N1C 2024 Annual Meeting

Identification of genetic variants amenable to ASO therapies

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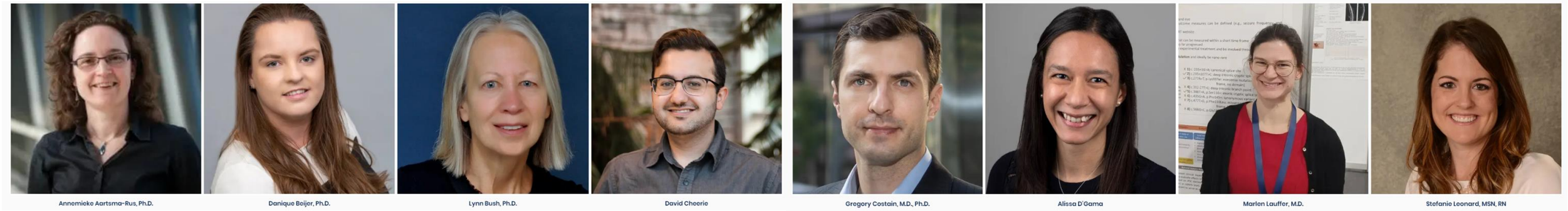
The Patient Identification Working Group



… and Aubrie Soucy Verran, Emma Sherill, Charu Kaiwar, Logan Newton



Who do we treat?



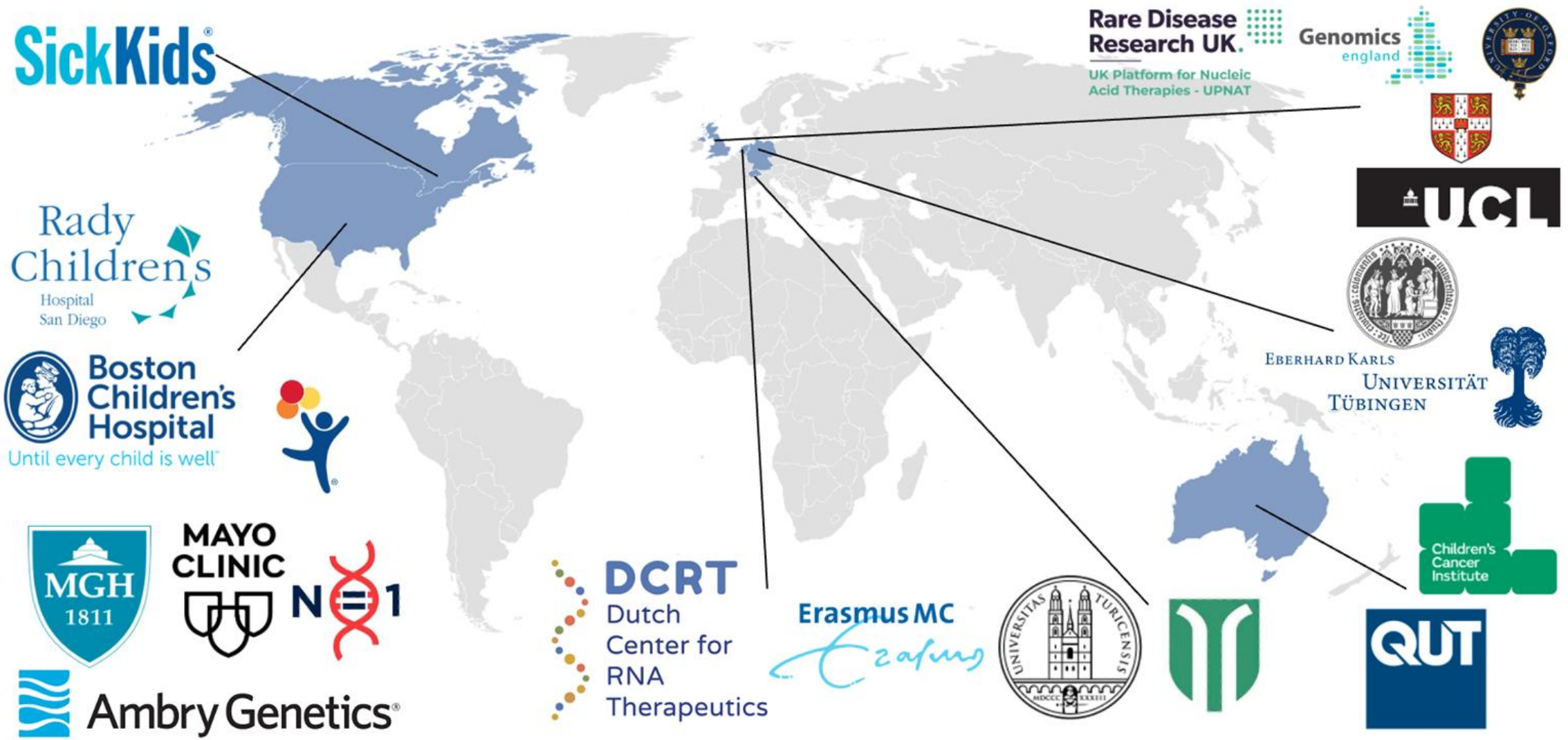
The Patient Identification Working Group

