FOXO6 gene confers protection against negative symptoms in schizophrenia
or: The Fantastic Mr. *FOXO6*

Joseph (Jake) Shenker[1,2,3,4]
Sarojini Sengupta[1,2], Ridha Joober[1,2], Ashok Malla[1,2],
M. Mallar Chakravarty[1,2,4], Martin Lepage[1,2,3]

1 McGill 2 Douglas Institut universitaire en santé mentale / Mental Health University Institute 3 CRISP Comprehensive research into schizophrenia and psychosis Research Group 4

Overview

- Studies investigating the **genetic risk** for **schizophrenia** have identified a large number of **risk genes**

- However, the **phenotypic contribution** of risk genes remains unclear

Background

- Schizophrenia characterized by **positive** and **negative** symptoms
  - **Positive symptoms** are psychotic behaviours not present in healthy people
    - Hallucinations, delusions, thought disorders
  - **Negative symptoms** are disruptions of normal emotions and behaviour
    - Flat affect, anhedonia (inability to experience pleasure), asociality, avolition/amotivation (lack of drive or motivation)

Background

- Negative symptoms also present in other forms of psychosis
  - “Similar symptom factors in schizophrenia and mood disorders suggest a continuity […] that appears to reflect an underlying dimension of psychotic processes.”[1]
- In addition, risk genes for psychotic disorders have been shown to overlap
  - "…specific SNPs are associated with a range of psychiatric disorders […]. 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: 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
- All participants: neuropsychological and symptom assessments; DNA extraction via blood or saliva sample
- A subset of participants also underwent T1 structural MRI scan

Methods: Recruitment

Participant demographics			
Total # of participants	Male: Female ratio	Age (mean)	Age (range)
133	3.75:1	22.5	16:32

Subset of participants with MRI T1 scan			
Total # participants w/ MRI T1 scan	Male: Female ratio	Age (mean)	Age (range)
61	3.35:1	23	17:31

Methods: Genes of interest

- Selected SNPs which were implicated in previous GWAS as contributing to risk for bipolar disorder
- Participants genotyped for 19 SNPs

SNP	Gene	Risk allele	Reference
rs1938526	ANK3	G	Ferreira et al., 2008
rs736408	ITIH3	C	Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011
rs2070615	CACNB3	G	
rs2175420	TENM4	T	
rs2176528	-	G	
rs3774609	CACNA1D	T	
rs3845817	LOC10536-166	T	
rs4660531	FOXO6	T	
rs6746896	-	G	
rs7296288	-	C	

SNP	Gene	Risk allele	Reference
rs7578035	LOC10192-070	G	Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011
rs9371601	SYNE1	T	
rs9804190	ANK3	C	
rs11168751	CACNB3	G	
rs4765913	CACNA1C	A	Psychosis Endophenotypes International et al., 2014
rs10896135	C11orf80	G	
rs10994336	ANK3	T	
rs10994397	ANK3	T	
rs12576775	TENM4	G	

Analysis: Negative symptoms

- Measurement of negative symptom severity: Scale for the Assessment of Negative Symptoms (SANS)
- Genotype coded 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

