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No association between common genetic variation in *FOXP2* and language impairment in schizophrenia

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A B S T R A C T

The *FOXP2* gene is hypothesised to be involved in schizophrenia by affecting speech and language development. Associations between common single nucleotide polymorphisms (SNPs) in *FOXP2* and language have been inconsistent. We tested five previously associated SNPs for association with language in the Western Australian Family Study of Schizophrenia (n = 709, including n = 333 with schizophrenia/spectrum disorder) and found no significant associations. When we included all common *FOXP2* variants, one SNP (rs2189008) was nominally associated with language. This is the most comprehensive analysis to date and indicates that common variants in *FOXP2* do not play a major role in speech and language development in a clinical family sample.

1. Introduction

The first gene implicated in speech and language was *FOXP2*, which encodes Forkhead box protein P2, a member of the FOX group of transcription factors. Linkage and molecular genetic analyses in a large family affected by severe speech and language deficits as well as orofacial dyspraxia led to the discovery of a heterozygous missense mutation (Fisher et al., 1998; Lai et al., 2001). This missense mutation disrupts the DNA-binding site of the FOXP2 transcription factor, interfering with its ability to bind to and regulate target genes (Vernes et al., 2006). Subsequent reports of cases heterozygous for other damaging *FOXP2* mutations (nonsense mutations, translocations and deletions), have confirmed that disruption of one gene copy appears sufficient to derail speech development (Graham and Fisher, 2013).The *FOXP2* gene is expressed in several brain structures (Lai et al., 2003) and genetic variants in several FOXP2 targets have also been implicated in language impairment (reviewed in Graham and Fisher (2013)).

Since its implication in speech and language, some studies have reported associations between common genetic variants (single nucleotide polymorphisms, SNPs) in *FOXP2* and speech and language phenotypes in healthy controls (Ocklenburg et al., 2013; Mozzi et al., 2017; Crespi et al., 2017), although no associations were observed with language impairment in a large population based study (Mueller et al., 2016). Associations have been reported between *FOXP2* SNPs and language- and reading-related neuropsychological and neuroanatomical phenotypes, including distinct developmental (e.g. dyslexia, Mozzi et al., 2017) and neurodegenerative disorders (e.g. fronto-temporal dementia Padovani et al., 2010) of language and communication. Abnormalities in speech and language processing play a central role in the key symptoms of schizophrenia (Bleuler et al., 1950) such as auditory verbal hallucinations (Badcock, 2010; Ford et al., 2014). In schizophrenia, variants in the *FOXP2* gene have been associated with grey matter concentration (Spaniel et al., 2011); auditory verbal hallucinations in those without a history of child abuse (McCarthy-Jones et al., 2014; Sanjuan et al., 2006) and poverty of speech (Tolosa et al., 2010). However, other studies have reported no association between SNPs in *FOXP2* and schizophrenia (Sanjuan et al., 2005) and auditory verbal hallucinations in schizophrenia (McCarthy-Jones et al., 2014). These studies have used various approaches to capture common genetic variation in *FOXP2*, genotyping between one and 27 SNPs across different parts of the gene, with little consistency between studies. While there is some evidence linking *FOXP2* SNPs with language impairment in schizophrenia, there has been substantial variation in the language measures employed, and little replication between studies.

Given the ongoing interest in the role of *FOXP2* in language impairment, and the fact that language impairment is central to schizophrenia spectrum disorders, we undertook a thorough investigation of

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Table 1
All *FOXP2* SNPs previously shown to be associated with language phenotypes after correction for multiple testing (all SNPs shown in bold were assessed for association with all WAFSS language measures shown in bold in this study).