- ❑ Variant Subgroup
- ❑ Disease Subgroup
- ❑ Patient Subgroup

Consensus guidelines for eligibility assessment of pathogenic variants to antisense oligonucleotide treatments

The N1C VARIANT Guidelines

2024
Annual Meeting | Montreal, Quebec




ROUND 1
Draft
Feedback
Revise
Pilot
ROUND 2
Draft
Feedback
Revise
Pilot
ROUND 3
Draft
Feedback
Revise
Pilot

ASO Strategies

- ❑ Splice correcting
- ❑ Canonical exon skipping
- ❑ Transcript knockdown
- ❑ Upregulation from the wildtype allele

STEP 1

Is the variant description correct?

What is the inheritance pattern of the variant?

STEP 2

STEP 3

Has the pathomechanism been described?

Have splicing effects been assessed?

STEP 4

STEP 5

What ASO strategies can be considered?

STEP 1

Is the variant description correct?

What is the inheritance pattern of the variant?

STEP 2

STEP 3

Has the pathomechanism been described?

Have splicing effects been assessed?

STEP 4

STEP 5

What ASO strategies can be considered?

Inheritance	Gain-of-Function	Loss-of-Function	Dominant-Negative
Autosomal Recessive	Exon skipping Knockdown	Exon skipping	
Autosomal Dominant	Exon skipping* Knockdown*	Exon skipping* Wildtype upregulation	Exon Skipping* Knockdown*
X-Linked Recessive	Exon skipping Knockdown	Exon skipping	
X-Linked Dominant	Exon skipping* Knockdown*	Exon skipping* Wildtype upregulation**	Exon Skipping* Knockdown*

* Considerations for allele specific ASO

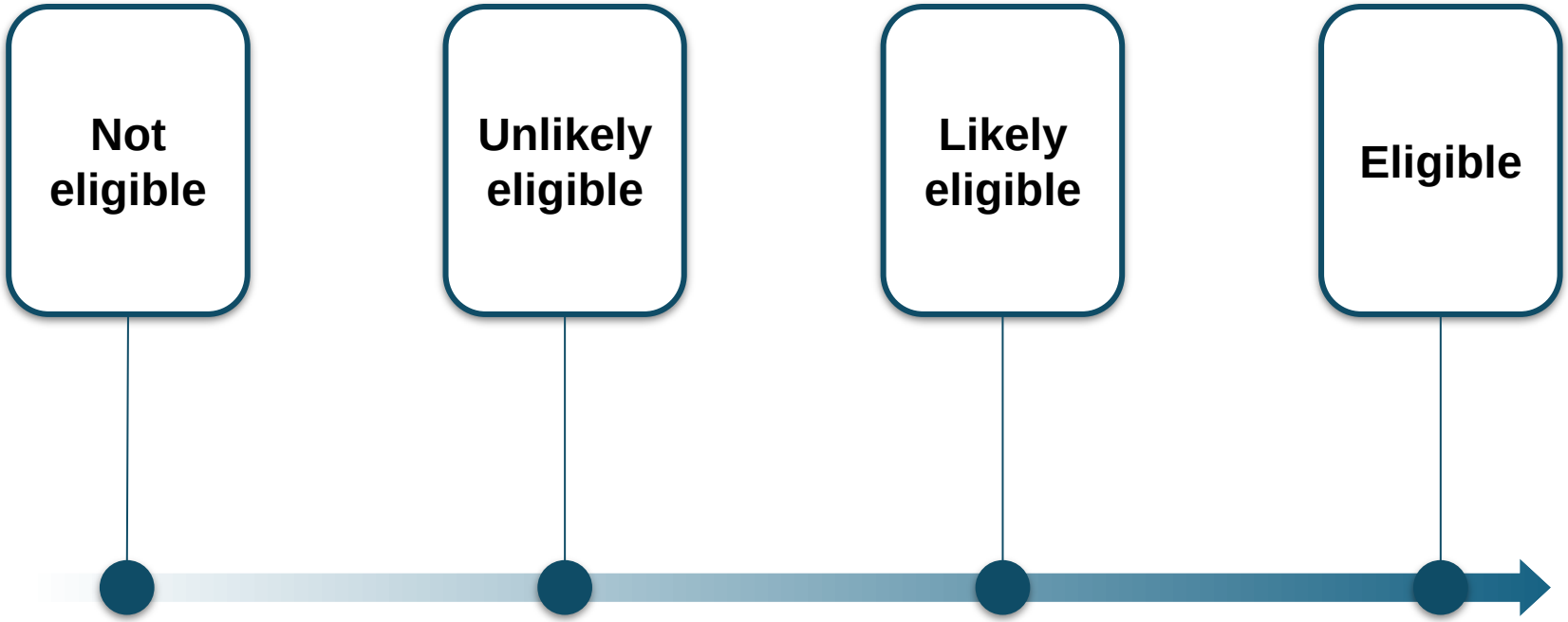
** Only applicable for individuals with two X chromosomes

