Metabolic and Genetic Explorations in Refractory Schizophrenia Project: Findings from whole genome and RNA sequencing in the first 10 participants

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BACKGROUND

- The **UBC MAGERS (Metabolic Explorations in Refractory Schizophrenia) Study** is an intensive pilot, multimodal –omics and psychiatric genetic counselling research project conducted in participants with **highly treatment-resistant schizophrenia (SCZ) or schizoaffective disorder (SZAD)** hospitalized in the tertiary provincial BC Psychosis Program Unit at UBC Hospital.
- We hypothesized that this cohort with "extreme phenotypes" of psychosis is likely to be enriched in potent, rare or novel genetic risk variants with potential precision medicine implications

METHODS

Inclusion and exclusion criteria

- Capability to provide informed, consistent consent, or assent with surrogate consent
- DSM-5 diagnosis of SCZ (schizophrenia), SAD (schizoaffective disorder), and/or Catatonia
- Exclusions included psychotic disorder due to substance use, medication, or a medical disorder

Phenotyping

- Structured birth histories and three-generation family histories (AD, PC)
- Mini-International Neuropsychiatry Interview (MINI) and Childhood Trauma Questionnaire (OL)
- Detailed reviews of medical records, labs, EEG and imaging data, physical exams (VW, RS)
- Structured neurological and dysmorphology exams (RS) ; neuropsychological assessment (MM, IT)
- DSM-5 diagnoses by multidisciplinary consensus and MINI (PC, AD, JL, HN, OL, RW, WH)
- Extensive biochemical screening for psychosis-associated inborn errors of metabolism
- Immunophenotyping utilizing a MesoScale cytokine and inflammatory marker panel (CWS, CB)

Genetic investigations Chromosomal microarrays were performed in the Royal Columbian Hospital Molecular Cytogenetics Lab (CT, AM, MH), and whole genome DNA (gDNA) and RNA sequencing (RNA-Seq) at BC's Genome Sciences Centre (AJM). gDNA reads were barcoded using 10xGenomics Chromium library kits to permit linked read sequencing. Effective average read depth was 32X. RNA-Seq was performed on 4 samples per case (≈ 40M reads per sample)

Bioinformatics

DNA and RNA analysis was performed in UBC's Michael Smith Labs (MSL). In the Pavlidis lab (GPM, SR, PP), linked, bar-coded gDNA reads were aligned to the reference hg19 genome using 10xGenomics' Long Ranger pipeline. *Single nucleotide variants (SNVs) and short indels* were called using GATK, quality-filtered, and annotated with Ensembl VEP 98, gnomAD allele frequencies, and ClinVar. *Gene-level annotation (GLA)* included gnomAD's mutation intolerance scores (pLI and Z-score), haploinsufficiency ranking, and various gene lists, including ACMG actionable genes; those with reported schizophrenia, bipolar disorder (BP), or frontotemporal dementia association; and metabolic pathway genes with psychiatric symptom association. SNVs and indels were filtered based on VEP-rated impact, rarity (MAF < 0.0005), and pathogenicity (CADD ≥ 20); prioritized using other available annotation, and reviewed in the IGV browser in conjunction with RNA-Seq data for independent evidence of validity. *Structural variants* were called using Long Ranger, and annotated using DGV, DGV Gold and gnomAD population frequencies, as well as GLA. Variants were visually inspected in 10xGenomics' Loupe browser and compared with chromosomal microarray calls

Gene expression was estimated from RNA-Seq data with an in-house pipeline based on RSEM and STAR. For allele-specific expression, RNA-seq data was aligned to phased personalized genomes generated from a high confidence subset of the genomic variants. The significance of allelic imbalance was inferred with ANEVA-DOT using GTEx whole blood background expression data

Additional prediction of *functional protein impact* in the MSL Gsponer lab (EW, JS) utilized their in-house tools, including LIST-S2 and IDRBind.

Variant curation

Variants were curated (JJ, AE, KB, RS) per ACMG guidelines and genotype-phenotype correlation, using web-based tools and databases, including SCHEMA (Schizophrenia Exome Sequencing Meta-analysis Consortium (Singh et al, 2020), SZGR2 (Schizophrenia Gene Resource 2); Varsome, ClinVar, DECIPHER, DGV, OMIM; GeneCards, GeneDistiller, STRING; UniProt, SWISS-MODEL; dbSNP, UCSC Genome Browser; PubMed, PMC; PER ; and mouse phenotyping data (IMPC, MGI).