SNP	BP	A1/A2	Global MAF	WAFSS MAF	Associated phenotype	study sample	Reference	WAFSS measure	N affected	N family members	N controls	N total
rs2253478	113977996	A/G	0.35	0.41	Poverty of speech	293 SZ/340 controls	Tolosa et al., 2010	**DIP**	**310**	**0**	**0**	**310**
								TLC	153	0	0	153
rs2396753	114148331	C/A	0.32	0.39	Speech perception	456 healthy controls	Ocklenburg et al., 2013					
					Auditory hallucinations	186/160 SZ with/without hallucinations	Sanjuan et al., 2006	**DIP**	**310**	**0**	**0**	**310**
rs6980093	114162740	A/G	0.31	N/A; r2 = 1 with rs2396753	Fluency	699 healthy children	Mozzi et al., 2017	**FAS**	**328**	**217**	**142**	**687**
								Semantic	152	2	106	260
					Comprehension	572 children with dyslexia	Mozzi et al., 2017					
rs12533005	114056055	G/C	0.38	N/A; r2 0.93 with rs2396753 and rs6980093	Speech perception	456 controls	Ocklenburg et al., 2013					
rs7799109	114199285	C/T	0.25	0.12	Speech fluency	882 HC	Crespi et al., 2017	**SPQ Q16, Q25, Q58**	**328**	**217**	**142**	**687**
								Semantic	152	2	106	260
rs17137124	114210814	T/C	0.48	0.44	Fluency and formal thought disorder	53 SZ/119 controls	Rossell et al., 2016	**DIP**	**310**	**0**	**0**	**310**
								TLC	153	0	0	153
rs1456031	114296102	T/C	0.41	0.45	Auditory verbal hallucinations	211/122 SSD with/without hallucinations	McCarthy-Jones et al., 2014	**DIP**	**310**	**0**	**0**	**310**
					Inner speech	882 HC	Crespi et al., 2017	**SPQ Q31,Q55,Q64**	**314**	**193**	**133**	**640**
					Verbal fluency and phonological fluency	210 frontal-temporal dementia and 200 age matched controls	Padovani et al., 2010	**FAS**	**328**	**217**	**142**	**687**

BP: base pair position on chromosome 7 based on genome build hg19. SNP: single nucleotide polymorphism. MAF: minor allele frequency. A1/A2: minor/major allele. global MAF: 1000 Genome phase 3 data from 2,500 worldwide individuals. TLC – Thought, Language and Communication Scale; DIP - Diagnostic Interview for Psychosis; SPQ – Schizotypal Personality Questionnaire

all genetic variation across the gene in a large sample of people from the Western Australian Family Study of Schizophrenia (WAFSS) which comprises participants with a diagnosis of schizophrenia spectrum disorder, their healthy biological family members and unrelated healthy controls (described in detail elsewhere; Hallmayer et al., 2005, Jablensky, 2006). The WAFSS participants have been richly phenotyped, allowing us to investigate associations between genetic variants in *FOXP2* and multiple, converging assessments of speech, language and language-related symptoms (Jablensky, 2006). These include *objective* (gained from standardised cognitive assessment), *subjective* (participant self-report) and *interviewer-rated* (clinical judgement from interview observation and case notes review) measures of speech and language abilities and language-related symptoms (Jablensky, 2006). Specifically, we assessed five measures for association with *FOXP2* single nucleotide polymorphisms (SNPs), based on their having shown previous associations with *FOXP2* variants (Table 1), and having been measured in sufficient WAFSS participants to allow adequately powered analysis. The language measures included (i) objective assessment of verbal (phonemic) fluency using the Controlled Oral Word Association Task (Benton and Hamsher, 1989); (ii) self-reported inner speech and (iii) subjective speech fluency, derived from the Schizotypal Personality Questionnaire (SPQ; Raine, 1991); and Interviewer-rated measures of (iv) global formal thought disorder and (v) auditory verbal hallucinations, assessed with the Diagnostic Interview for Psychosis (DIP; Castle et al., 2006). Genetic data was available for all WAFSS participants, from either genome wide genotyping with imputation, or whole genome sequence data, both of which result in rich genetic data across the *FOXP2* gene.

We hypothesised that the *FOXP2* SNPs found previously to be associated with language phenotypes would be significantly related to the subjective, objective and interview measures of language in our study. We hypothesised further that other common SNPs in the *FOXP2* gene would also be significantly associated with our language measures.

To test these hypotheses, we first reviewed all *FOXP2* SNPs investigated to date, and attempted to replicate any significant associations from these previous reports with our WAFSS language phenotypes. Our second aim was to extend and refine any genetic signals identified in aim one by incorporating our rich genetic data across the entire *FOXP2* gene.