Unable to assess

Guidelines not applicable to variant

Variant not diagnostic

Inheritance and pathomechanism unknown



Eligibility towards specific ASO strategy

29 practice examples for trialing our guidelines

Piloting Round	Transcript	Gene	Variant
1	NM_025152.3	*NUBPL*	c.815-27T>C
1	NM_000350.3	*ABCA4*	c.769-784C>T
1*	NM_024312.5	*GNPTAB*	c.3505_3504del
2	NM_020366.3	*RPGRIP1*	c.1468-128T>G
2	NM_000329.3	*RPE65*	c.1430A>G
2	NM_001086521.2	*NDUFAF8*	c.195+271C>T
2	NM_003907.3	*EIF2B5*	c.1156+13G>A
2	NM_206933.4	*USH2A*	c.2692C>T
2	NM_000391.4	*TPP1*	c.225A>G
2	NM_024298.5	*MBOAT7*	c.758_778del
2	NM_018075.5	*ANO10*	c.289del
2*	NM_024312.5	*GNPTAB*	c.3503_3504del
2	NM_003650.4	*CST7*	c.2035-946G>A
2	NM_000303.3	*PMM2*	c.640-15479C>T
2	NM_000202.8	*IDS*	c.1122C>T
3	NM_018075.5	*ANO10*	c.1025G>A
3	NM_001127222.2	*CACNA1A*	c.4174G>A
3	NM_024312.5	*GNPTAB*	c.3488del
3	NM_133433.4	*NIPBL*	c.5329-15A>G
3	ENST00000361390.2	*MT-ND1*	m.4142G>T
3	NM_014727.3	*KMT2B*	c.8079delC
3	NM_024312.5	*GNPTAB*	c.1123C>T
3	NM_005859.5	*PURA*	c.159dup
3	NM_001167623.2	*CACNA1C*	c.1216G>A
3	NM_000561.4	*HEXB*	c.1509-26G>A
3	ENST00000435607.3	*SCN4A*	c.3891C>A
3	NM_001244008.2	*KIF1A*	c.914C>T
3	NM_000492.4	*CFTR*	c.2989-313A>T
3	NM_001194.4	*HCN2*	c.736G>A
3	NM_000170.3	*GLDC*	c.538C>T