RESULTS

In these 10 participants, we identified 195 moderate-high impact DNA sequence variants (mean 19.5, range 12-42). 3 cases harbored LoF (predicted loss-of-function) mutations (1 each) relevant to their neuropsychiatric phenotypes. There were 5 (2.6%) in-frame insertions/deletions. 177 (90.8%) of mutations were missense; 12 (6.2%), in 8 participants, mapped into functional protein domains, 1 to core, and 5 into buried regions, where germline mutations are more likely to be pathogenic. The rest, residing mainly in intrinsically disordered protein regions, are likely benign, but 24 mapped to protein-protein interaction sites (2 to core, 3 to buried, and 4 to rim interaction interface features).

Table 1. Clinical overview and notable variants

Study ID	Sex, age	Main Psychiatric Diagnoses	Adm. PANSS[1] Score (30-120)	Discharge. PANSS[1] Score (30-120)	DOR[2] Score (1- 6)	Relevant Family History	Chromosomal Microarray Results	Genome Sequencing Results (some highlights listed)
P1	M, 38	SCZ	63	59	5	Brother with MDD, maternal aunt and great-grandfather with alcoholism, cousins with BPD/SAD	Normal male microarray	35 prioritized variants VUS[3] in MDGA1 (p.T152M)
P2	M, 27	Psychosis NOS, most likely SCZ; r/o OCD	102	92	5	Maternal half-brother with DD/ID and ASD caused by unshared chromosome abnormality (including auditory/visual hallucinations)	Xp22.33 deletion (SHOX) from mildly affected mother	19 prioritized variants P[4] variant in GRK2 (p.K319X) VUS in MGAT5 (p.V368L) Compound heterozygosity for VUS in LRP1B
P3	M, 64	SAD and OCD	31	NC	6	2 sisters with OCD, 1 with MDD. Multiple sibs with possible psychosis.	Normal male microarray	50 prioritized variants VUS in ATR (p.D1687G) VUS in RET (p.R982H): ACMG SF gene[5]
P4	M, 37	SAD-BP sub type	99	62	4	None reported	15q13.3 deletion (CHRNA7) from unaffected mother	20 prioritized variants VUS in WDR20 (p.G80R)
P6	M, 37	SAD-BP sub type	123	83	5	Father with addiction + MI; brother with MI	Normal male microarray	13 prioritized variants P LoF[6] variant in SETD1A (p. Q484X)
P7	M, 55	SAD	102	61	5	Father with alcoholism; maternal uncle had a psychotic episode; maternal aunt, BPD and anxiety	Normal male microarray	16 prioritized variants VUS in PI4KA (p.L2040I), NRXN3 (p. G426D), and ARHGEF17 (p. Q1090L)
P8	M, 41	SCZ	88	88	5	Maternal: 2 aunts with SCZ or SAD; 2 cousins with MI. Paternal uncle BPD, grandfather, depression.	Normal male microarray	17 prioritized variants VUS in NR2E1 (p.K53R)
P9	F, 28	SCZ	97	87	5	Alcoholism in both parents, ?BPD in father	Normal female microarray	12 prioritized variants VUS in CNOT1 (p.Q821H) and CHD7 (Q1701E)
P10	M, 25	SCZ	112	91	5	Mother has SCZ	22q11.2 duplication (TOP3B, maternal inheritance)	22 prioritized variants. Heterozygous P LoF variant in ATP7B (recessive gene for Wilson's disease, p. Q717X). VUS in SETD1B (p.R262C) and GGA1 (p.A192T)
P11	M, 36	SCZ	86	69	5	Maternal uncle with depression	Normal male microarray	18 prioritized variants Possible P LoF variant in FOXP1 (p.S561X) Likely P variant in CNOT1 (p.E884G)

[1]Pos. & Neg. Symptom Scale in SCZ. [2]Degree of Resistance to Rx Scale. [3]VUS = Variant of Uncertain Significance. [4]Pathogenic. [5]ACMG Secondary Finding [6]Loss-of-Function.