2. Methods

2.1. Literature review

We conducted a literature search using the search terms 'FOXP2' and 'language' on the 22/11/2017, which identified eight previous *FOXP2* studies assessing language phenotypes that were the same or very similar to those measured in the WAFSS (Table 1). We included all SNPs from these studies that were significantly associated with any language phenotype after correction for multiple testing (Table 1). Where a group of previously associated SNPs was highly correlated with each other ($r^2 > 0.9$), only one representative SNP was included, resulting in five *FOXP2* SNPs to be replicated in this study.

2.2. Participants

Participants (total sample n = 709, comprised of 333 participants with a diagnosis of schizophrenia/spectrum disorder, 232 unaffected first degree family members, and 144 healthy controls; Table 1) in this study were sampled from the WAFSS – a long running study of genetics and cognition in schizophrenia which has been described in detail elsewhere (Hallmayer et al., 2005; Jablensky, 2006). Patients were recruited from inpatient and outpatient services of Graylands Hospital, Perth, whilst healthy controls were recruited via advertising or telephone screening in the local area. Diagnostic evaluation and symptom profile of patients was undertaken with a semi-structured clinician-rated interview, the Diagnostic Interview for Psychosis (DIP; Castle et al., 2006). All patients met ICD-10 and DSM-IV criteria for lifetime diagnosis of schizophrenia or schizophrenia spectrum disorder (schizophrenia, schizoaffective disorder, schizotypal disorder and acute transient psychosis), established by consensus of two senior clinicians. Patients were maintained on their usual medication and were clinically stable at the time of testing. Control families were screened for psychopathology with a brief Psychosis Screen (Jablensky, 2006) and excluded if they or a first-degree relative had been diagnosed with schizophrenia/schizophrenia spectrum disorder or bipolar affective disorder. Additionally, exclusion criteria for all participants included a history of neurological disorder, head injury, substance abuse or dependence, and poor English fluency.

All WAFSS participants were administered a cognitive and personality test battery by research psychologists. The battery was chosen on the basis of showing evidence of heritability (Cannon et al., 2000) and reasonable effect sizes of test measures (Heinrichs and Zakzanis, 1998) at the time of the study design. The total sample of 709 participants was 63% male (449 males and 260 females). The mean age at assessment of the total sample was 40.8 years (SD 14.4, range 18–85 years). The 333 participants with a diagnosis of schizophrenia/spectrum disorder were 78% male (261 men and 72 women), with mean age 34.9 years (SD 10.6, range 18–79). The unaffected participants were 50% male (188 females and 188 males) with mean age 45.9 years (SD 15.237, range 18–85).

The study was approved by the Human Research Ethics Committee at the University of Western Australia (file reference RA/4/1/4339), and by the institutional ethics committee of the North Metropolitan Health Service, Perth (project number 06_2011). Participants were treated in accordance with the declaration of Helsinki. Written, informed consent was obtained from all participants prior to testing.

2.3. Language measures

We chose language measures for analysis in the study based on their (i) having been measured in sufficient numbers of WAFSS participants to assess their associations with *FOXP2* SNPs with sufficient power (see power section) and (ii) being comparable to a language measure that has previously been reported to be associated with a *FOXP2* SNP.

2.3.1. Objective measures

Verbal fluency was assessed using the FAS version of the phonemic Controlled Oral Word Association Task (FAS; Benton and Hamsher, 1989). In this timed task, participants are given one minute to say as many words as they can think of beginning with a particular letter (F, A, and S). Participants are instructed not to produce proper nouns or to repeat words. As in previous studies (Crespi et al., 2017; Padovani et al., 2010), scores on the FAS refer to the total number of correct words produced on each trial, which provides an objective index of effortful lexical retrieval.

