Genomic characteristics of Rett Syndrome modifier genes

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Introduction

- Genetic modifiers are non-primary disease-causing genes that alter the severity of genetic diseases and thus may act as a therapeutic target.
- Rett syndrome (RTT) is a rare neurodevelopmental disorder typically caused by mutations in the X-linked *MECP2* gene.
- There are currently no therapeutic options for RTT patients.
- Skewed X inactivation has been implicated in RTT but cannot fully explain the variable severity of RTT. Therefore, genetic modifiers may also play a role.
- Recently, a large RTT modifier screen in *Mecp2*/Y mice assessed phenotype improvement following mutagenesis (Enikanolaiye *et al.*). This analysis identified 31 genes that improved the RTT phenotypes.
  - The resultant gene set was enriched for genes involved in transcriptional regulation and DNA damage.

Methods

- Genetic constraint was examined via gnomAD observed/expected metric and gene variation intolerance rank (Abramovs *et al.*)
- The developmental expression pattern of each modifier was compared to *MECP2* using RNA sequencing data from human tissue from the BrainSpan database.
- Biological processes associated with this set of genetic modifiers in the context of RTT were determined via the GeneWalk program (Ietswaart *et al.*)
- To determine which of these genes are most tractable as human drug targets, the human phenotypes associated with these 31 genes were assessed using the Open Targets Genetics v6 database.
- Results were filtered programmatically for signals which were most likely attributable to these genes (i.e., L2G score of 0.5 or greater).

Results

- *CD22*, *FAN1* and *APOA5* were the least constrained genetic modifiers.
- *BIRC6*, *DENND4A*, *RAD50* and *FAN1* showed similar temporal expression patterns to *MECP2* and thus are likely involved in similar processes.
  - 75% of these genes are involved in DNA damage response (DDR)
- The top biological processes associated with the candidate RTT genetic modifiers were double-strand break repair and transcriptional regulation.
- *BIRC6* was associated with multiple cognitive traits such as cognitive performance and aspects of educational attainment.

Genetic Constraint

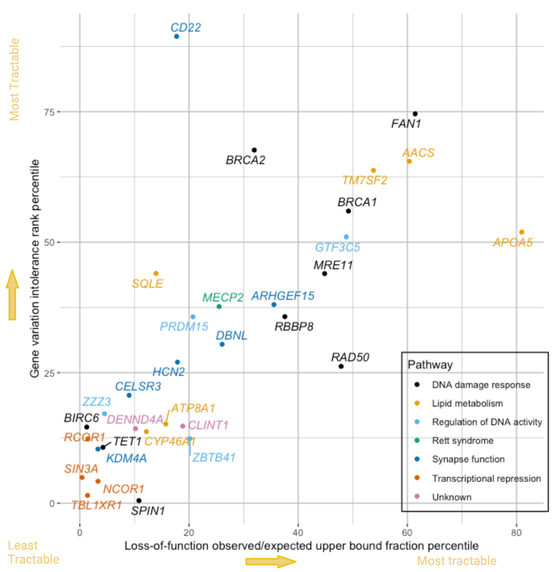


Figure 1. Exploring tractable drug targets based on genetic constraint. The gnomAD loss of function observed/expected metric was plotted against gene variation intolerance rank (Abramovs *et al.*) to visualize theoretical tractable drug targets of the potential RTT modifier genes. Genes with higher percentiles are less constrained and thus better drug targets.

Temporal Expression

BrainSpan stage

Stage	Age
2	8PCW ≤ Age < 10PCW
3	10PCW ≤ Age < 13PCW
4	13PCW ≤ Age < 16PCW
5	16PCW ≤ Age < 19PCW
6	19PCW ≤ Age < 24PCW
7	24PCW ≤ Age < 38PCW
8	birth ≤ Age < 6M
9	6M ≤ Age < 19M
10	19M ≤ Age ≤ 5Y
11	6Y ≤ Age ≤ 11Y
12	12Y ≤ Age ≤ 19Y
13	21Y ≤ Age < 40Y

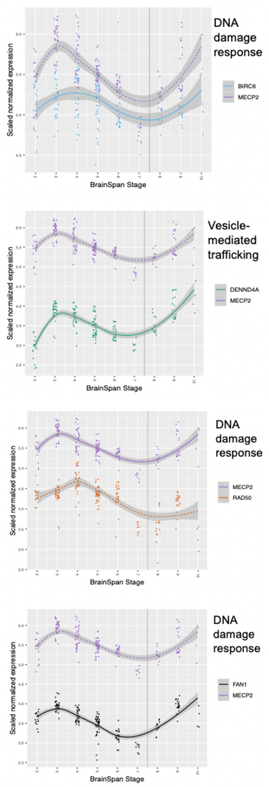


Figure 2. Developmental expression patterns of the RTT candidate modifiers and *MECP2*. Of the 31 candidate RTT modifiers *BIRC6, DENND4A, RAD50* and *FAN1* showed similar developmental expression patterns to *MECP2*.