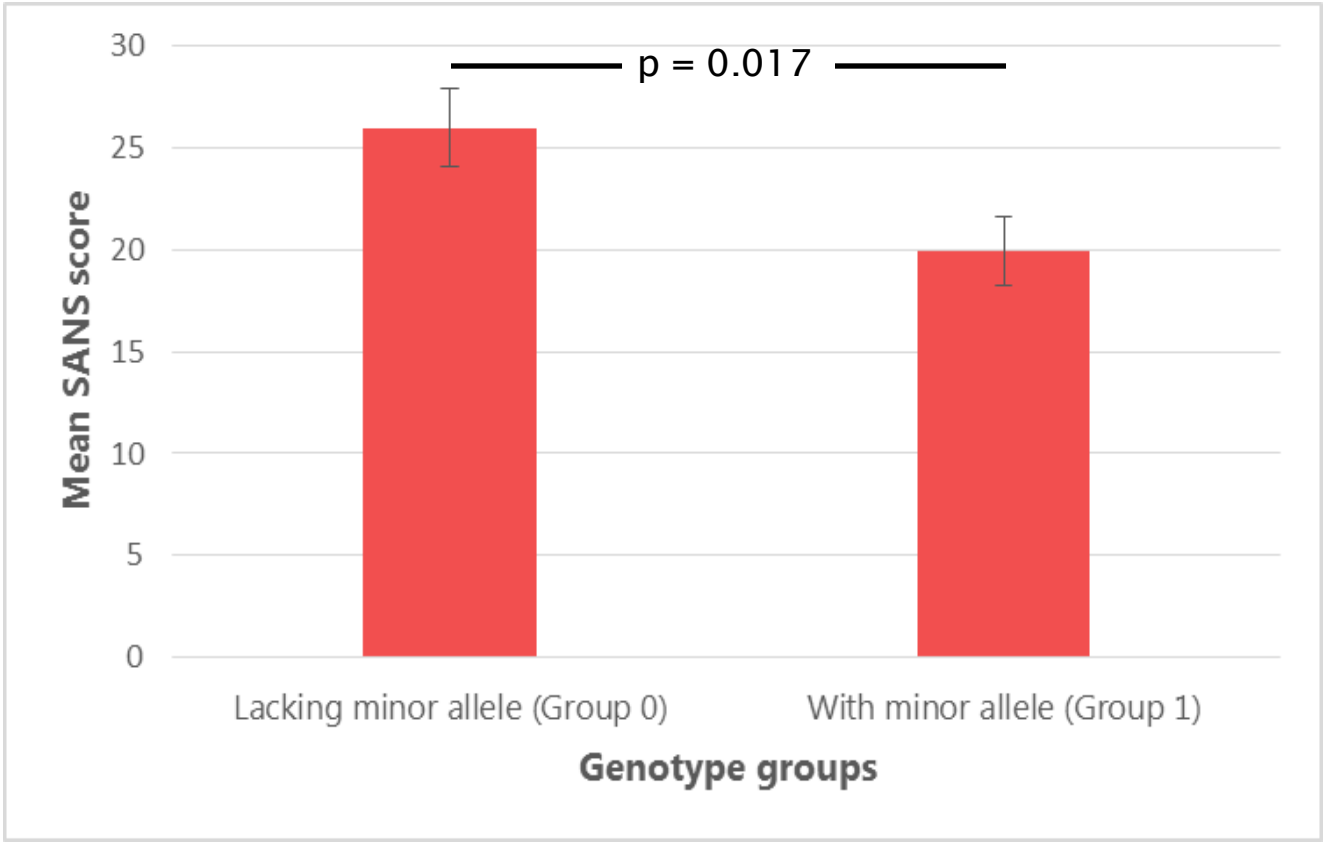
Results: Genotype & SANS

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.

What is *FOXO6*?

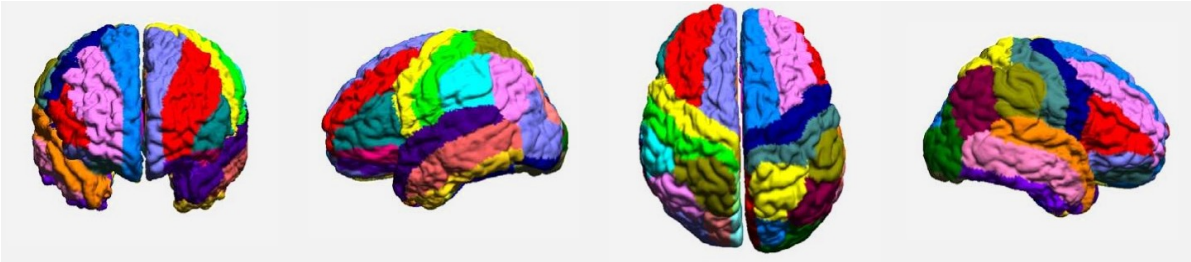
- Gene that codes for Fox-O6 transcription factor, a member of the O-group of forkhead (Fox) transcription factor family

- Fox-O6 differs from other Fox-O isoforms:

    - Mainly specific to neurons[3]
    - Highly enriched in the hippocampus - may be important for memory consolidation[4]
    - Relationship with mood disorders: some evidence that BDNF and lithium can suppress Fox-O6 activity[5]

Analysis: Neuroanatomical changes

- Subset of participants also underwent T1 structural MRI scan (n=61)
- Scans pre-processed through CIVET pipeline[6]
- Using LONI Probabilistic Brain Atlas (LPBA40), calculated mean cortical thickness and total surface area at 24 regions of interest[7]

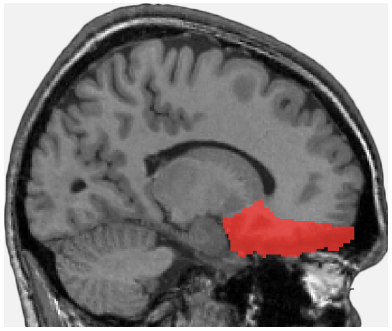


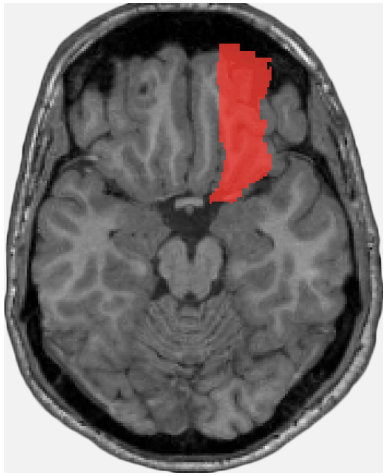
- MANCOVA (with age and gender as covariates) performed to examine structural differences by genotype

