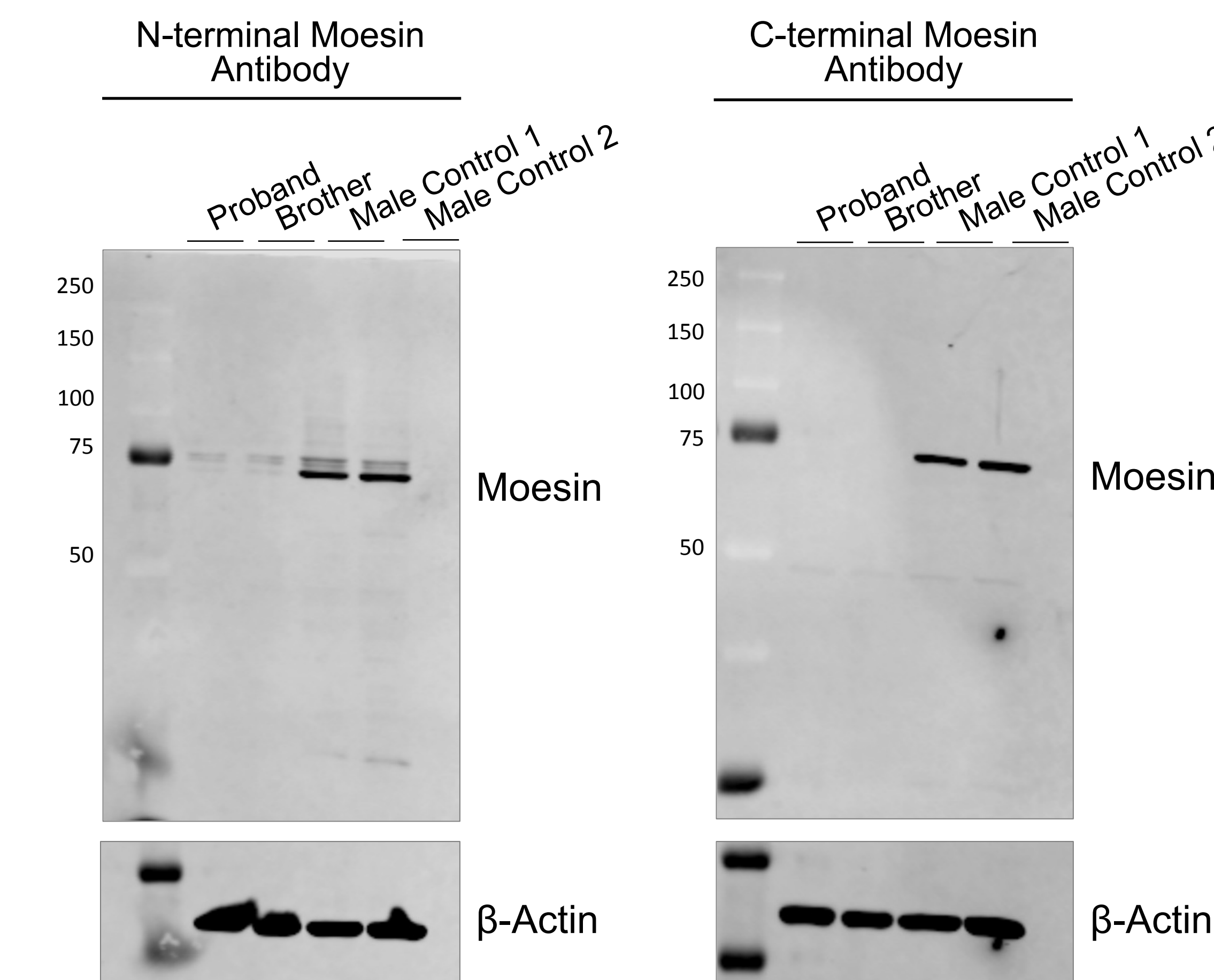


FIGURE 5: PBMCs were isolated from blood samples collected from the brothers and two healthy male controls, and lymphoblastoid cell lines (LCLs) were generated using the Epstein-Barr virus. Moesin production in LCLs was assessed through immunoblotting experiments using anti-moesin antibodies (N-terminal and C-terminal). We observed that moesin expression was absent in the brothers' LCLs, in contrast to LCLs from healthy male controls.

CONCLUSIONS

- This research highlights that a synonymous variant can cause moesin deficiency and X-MAID.

- Additionally, this research ends the diagnostic odyssey for the brothers and makes them eligible for a potentially curative hematopoietic stem cell transplant.

ACKNOWLEDGMENTS

We want to thank the patients and their family for trusting us.