RNA4RD Ascertainment Pathway

1. **Context 1 ~ 75/100 cases. Splicing VUS.**
   - presumed monogenic disorder; AND
   - putative splicing variant(s) classified VUS in a phenotypically concordant gene; AND
   - variant allele frequency consistent with disease incidence; AND preferably
   - variant segregates with disease; AND
   - associated gene amenable to examination by RNA-Seq or RT-PCR in an available biospecimen

2. **Context 2 ~ 25/100 cases. No hit or single hit with another test strongly indicative of a known associated gene.**
   - presumed monogenic disorder; AND
   - no identified variant; OR monoallelic variant in a phenotypically concordant recessive gene with an allele frequency consistent with disease incidence; AND
   - another biochemical or diagnostic test, or a reliable functional assay, is strongly indicative of a genetic disorder with one (or only a few) associated genes; AND
   - associated gene(s) are amenable to examination by RNA-Seq or RT-PCR in an available biospecimen

Q&A defined so far:

1. *Are variants already classified LP/P eligible for RNA testing via RNA4RD?*
   → **YES:** With clinical justification explaining why RNA testing will help to inform diagnosis or clinical management of the affected individual.

2. *Are essential splice-site variants eligible for RNA testing via RNA4RD?*
   → **YES:** With clinical justification explaining why RNA testing will help to inform diagnosis or clinical management of the affected individual.

3. *Is the degree of genotype-phenotype concordance important for eligibility for RNA4RD?*
   → **YES:** This informs the triage process into RNA4RD. The submission portal has been updated to record a clinical description of genotype-phenotype correlation
   a. Phenotype highly specific for a disease with a single genetic aetiology
   b. Phenotype highly specific for a disease with only a few associated disease genes
   c. Phenotype strongly consistent with gene but high genetic heterogeneity of disorder
   d. Phenotype consistent with gene, but not highly specific, and condition can be heterogeneous
   e. Phenotype not wholly consistent with gene.

4. *Will RNA4RD accept cases to help exclude pathogenicity of an identified variant?*
   → **YES:** With clinical justification explaining why RNA testing will help to inform diagnosis or clinical management of the affected individual.

5. *Will RNA4RD accept cases where a variant is not strongly predicted to alter splicing?*
   → **YES:** Triage into RNA4RD is based on the clinical merit of RNA testing. No splicing prediction tool is 100% accurate. RNA testing to confirm splice-neutral impact of a variant can be of equal importance as confirming splice-altering behaviour. Pathology laboratories will depend upon having access to a cohort of splice-neutral cases to validate their RNA testing pipelines – so ascertainment of some splice-neutral cases is important.

6. *How does ascertainment into RNA4RD work?*

   Phase 1: **Submit case** and variant (if known) into the submission portal. Contact us if your organisation's firewall prevents the link from working. (http://www.kidsneuroscience.org.au/splice_site_submission)

   Phase 2: ***In Silico* assessment**: Cooper team conduct *in silico* assessment of each variant and reply (typically within 7 days) to the referring clinical team with our opinion on the likelihood of being a splice-altering variant and the technical feasibility of RNA testing using a clinically accessible specimen(s).

   Phase 3: **Automatic Triage:** Context 1 cases with *genotype-phenotype concordance* rated **a**, **b**, or **c** are eligible for automatic triage into RNA testing. Before RNA testing can proceed, the Cooper team require Research Consent and details from the Genetic Testing Diagnostic Laboratory regarding all other variants in the target gene (exonic or intronic, common or rare).

   Phase 3b: **Priority access for Aboriginal or Torres Strait Islander peoples**. Context 1 **or** 2 cases with phenotypic concordance rated **a**, **b**, or **c** are eligible for automatic triage into RNA testing.

   Phase 4: **Clinical Consult:** Context 1 cases with *genotype-phenotype concordance* rated **d** or **e** are referred to an RNA4RD 'Investigator pod' (2x Clinical Geneticists + 1x Diagnostic Laboratory Representative) to assess clinical merit of RNA testing, or another diagnostic investigation, as best next step. The RNA4RD Investigator pod can triage the case directly into RNA4RD or refer for further discussion via MDT.

   Phase 5: **Multi-disciplinary Team discussion** for Context 2 cases and Context 1 cases where there is some complexity.