FOXO6 Gene Confers Protection Against Negative Symptoms in Schizophrenia

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Background

- Schizophrenia is characterized by positive and negative symptoms
  - Positive symptoms are psychotic behaviours not present in healthy people (e.g. hallucinations, delusions, thought disorders)
  - Negative symptoms are disruptions of normal emotion and behaviour (e.g. flat affect, anhedonia, asociality, avolition/amotivation)
- Negative symptoms also present in other forms of psychosis
  - "Similar symptom factors in schizophrenia and mood disorders suggest a continuity in the major affective and psychotic disorders that appears to reflect the underlying dimension of a psychotic process."[1]
- In addition, risk genes for psychotic disorders have been shown to overlap
  - "...specific SNPs are associated with a range of psychiatric disorders of childhood or adult onset. [...] These results provide evidence relevant to the goal of moving beyond descriptive syndromes in psychiatry, and towards a nosology informed by disease cause."[2]

Question: Do genes implicated in risk for bipolar disorder confer significant risk towards negative symptoms in schizophrenia?

Methods: Genes of interest

Genes selected were implicated in previous GWAS as contributing to risk for bipolar disorder

SNP	Chromosome	Position	Gene	Risk allele	Reference
rs1938526	10	60540625	ANK3	G	Ferreira et al., 2008
rs736408	3	52801338	ITIH3	C	Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011
rs2070615	12	48824388	CACNB3	G	
rs2175420	11	79412839	TENM4	T	
rs2176528	2	194007459	-	G	
rs3774609	3	53798876	CACNA1D	T	
rs3845817	2	65531391	-	T	
rs4660531	1	41374150	FOXO6	T	
rs6746896	2	96745212	-	G	
rs7296288	12	49086185	-	C	
rs7578035	2	98766429	-	G	
rs9371601	6	152469438	SYNE1	T	
rs9804190	10	60080073	ANK3	C	
rs11168751	12	48825355	CACNB3	G	
rs4765913	12	2310730	CACNA1C	A	Psychosis Endophenotypes International et al., 2014
rs10896135	11	66783531	C11orf80	G	
rs10994336	10	60420054	ANK3	T	
rs10994397	10	60519366	ANK3	T	
rs12576775	11	79366149	TENM4	G	

Methods: Recruitment

- Participants diagnosed with a first-episode of schizophrenia (spectrum) recruited from the Prevention and Early Intervention Program for Psychosis (PEPP-Montréal) at the Douglas Mental Health University Institute (Montreal, Canada)
- All participants underwent neuropsychological and symptom assessments, DNA extraction via blood or saliva sample
- A subset of participants also underwent T1 structural MRI scan

Gene analysis: Genotyping was performed at the McGill University and Génome Québec Innovation Centre using Sequenom iPlex Gold Technology[6]

MRI protocol: 1.5T Siemens MRI scanner. TR=22ms; TE=9.2ms; flip angle=30; FOV=256mm SI x 204mm AP; 180 sagittal slices; voxel size=1mm3

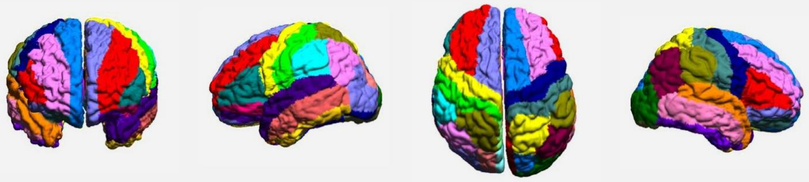
Participant demographics				Subset of participants with MRI T1 scan			
Total # of participants	Male: Female ratio	Age (mean)	Age (range)	**Total # participants w/ MRI T1 scan**	Male: Female ratio	Age (mean)	Age (range)
133	3.75:1	22.5	16:32	**61**	3.35:1	23	17:31

Analysis: Negative symptoms

- Measurement of negative symptom severity: Scale for the Assessment of Negative Symptoms (SANS)
- Coding genotype as binary: presence or lack of minor "risk" allele in given individual
  - Major allele homozygous: no risk (coded 0)
  - Heterozygous or minor allele homozygous: presence of risk (coded 1)
- ANCOVA (with age and gender as covariates): SANS scores by genotype

Analysis: Neuroanatomical changes

- Subset of participants also underwent T1 structural MRI scan (n=61)
- Scans pre-processed through CIVET pipeline[7]
- Using LONI Probabilistic Brain Atlas (LPBA40), calculated mean cortical thickness and total surface area at 26 regions of interest[8]
- MANCOVA (with age and gender as covariates) performed to examine structural differences by genotype



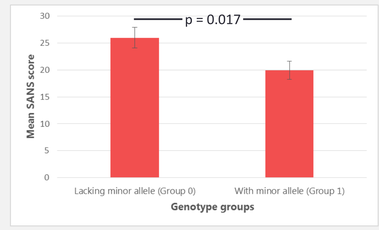
Results: Genotype and SANS

ANOVA uncovered one gene with significant effects on SANS scores:

- The gene: ***FOXO6* (rs4660531)**
- rs4660531 genotype showed a significant effect on SANS scores (Cohen's d=0.46, F=5.854, p=.017)
- Lack of minor allele = higher SANS scores

	Without minor allele	With minor allele
Number of participants	72	61
Male:Female ratio	3.5:1	4.08:1
Gender chi-square	p = 0.719	
Age range, mean	16-31, 22.65	16-32, 22.35
Age t-test	p = 0.663	
IQ t-test	p = 0.245	
Calgary Depression Score	p = 0.760	

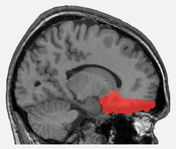
Mean SANS scores per genotype group



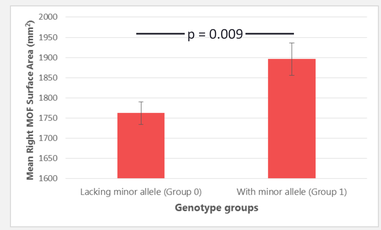
Scores on the Scale for the Assessment of Negative Symptoms (SANS) can range from 0-90, where a higher score denotes more severe negative symptoms.

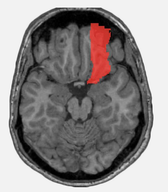
Results: *FOXO6* and neuroanatomy

- rs4660531 genotype showed a significant effect on frontal lobe surface area (F=1.727, p=.049) and, more specifically, on the **right middle orbitofrontal gyrus (MOF)** (Cohen's d=0.69, F=7.289, p=.009, Bonferroni-corrected)
- Lack of minor allele = smaller MOF surface area



Mean right MOF surface area per genotype group





Discussion

- Patients lacking the *FOXO6* minor allele exhibited more negative symptoms and reduced MOF surface area
  - Middle orbitofrontal gyrus is part of the orbitofrontal cortex (OFC), which has been previously associated with negative symptoms in schizophrenia[9]
- The *FOXO6* minor allele may be protecting schizophrenia patients from more severe negative symptoms and associated OFC surface area reduction

Possible mechanism of *FOXO6* protection: Fox-O6 protein protects against oxidative stress by increasing manganese superoxide dismutase (MnSOD)[10]; decreased MnSOD in schizophrenia linked to increased negative symptoms[11]

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