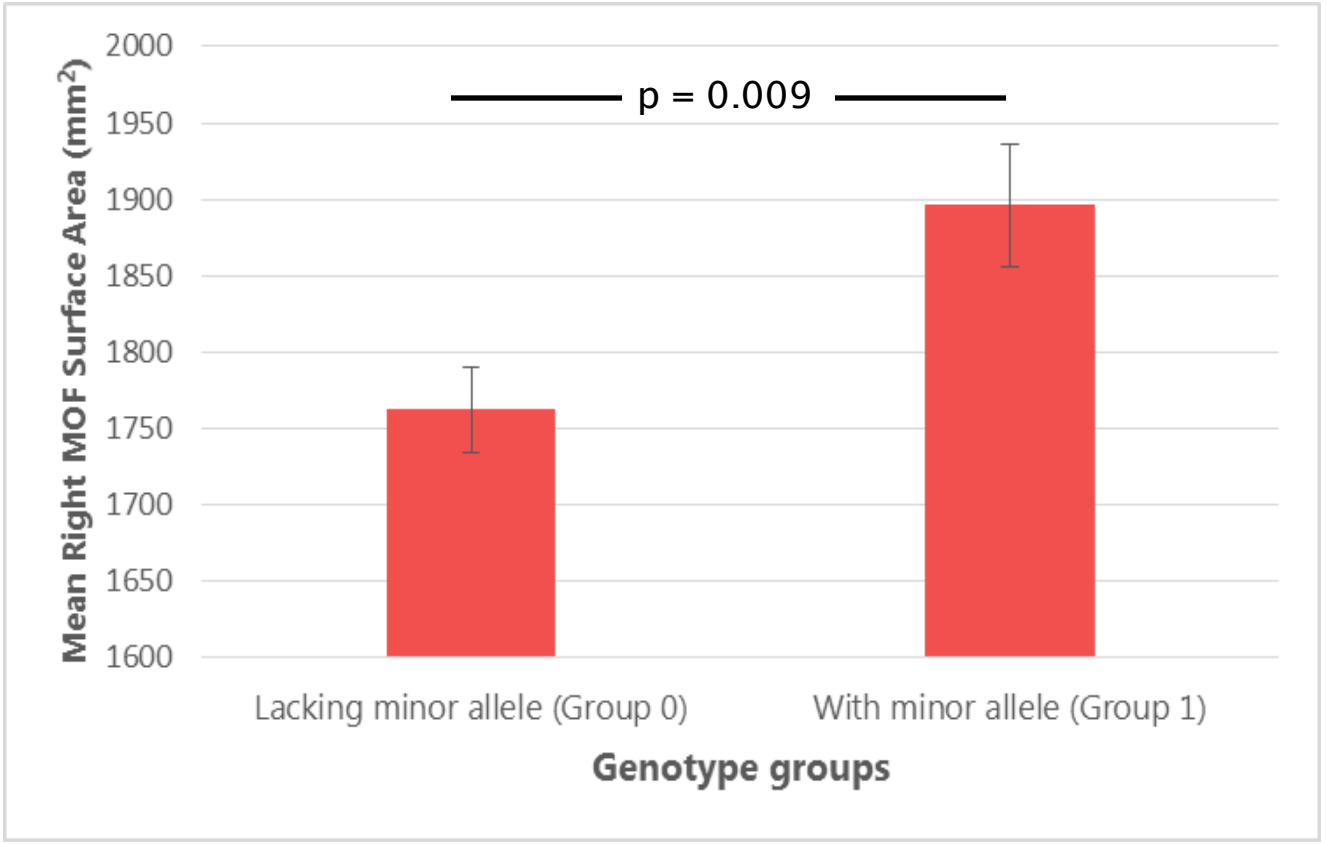
Results: *FOXO6* and neuroanatomy

- rs4660531 genotype showed a significant effect on frontal lobe surface area ($F=1.727$, $p=.049$)
- Effect localized to 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
- MOF is part of the orbitofrontal cortex (OFC)
    - Reductions in this region previously associated with greater negative symptoms in schizophrenia[8]

Mean right MOF surface area per genotype group







Discussion

- Patients lacking the *FOXO6* minor allele exhibited more negative symptoms and reduced MOF surface area

- The *FOXO6* minor allele may be protecting schizophrenia patients from more severe negative symptoms and associated MOF/OFC surface area reduction

Discussion

Possible mechanism of *FOXO6* protection

- Fox-O6 protein protects against oxidative stress by increasing manganese superoxide dismutase (MnSOD)[9]
- Decreased MnSOD in schizophrenia linked to more severe negative symptoms[10]

Contribution to manic phenotype in BPD?