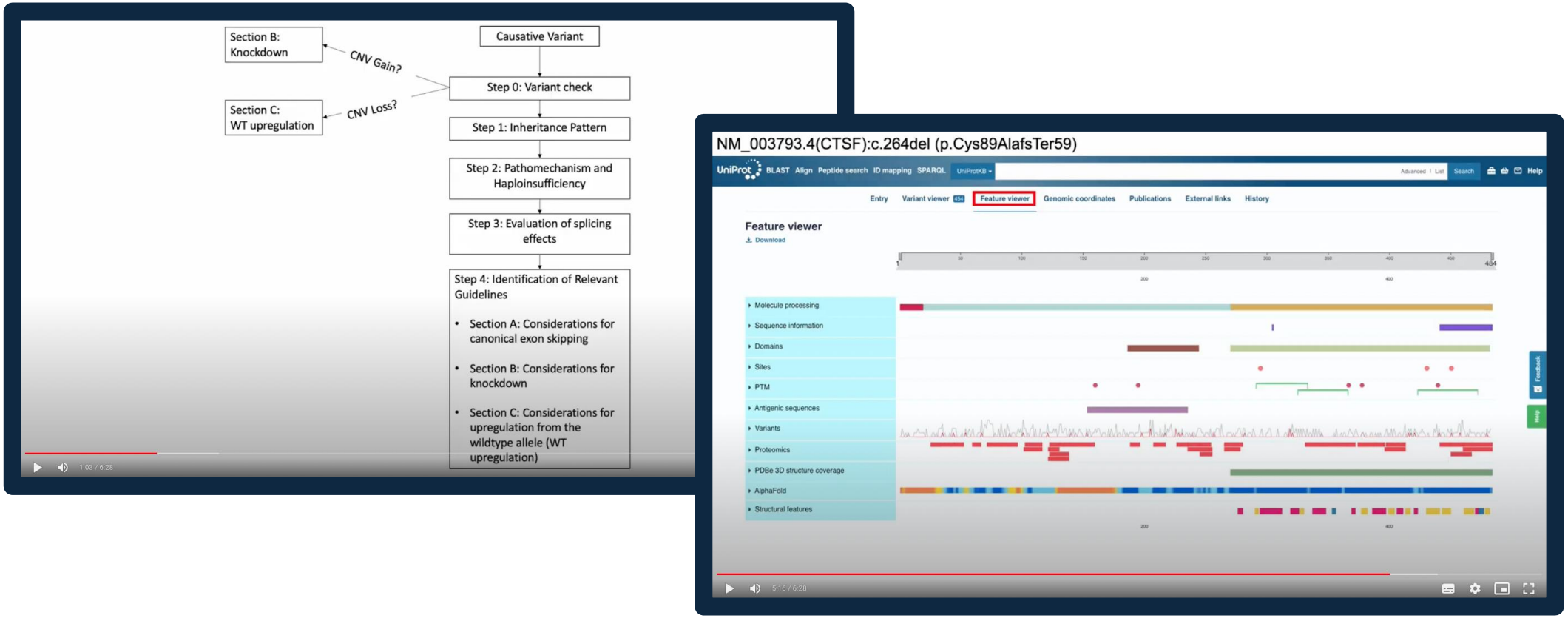
Variant 3: *GNPTAB* - NM_024312.5:c.3503_3504del

- Variant description is correct, ASO has been studied (https://doi.org/10.1089/hum.2020.034), however, this paper did not validate the effect of exon-19 skipping on protein function. In their discussion, the authors noted that the effect of this ASO on protein expression, subcellular localization, cleavage of the GlcNAc-1-phosphotransferase, and correction of mis-sorting of lysosomal enzymes needs to be studied.
- Additionally, exon 19 codes for a repeat stealth domain. However, this domain does not meet the criteria for exclusion (i.e., it is not the only domain, it is not a mutation hotspot, and it is not functionally proven to be important).
- The following was noted in the discussion:
  - *"However, unlike the Stealth domains 1-3 harboring a high number of ML II-causing missense mutations, only one ML III alpha/beta causing amino-acidic substitution has been reported on the fourth Stealth domain [c.3458A>G; p.(Asn1153Ser)]. Furthermore, a recent combined in vitro and in silico analysis of missense GNPTAB mutations has provided new insights into the role of these conserved Stealth regions for catalytic activity of GlcNAc-1-phosphotransferase, showing that the amino acid*
- **Therefore**