2.3.2. Subjective measures

Measures of inner speech and spoken speech fluency were derived from the Schizotypal Personality Questionnaire (SPQ, Raine, 1991), following item selection described by (Crespi et al., 2017). The SPQ is a valid and reliable self-report measure of schizotypal traits and has been regularly used in both clinical and healthy populations (Badcock and Dragovic, 2006; Fonseca-Pedrero et al., 2018; Hallmayer et al., 2005). Inner speech was represented by summing the scores from three questions: (1) (*Are your thoughts sometimes so strong that you can almost hear them? SPQ item 64)*, (2) (*Have you ever felt that you are communicating with another person telepathically (by mind reading)? SPQ item 55)*, and (3) (*I often hear a voice speaking my thoughts aloud. SPQ item 31*). These items specifically target the subjective experience of thought in the absence of overt spoken language (Crespi et al., 2017). Spoken speech fluency was assessed by summing three items from the Odd speech

subscale of the SPQ: (1) (*I sometimes jump quickly from one topic to another when speaking. SPQ item 16*), (2) (*Do you tend to wander off the topic when having a conversation? SPQ item 58*), and (3) (*I sometimes forget what I am trying to say. SPQ item 25*). These three items index the subclinical expression of formal thought and language disturbance, reflected in overt speech (Crespi et al., 2017).

2.3.3. Interviewer-rated measures

Global formal thought disorder in patients with a diagnosis on the schizophrenia spectrum was assessed using the sum of responses to questions 93-97 on the DIP (Castle et al., 2006). This method is broadly analogous to using the Thought, Language and Communication Scale, which Rossell et al. (2017) reported to be associated with a *FOXP2* SNP. Auditory verbal hallucinations were also assessed with the DIP (total sum of responses to questions 51-53). Previous *FOXP2* studies have used either the DIP (McCarthy-Jones et al., 2014), the Psychotic Symptoms Ratings Scales (Sanjuán et al., 2006) or the Positive and Negative Syndrome Scale (Španiel et al., 2011) to assess auditory verbal hallucinations. All these studies are similar in assessing thought disorder and/or auditory verbal hallucinations on the basis of a clinician-rated semi-structured interview.

Measures of inner speech, spoken speech fluency, verbal fluency, and formal thought disorder yielded quantitative (continuous) data, which was collected from schizophrenia cases and controls using the same methodology. In contrast, auditory verbal hallucinations were coded as a binary (yes/no) variable. Schizophrenia cases without hallucinations were included in the analysis with the same phenotypic coding as the healthy controls, all of whom were free from hallucinations.

2.4. WAFSS genotyping

The WAFSS cohort has previously undergone genotyping for genome wide association studies (GWAS) (n = 592 unrelated individuals) and whole genome sequencing (WGS) (n = 317 individuals from the familial sample, described in (McCarthy et al., 2017). These genetic data were cleaned using standard genotype quality control (QC), including removal of highly missing (> 5%) SNPs and individuals. Imputation to 1000 genomes phase 3 was performed for all n = 732 individuals with either GWAS or WGS data using Beagle version 4.1 (Browning and Browning, 2016). This resulted in 17,113 SNPs imputed across the *FOXP2* gene +/- 20kb (chr 7: 113706365..114353827 on genome build hg19) in all individuals. Quality control of the imputed data involved removing all variants where the imputed genotype dosage differed substantially from the imputed genotypes (calculated as [abs(DS-GT)/GT], with variants with a QC value > 5 excluded (total n = 222; n = 221 from the WAFSS group and n = 1 from the WGS group). After QC, 3,883 variants were polymorphic in the WAFSS across the *FOXP2* gene in the WGS data, of which n = 1,336 were successfully imputed in both the WGS and the GWAS data, including three of the five candidate SNPs. The remaining two candidate SNPs were successfully imputed in the WGS, but not the WAFSS data, meaning that these two SNPs were assessed in 317 participants with WGS data only.

2.5. Population substructure

In order to avoid potential confounding of the results due to systematic differences in ethnicities within the WAFSS, the genome-wide genetic data were used to perform principal component analysis (PCA). When the first two principal components (PCs) were plotted, 5 individuals who did not cluster with the HapMap European reference population were excluded from further analyses. In addition, the first 10 principal components were included as covariates in the association analysis to control for the effects of any residual population substructure.

2.6. Association analysis

All analyses were performed in R version 3.3.1 (R Development Core Team, 2008). Data were standardised (for every observation, the mean was subtracted and it was then divided by the SD) prior to analysis to make SNP associations across the different language measures comparable. Beta values reported are the 'standardized' regression coefficients, interpreted as a change in language measure in units of SDs, for a one SD change in SNP genotype.

Transformation to a normal distribution was attempted using 'rntransform' in the genABEL R package for all measures prior to analysis. Normality of the error distributions was tested for each language measure using the Shapiro–Wilk test.