Table 2. Summary of selected mutations highlighted in next panel

Study ID	Gene	Relevant Roles	CADD Score	Mutation	ACMG Class	Classification Rationale	Other Evidence
P1	MDGA1	Encodes protein critical for regulating perisomatic inhibitory synapses in hippocampal CA1 pyramidal neurons, and synaptic adhesion via neurexin-neuroligin-2 interaction (Connor et al, 2017). Necessary for normal LTP, spatial learning, and memory.	28.7	Rare heterozygous missense: p.Thr152Met. LIST-S2 0.970303.	VUS	Mutation maps into the Ig2 domain of the protein critical for Mdga1's interaction with neuroligin 2; residue is phylogenetically conserved in MDGA1 and MDGA2.. However, no variants in MDGA1 curated in ClinVar as pathogenic to date, and conflicting predictions re haploinsufficiency	p.Thr152Met found in 3/48,496 SCZ alleles, but in none of 194,644 control alleles in SCHEMA. Located in the 6p22.3-p21.1 region linked to SCZ, and identified as a SCZ and/or bipolar disorder risk gene in several studies.
P6	SETD1A	Encodes subunit of a histone lysine methyltransferase implicated in dynamic chromatin regulation & maintenance. Heterozygous mutant mice show reduced parvalbumin +ve interneurons in cortical layer 5, and Reelin +ve interneurons in layers 2, 3, and 5 in medial PFC	36	Frameshift: p.Gln484Ter. (Novel - not found in any database) Heterozygous protein truncating (frameshift, leading to premature stop codon).	Pathogenic	Novel (i.e., not in any database) protein-disrupting variant in a clinical NDD-associated dominant gene.	Participant's neurodevelopmental, psychiatric, and facial morphological phenotype consistent with haploinsufficiency. LoF mutations are strong genetic risk factor for schizophrenia.
P8	NR2E1	Encodes orphan nuclear receptor 2E1 (a.k.a. TLX), a transcription factor critical for neural development and adult neurogenesis, including development of the hippocampus and amygdala. Neurons from mice deficient in Tlx prematurely differentiate in a gene dosage-sensitive manner in early cortical neurogenesis	25.8	Rare heterozygous missense mutation: p.Lys53Arg. Novel.	VUS	Maps into the NR C4-type zinc finger region nuclear receptor domain of the encoded protein and may result in haploinsufficiency (NR2E1 appears to be dosage sensitive). However, no ClinVar pathogenic variants or human phenotype yet reported.	Nr2e1/Tlx mutant mice exhibit gene-dosage sensitive limbic system abnormalities and abnormal social behavior, including aggression ameliorated by clozapine, and by the 5-$HT2_{A/C}$ antagonist ketanserin (Kumar et al, 2008). NR2E1/TLX is downregulated in a patient-derived neuronal IPSC model of schizophrenia (Murai et al, 2016).
P11	FOXP1	Encodes a forkhead box P family multifaceted transcriptional repressor implicated in development of midbrain dopamine and GABAergic medium spiny neurons.	N/A	Heterozygous frameshift indel (13 bp deletion), resulting in p.Ile561MetfsTer5.	Pathogenic	Truncating mutation in a haploinsufficient gene; however, previously reported cases with FOXP1 syndrome have mutations that disrupt or truncate before the FOX domain, whereas this maps just past it in the protein.	Strong support for FOXP1 as a SCZ risk gene in GWAS, although not for LoF variants in SCHEMA. LoF mutations cause language and speech delay/apraxia and typically DD/ID and/or ASD, aggression, anxiety, and OCD . Participant was shy but not overtly autistic, and was diagnosed with ADHD, but completed 4 years of university in a B.Sc. program.
P11	CNOT1	Encodes a subunit of the CCR4-NOT transcription complex, a master regulator, orchestrating gene expression, RNA deadenylation, and protein ubiquitination. Mutations in the orthologous Drosophila gene Not1 cause learning and memory defects.	32	Heterozygous missense mutation: p.Glu884Gly. Novel.	Likely pathogenic	Novel; mutation maps into a region interacting with ZPF36 (important for regulating protein synthesis and degradation), where 2/2 coding variants are curated as pathogenic; predicted pathogenic by 11/12 algorithms; highly missense-intolerant gene with many clinically pathogenic missense mutations.	Mutations of all classes in CNOT1 were associated in 39 individuals with developmental delay and/or learning and intellectual disability, dysmorphic features, and skin and skeletal abnormalities, without a recognizable gestalt (Vissers et al, 2020, Strong support for CNOT1 as a SCZ risk gene in GWAS (Pascal p = 1.962 x 10-7), although not for LoF or missense mutations in SCHEMA.