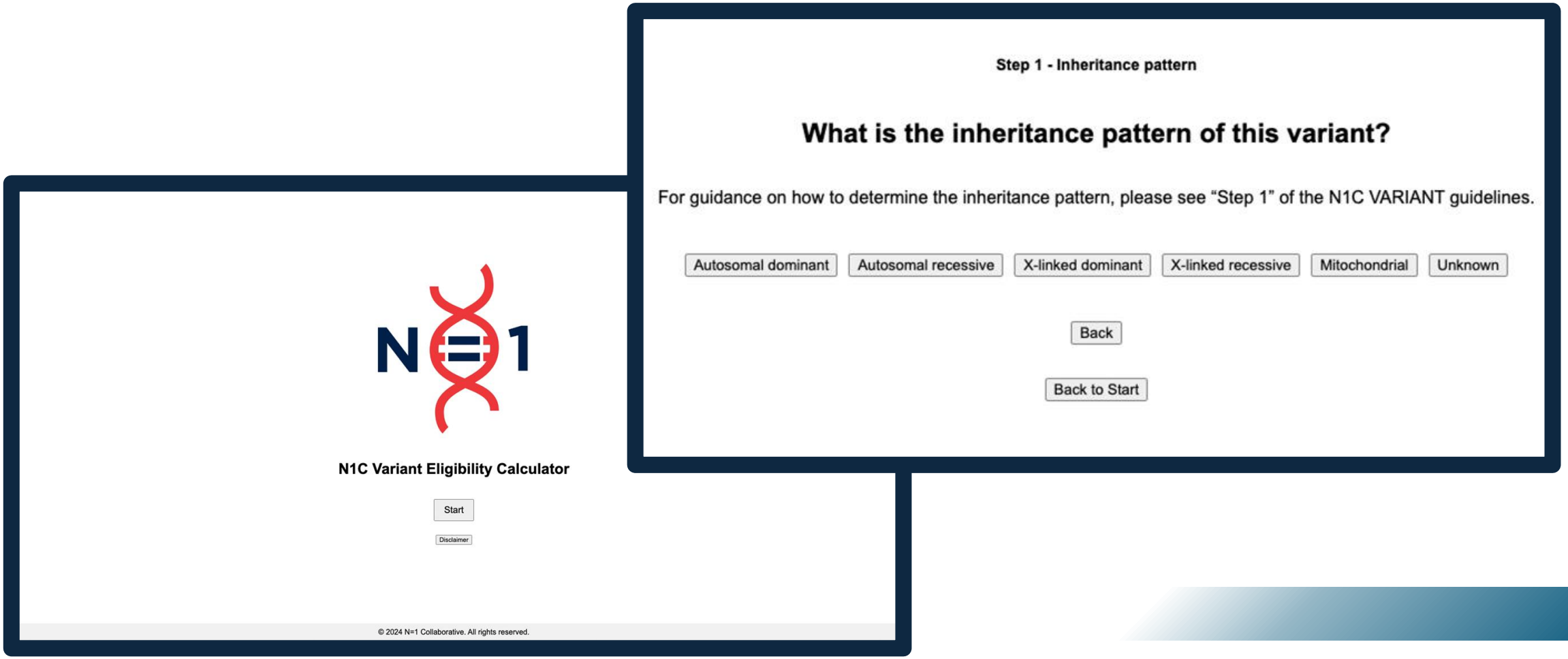
Variant 3: *NDUFAF8* - NM_001086521.2:c.195+271C>T

- Variant description is correct
- No ASO exists
- Variant is inherited in an autosomal recessive manner and variant is loss-of-function variant
- Functional evidence of aberrant splicing, determined using cDNA studies on RNA derived from patient fibroblasts (https://doi.org/10.1016/j.ajhg.2019.12.001)
  - Traces of wildtype transcript can be seen in the supplementary figures.
  - The variant meets all criteria to be established as a "probably" (intronic, not within 15 base pairs).
  - Note: It's important to make your own judgement using available data. The article stated degradation of the transcript is associated with this variant, but trace amounts can be seen in the supplementary. Furthermore, consider the location of the variant and whether its position can possibly destroy a branchpoint (typically found within 40-80bp of the 3' of the intron) or canonical splice site.
- **Therefore, this variant is likely eligible for splice correction**

12 instructional video examples for assessment of different variants and ASO strategies



Variant Eligibility Calculator walks users step-by-step through the N1C VARIANT Guidelines



Summary

- ❑ Guides the rare disease community in identifying candidates for variant-specific ASO therapies
- ❑ Supports integrating these assessments into clinical practice for improved patient care
- ❑ Enables standardized assessment of pathogenic variants to prioritize cases for ASO therapy development

Future Work Variant Subgroup

- ❑ provide guidance (workshops) on how to use and implement guidelines
- ❑ improve and refine guidelines
- ❑ automate variant identification and eligibility assessment

Future Work Patient Identification Group

- ❑ Communication guidelines for clinicians and genetic counsellors
- ❑ Roadmap to treatment

Acknowledgements

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N=1 Collaborative members

Patients and their families

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