We assessed associations between SNP genotypes and language measures adjusted for age, sex, years of formal education, and the first 10 genetic PCs (to correct for stratification). In addition, the familial relationships between some members of the study cohort were accounted for in all analyses by fitting linear mixed models including the kinship matrix as a random effect using restricted maximum likelihood (*REML*) estimation in R. The kinship matrix was directly estimated from recorded pedigrees using the "gap" package version 1.1-16 (Zhao J.H., 2007). The null model, describing only the relationship between the language measure (adjusted for covariates) and genetic relatedness, was compared to the same model including SNP genotypes (coded according to an 'additive' model, i.e. the number of minor alleles – 0,1,2) using log likelihood ratio tests. Bonferroni correction was applied to account for multiple testing (including all SNPs and all phenotypes), with p values < 0.002 considered statistically significant (0.05/25 tests).

2.7. Possible confounding due to schizophrenia case status

The primary analyses of association between language measures and *FOXP2* SNPs were conducted in the entire sample (schizophrenia cases, their unaffected relatives and healthy controls). However, there is the potential for confounding due to (chance) allele frequency differences between schizophrenia cases and controls, resulting in false positive associations between SNPs and language measures (which are themselves associated with schizophrenia). Therefore, for any significant SNP associations, a sensitivity analysis adjusting for case status was also performed.

2.8. Power

All SNPs included in this study had an expected minor allele frequency of > 0.2, therefore power calculations were calculated assuming a MAF of 20%. Power was calculated in Quanto (Gauderman, 2006) assuming an additive genetic model and a continuous trait. Type I error rate was set at 0.002 (0.05/25 tests (assuming 5 SNPs and 5 phenotypes)) assuming a two-sided test. Under these parameters, to achieve 80% power a sample size of 139 individuals is required to detect a difference of 0.5 SD change in language measure per copy of the minor allele, and 581 individuals to detect a 0.25 SD change in language measure. Most previous studies have reported effect sizes around 0.25 SD.

2.9. Posthoc analysis

As we have rich SNP information across the *FOXP2* gene from the 1000 genomes imputation in the WAFSS sample, where a previously associated *FOXP2* SNP is nominally associated ($p < 0.05$) with a language phenotype in this study, a regional fine mapping association analysis was performed using all 17,113 imputed SNPs across the gene to elucidate more precisely the source of the genetic signal within the *FOXP2* gene.

Table 2
Association results between the five candidate *FOXP2* SNPs and five language measures.

	SPQ speech fluency (n = 640/303)			SPQ inner speech (n = 640/303)			FAS (n = 687/315)			DIP FTD (n = 310/108)			DIP AVH (n = 310/108)		
	beta	se	pval	beta	se	pval	beta	se	pval	beta	se	pval	beta	se	pval
rs2253478_G (all)	−0.03	0.06	0.78	−0.05	0.05	0.34	0.05	0.05	0.71	0	0.08	0.83	−0.17	0.08	0.06
rs2396753_C (wgs)	0.03	0.06	0.65	−0.04	0.06	0.46	0.1	0.05	0.13	0.02	0.1	0.73	0.04	0.1	0.66
rs7799109_C (all)	−0.12	0.09	0.21	−0.06	0.09	0.64	0.1	0.08	0.36	−0.14	0.13	0.33	−0.06	0.13	0.78
rs17137124_T (wgs)	−0.05	0.06	0.58	−0.07	0.06	0.38	0.19	0.05	6.00E-03	−0.01	0.1	0.63	−0.07	0.1	0.92
rs1456031_T (all)	−0.1	0.06	0.08	−0.06	0.05	0.28	0.03	0.05	0.99	−0.05	0.08	0.71	0.1	0.08	0.16

Standardised values are reported; all analyses were adjusted for age, sex, education, the first 10 PCs, and the genetic relatedness matrix. n refers to the number of people analysed for the total sample/the whole genome sequence (wgs) sample. Three of the SNPs were successfully imputed in the whole sample (indicated by 'all'), the remaining two candidate SNPs were successfully imputed in the wgs data only (indicated by 'wgs'). SPQ - Schizotypal Personality Questionnaire. FAS - FAS version of the phonemic Controlled Oral Word Association Task. DIP - Diagnostic Interview for Psychosis. FTD - Formal Thought Disorder. AVH - Auditory Verbal Hallucinations.