Implicated Biological Processes

GO Name	n
Regulation of transcription by RNA polymerase II	7
Double-strand break repair via homologous recombination	5
Negative regulation of DNA-templated transcription	5

Figure 3. Top biological processes attributed to the canidate RTT genetic modifiers. The GeneWalk program was used to determine the biological processees implicated in this specific biological context.

Human Phenotypes Associated with DDR Genes

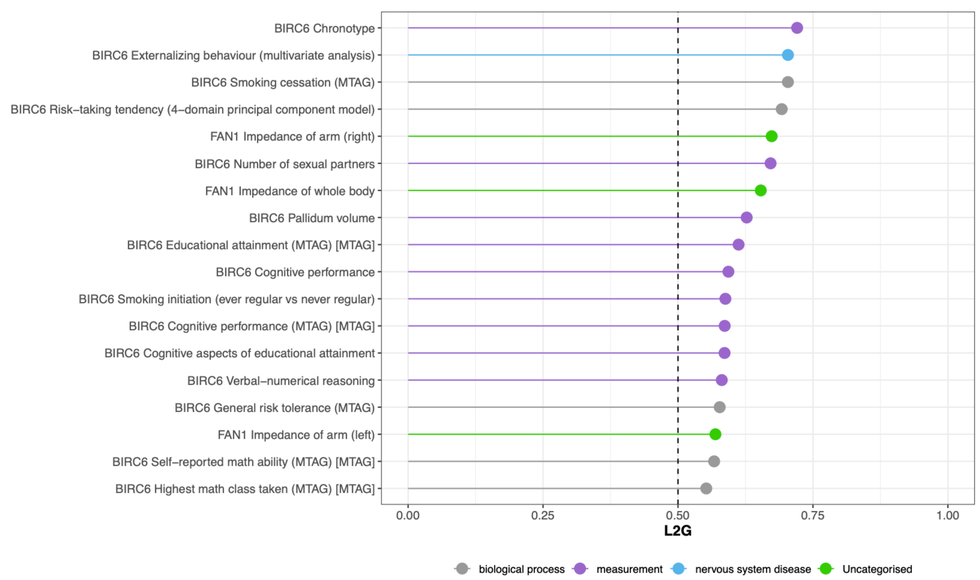
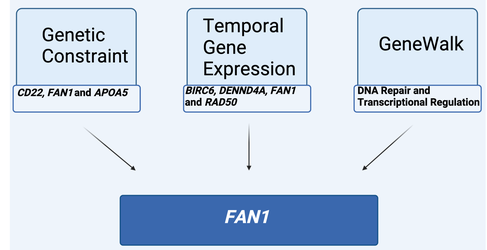


Figure 4. Human trait-gene associations from GWAS for DNA repair genes. Trait associations with an L2G score of 0.5 or greater were plotted by gene and significance. *BIRC6* made up most of the most likely attributable associations in the DNA damage repair pathway and was associated with various cognitive traits.

Abramovs, N., Brass, A. & Tassabehji, M. GeVIR is a continuous gene-level metric that uses variant distribution patterns to prioritize disease candidate genes. Nat Genet 52, 35–39 (2020).
Enikanolaiye, A., et al. Suppressor mutations in Mecp2-null mice implicate the DNA damage response in Rett syndrome pathology. Genome research 30.4, 540-552 (2020).
Ietswaart, R. et al. *GeneWalk identifies relevant gene functions for a biological context using network representation learning.* Genome Biology **22,** 55 (2021)
Mountjoy, E., Schmidt, E.M., Carmona, M. et al. An open approach to systematically prioritize causal variants and genes at all published human GWAS trait-associated loci. Nat Genet 53, 1527–1533 (2021).

Conclusion



- Human-relevant information for RTT genetic modifiers was identified.
- This data points to *FAN1* as a strong RTT genetic modifier candidate.
- Therefore we are currently using functional genomics to examine *FAN1* as a RTT genetic modifier in human induced pluripotent stem cells (iPSCs).
- This will allow us to confirm the modifier effect and elucidate the underlying biological mechanisms.

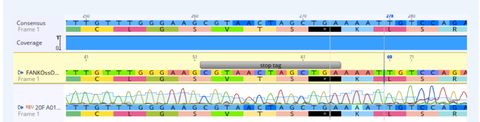


Figure 5. Confirmed *FAN1* KO in RTT iPSCs. Sanger sequencing chromatogram showing *FAN1* has been knocked out (KO) completetly in patient derived RTT induced pluripotent stem cells (iPSCs) via a stop tag CRISPR-Cas9 protocol.

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
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