- *FOXO6* minor allele may be protective in schizophrenia, what about bipolar disorder?
- Minor allele protects against negative symptoms; perhaps increases risk of manic phenotype

Acknowledgements

- **Supervisors: Mallar Chakravarty & Martin Lepage**
- Members of CRISP Group, Cobra Lab, and PEPP-Montréal
- Sarojini Sengupta, Ridha Joober, Kathleen MacDonald
- Funding provided by the Canadian Institutes of Health Research (CIHR)

McGill

comprehensive research into schizophrenia and psychosis
CRISP
RESEARCH GROUP

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

Douglas
INSTITUT UNIVERSITAIRE EN SANTÉ MENTALE
MENTAL HEALTH UNIVERSITY INSTITUTE

>cobraLab_
computational brain anatomy

Methods: Protocols

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

LPBA40 Regions

Rostral middle frontal gyrus inferior tier	Angular gyrus
Caudal middle frontal gyrus	Precuneus
Superior frontal gyrus	Superior occipital gyrus
Rostral middle frontal gyrus superior tier	Middle occipital gyrus
Inferior frontal gyrus	Inferior occipital gyrus
Precentral gyrus	Cuneus
Middle orbitofrontal gyrus	Superior temporal gyrus
Lateral orbitofrontal gyrus	Middle temporal gyrus
Gyrus rectus	Inferior temporal gyrus
Postcentral gyrus	Parahippocampal gyrus
Superior parietal gyrus	Lingual gyrus
Supramarginal gyrus	Fusiform gyrus

References

1. Toomey, R. et al (1998). Negative, positive, and disorganized symptom dimensions in schizophrenia, major depression, and bipolar disorder. J Nerv Ment Dis, 186(8), 470-476.
2. Cross-Disorder Group of the Psychiatric Genomics Consortium. (2013). Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet, 381(9875), 1371-1379.
3. Nho, R. S., & Hergert, P. (2014). FoxO3a and disease progression. World J Biol Chem, 5(3), 346-354.
4. Salih, D. A., Rashid, A. J., Colas, D., de la Torre-Ubieta, L., Zhu, R. P., Morgan, A. A., . . . Brunet, A. (2012). FoxO6 regulates memory consolidation and synaptic function. Genes Dev, 26(24), 2780-2801.
5. Polter, A., Yang, S., Zmijewska, A. A., van Groen, T., Paik, J. H., Depinho, R. A., . . . Li, X. (2009). Forkhead box, class O transcription factors in brain: regulation and behavioral manifestation. Biol Psychiatry, 65(2), 150-159.
6. Ad-Dab'bagh, Y. et al. (2006). The CIVET Image-Processing Environment: A Fully Automated Comprehensive Pipeline for Anatomical Neuroimaging Research. In Proceedings of the 12th Annual Meeting of the Organization for Human Brain Mapping, M. Corbetta, ed. (Florence, Italy, NeuroImage).
7. Shattuck, D. W. et al. (2008). Construction of a 3D probabilistic atlas of human cortical structures. Neuroimage, 39(3), 1064-1080.
8. Kanahara, N. et al. (2013). Orbitofrontal cortex abnormality and deficit schizophrenia. Schizophr Res, 143(2-3), 246-252.
9. Ponugoti, B. et al. (2012). Role of forkhead transcription factors in diabetes-induced oxidative stress. Exp Diabetes Res, 2012, 939751.
10. Sirota, P. et al (2003). Overproduction of neutrophil radical oxygen species correlates with negative symptoms in schizophrenic patients: parallel studies on neutrophil chemotaxis, superoxide production and bactericidal activity. Psychiatry Res, 121(2), 123-132.
11. Psychiatric GWAS Consortium Bipolar Disorder Working Group. (2011). Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet, 43(10), 977-983.
12. Ferreira, M. A. et al. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet, 40(9), 1056-1058.
13. Psychosis Endophenotypes International Consortium et al. (2014). A genome-wide association analysis of a broad psychosis phenotype identifies three loci for further investigation. Biol Psychiatry, 75(5), 386-397.
14. Ehrich, M. et al. (2005). Multiplexed discovery of sequence polymorphisms using base-specific cleavage and MALDI-TOF MS. Nucleic Acids Res, 33(4), e38.