3. Results

Our literature review revealed that seven *FOXP2* SNPs have previously been significantly associated with various language measures after correction for multiple testing (Table 1). Of these seven SNPs, two were highly correlated ($r^2 > 0.9$) with at least one of the other seven SNPs, and were excluded from this analysis. We tested the remaining five independent SNPs that have previously been included in candidate gene analysis (shown in bold in Table 1) for association with the five language measures in the WAFSS. All five SNPs were common (Table 2) and were located in intronic regions of the *FOXP2* gene (Supplementary Fig. 1).

The Shapiro–Wilk test indicated that the error distributions were normally distributed for some (including FAS total), but not all, of the five WAFSS language measures tested for association with the five *FOXP2* SNPs. The non-normal error distribution for some measures was due to many ties present in some language measures, which no transformation was able to address. The sample sizes used in this study make the REML reasonably robust to false positive findings due to the non-normally distributed error. To ensure that any associations we did see were not spurious findings however, we also conducted an (unadjusted) non-parametric Kruskal–Wallis analysis and found that *p* values from the Kruskal-Wallis tests are in agreement with the REML results reported here (Kruskal-Wallis results shown in full in Supplementary

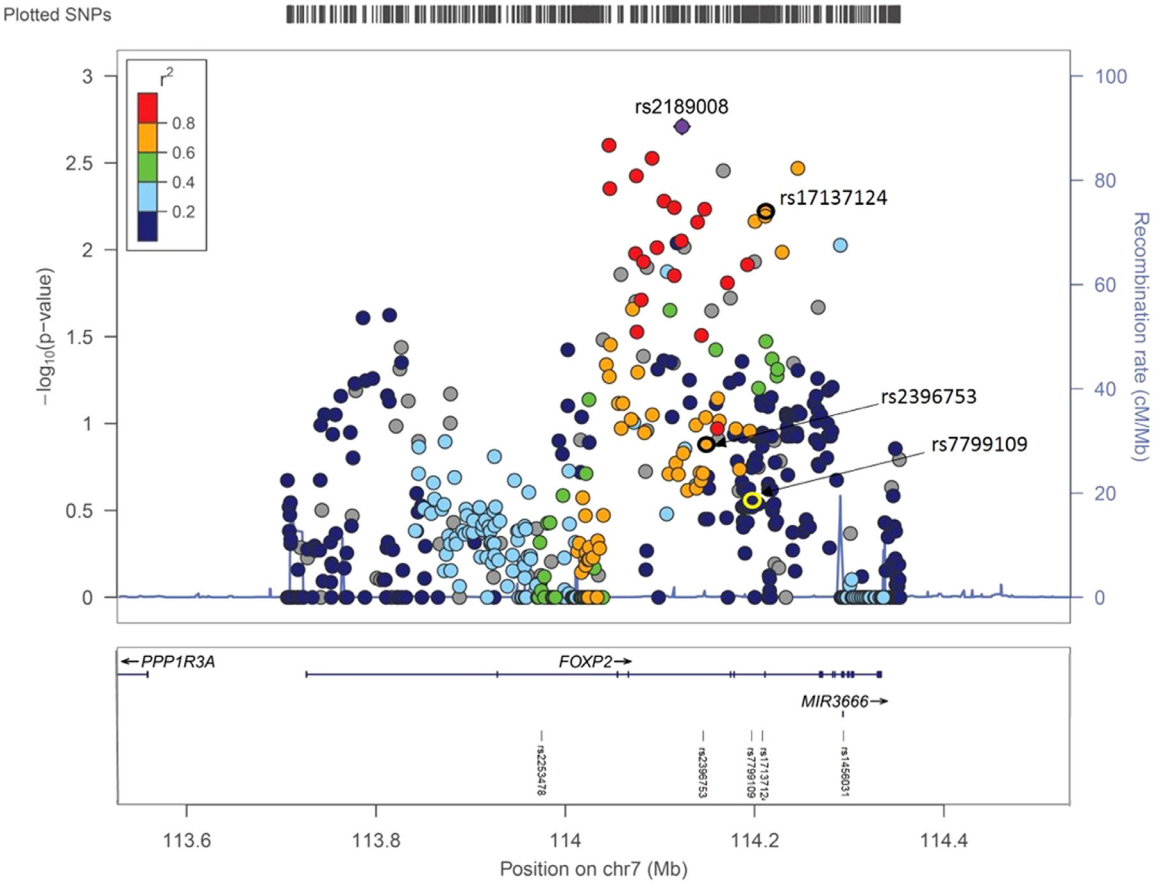

Fig. 1. Associations between all SNPs genotyped in this study in the *FOXP2 gene*, + / − 20 kb (hg19: 113706365..114353827) and FAS. From locuszoom (http://locuszoom.org/; Pruim *et al.*, 2010). Each dot represents a SNP, organised by p-value for their association with FAS (y axis) and chromosomal position on the x axis. The top hit SNP, rs2189008, is shown in purple. Correlation (r^2) between rs2189008 and the other SNPs is indicated by the colour of the dots. Surrounding SNPs from the five candidate SNPs are also marked. FAS: FAS version of the phonemic Controlled Oral Word Association Task.

Table 1).

None of the five candidate *FOXP2* SNPs was significantly associated with any of the language measures after Bonferroni correction ($p < 0.002$). An association between rs17137124 and FAS was nominally significant ($p = 0.006$) (Table 2). Effect sizes were broadly in keeping with previous reports (0.05–0.20 SDs), and the study had good power at the upper end of this range. However, it should be noted that power differed between the five candidate SNPs in this study, due to the fact that two SNPs (including rs17137124) were only well imputed in approximately half of the WAFSS dataset and power was lower for these two SNPs (Table 2).

In order to investigate whether the association with rs17137124 was driven by schizophrenia case status (itself associated with the FAS measure), we repeated the association analysis adding schizophrenia status to the model (Supplementary Table 1). Adjusting for schizophrenia case status, rs17137124 remained significantly associated with FAS. When subanalyses were conducted in schizophrenia cases and controls separately, the association was pronounced in cases and non-significant in controls (Supplementary Table 1).