Case examples

MDGA1 Participant P1 had aggression and conduct disorder in childhood, and onset of psychosis by 18, initially labelled schizoaffective, later schizophrenia. Auditory hallucinations, nihilistic and referential delusions were refractory to numerous antipsychotics, including clozapine with augmentation by aripiprazole, loxapine, sulpiride, ECT, SSRIs, and lamotrigine. He has a rare VUS in MDGA1, a good SCZ and NDD (neurodevelopmental disorders) candidate gene (see table 2).

SETD1A Participant P6 with childhood-onset schizophrenia has a pathogenic mutation in *SETD1A*, the first gene in which LoF (loss-of-function) mutations were enriched in SCZ cohorts at a genome-wide significance level. SETD1A remains the most significant (p=2.00 x10-12) risk gene in the SCHEMA SCZ exomes meta-analysis, with an odds ratio for LoF variants of 20.1 (5.68- 108).

- *SETD1A* haploinsufficiency confers a syndromic neurodevelopmental (NDD) and psychiatric phenotype (Singh, 2016). Overlapping phenotypic features in P1 include a high forehead, downslanting palpebral fissures, and fleshy/broad tapered fingers; verbal learning disability and ADHD; behavioral problems, including aggression, in childhood; and very early-onset psychosis, with auditory hallucinations beginning at age 9.
- *In adult mice* with Setd1a haploinsufficiency, which impairs active cortical neuronal axonal branching and synaptic dynamics, lysine demethylase 1 inhibitors (including tranylcypromine, a licensed MAOI antidepressant*) reversed working memory deficits* (Mukai, 2019).

NR2E1 Participant P8 with schizophrenia since his early 20s has a novel missense mutation (p.Lys53Arg) that maps into a critical domain of *NR2E1*, encoding an orphan nuclear receptor 2E1 (a.k.a. Tlx), a transcription factor critical for limbic system development and adult neurogenesis.

- Despite being extremely intelligent (premorbid IQ 142), and the offspring of highly educated professionals, with no evidence of sociopathy (volunteering with NGOs and homeless shelters), he sustained a minor TBI (without MRI sequelae) at age 12 in a fight, and once tried to choke a sibling (who described him as having a lifelong "violent temper").
- Aggression in NR2E1 ("Fierce") mutants *is attenuated by clozapine and the 5-$HT2_{A/C}$ antagonist ketanserin* (Juárez et al, 2013).

FOXP1 and CNOT1 Participant P11 (our 10th case, one was withdrawn for change in diagnosis) was diagnosed with ADHD in childhood, depression at 13, and SCZ at 19.

- He has extreme myopia, subtle finger chorea, macrocephaly (with childhood turricephaly), and multiple compound melanocytic nevi. Mild dysmorphic features include a broad-based nose, inward angling of his molars, mildly abnormal pinnae, asymmetrical nipple placement, mild pectus excavatum, pes planus with a sandal gap, and very broad great toes.
- A truncating FOXP1 mutation, classed as "Pathogenic" by ACMG rules, seems less compelling than a "Likely pathogenic" missense mutation in CNOT.

CONCLUSIONS

- Our preliminary results provide encouraging support for the hypothesis that patients with severe psychosis (mean admission and discharge PANSS scores of 90.3 and 69.2, respectively) are a good place to look for ultra-rare and potent genomic risk factors for SCZ and SAD.
- Consistent with the literature, we found SCZ-associated ultra-rare variants (URVs) impacting pleiotropic ("broad-spectrum") NDD risk genes such as SETD1A, and conferring substantial risk for psychosis. Because of their potent effects on risk, identifying genes targeted by these URVs may be tractable precision medicine targets, as well as illuminating key pathogenetic mechanisms.

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