Posthoc association analysis included all imputed SNPs across the *FOXP2* gene passing quality control, which were tested for association with FAS. A common SNP, rs2189008, located upstream of rs17137124, was the most strongly associated SNP in the region (Supplementary Table 2), $p = 0.0019$. These two SNPs, rs17137124 and rs2189008, are in reasonably high linkage disequilibrium with each other ($r^2 = 0.63$, Fig. 1). Although also somewhat correlated with rs2189008 ($r^2 = 0.52$) and located physically closer to it, rs2396753 was not associated with FAS in this study (Table 2, and Fig. 1). This is as expected, as despite both being somewhat correlated with rs2189008, rs2396753 and rs17137124 are not well correlated with each other ($r^2 = 0.33$). Although located close to rs17137124, rs7799109 is not correlated with the top SNP, rs2189008 ($r^2 = 0.10$), nor is it associated with FAS in this study (Table 2, and Fig. 1).

4. Discussion

FOXP2 has been examined as a candidate gene for language expression in humans for nearly two decades but studies with adequate samples are only now becoming available to examine common variants across the gene. This study aimed to replicate all previous findings of the relationship between *FOXP2* SNPs and language phenotypes (based on a systematic review of the peer-reviewed published literature) in our sample of people living with schizophrenia spectrum disorders and unaffected controls. The results revealed no significant associations between any of the previously investigated *FOXP2* SNPs and any of the language phenotypes after correction for multiple testing. We then extended previous findings by interrogating our comprehensive genetic data to refine the genetic signals across the entire *FOXP2* gene and found that the mostly strongly associated SNP in the *FOXP2* gene was a SNP which had not previously been investigated (rs2189008).

A strength of the current study compared to previous *FOXP2* association analyses in the literature was the availability of a comprehensive array of language measures–language phenotypes assessed across the spectrum of reporting methods via an objective lexical retrieval task (verbal fluency), subjective self-report questionnaire (SPQ), and clinician-rated measures (DIP), resulting in five measures of language for association analysis. In addition, our comprehensive genetic data allowed us to investigate all previously associated *FOXP2* SNPs within the same sample, thereby providing a valid comparison of their associations with language functioning. Of note, the WAFSS sample size was larger than previous studies of *FOXP2* associations with speech and language in schizophrenia (Tolosa et al., 2010 (n = 633), Sanjuan et al., 2006 (n = 346), Rossell et al., 2017 (n = 252) and McCarthy-Jones et al., 2014 (n = 333)).

Our failure to replicate previous associations could be due to a number of reasons. Firstly, because we tested five *FOXP2* SNPs for association with five language measures, we applied a more stringent correction for multiple testing (25 tests in total) than that used in previous studies. Although this study is larger than many of the previous association analyses, the additional burden of multiple testing meant that it was still underpowered for smaller effect sizes. Bonferroni correction to account for multiple testing has been posited to be overly conservative when variables are correlated, as the language measures are. However, using the less conservative false discovery rate (FDR) to correct for multiple testing does not change the pattern of results. This problem of lack of power is exacerbated by the fact that true effect sizes of the associations between *FOXP2* SNPs and language measures are likely smaller than those previously reported due to a phenomenon known as 'winners curse', in which the requirement for candidate associations to surpass a significance threshold in the discovery study means that discovery studies are more likely to have biased overestimates of the variant's true association in the sampled population (Palmer and Pe'er, 2017). A second possible reason for the lack of replication of previous findings is that, assuming these candidate SNPs are not causative themselves, but are correlated with a true causal variant located elsewhere in the *FOXP2* gene, differences in genetic correlation (LD) structure between study populations may mean that the candidate SNPs are correlated with the true causal variant (and therefore associated with language) in some populations but not in others.

The only previous association which was even nominally significant in the WAFSS was between rs17137124 and phonemic verbal fluency (FAS). Previous studies utilizing this measure reported a significant association with verbal fluency and rs6980093 ($p = 0.01$) in healthy children (Mozzi et al., 2017) and for rs1456031 ($p < 0.001$) in adults with frontotemporal dementia (Padovani et al., 2010). Failure on our part to replicate these findings may be due to our use of phonemic verbal fluency rather than the SPQ and semantic fluency tasks utilized by Mozzi *et al.* and neurological differences between schizophrenia and frontotemporal dementia. Padovani *et al.* also reported a suggestive association with rs17137124 ($p = 0.01$). Our finding of a suggestive association with rs17137124 and not with rs1456031 may be due to the fact that rs17137124 did not impute well in our sample, and therefore was only examined in a subset of the WAFSS (which was enriched for people with schizophrenia and their family members), whereas rs1456031 was examined in the whole sample, which included a higher proportion of healthy controls.

The posthoc analysis in this study shows that the SNP most strongly associated with FAS, rs2189008, is located upstream of the closest previously examined SNP, rs17137124, and is only in moderate linkage disequilibrium with it. This finding suggests that *FOXP2* is not comprehensively covered by the 'haplotype tagging' variants previously chosen for association analysis.

Large GWAS of language phenotypes have not yet been published. A word production task which assesses phonemic verbal fluency was performed by a small number of participants in the pilot UK Biobank data collection, but was not included in the published genetic analyses of cognitive functions (Davies et al., 2016), presumably due to the small sample size. One GWAS of language in a modest sample of people with Alzheimer's disease has recently been published (Deters et al., 2017), and no *FOXP2* SNPs were significantly or 'suggestively' associated with language in this study. However, we can't rule out the possibility that future studies in larger samples may identify *FOXP2* SNPs that are significantly associated with language phenotypes, although they are likely to have individually small effect sizes.

Although much of the research has focussed on the ability of *FOXP2* to modulate language in humans, the *FOXP2* transcription factor has been linked to the regulation of numerous functions (Nudel and Newbury, 2013) and as such may be involved in functional processes beyond the specific domain of language competence; for example it may have a broader role in the executive control of language. Objective measures of verbal fluency, such as FAS, place high demand on

cognitive flexibility as they require the ability to adaptively select, coordinate and regulate responses based on newly learned rules. Such domain-general executive-control abilities may be central to language (and action) learning more broadly. Evidence for this idea has been proposed by Schreiweis et al. (2014), who reported that humanised *FOXP2* may be directly involved in adapting brain development for language learning. The association between *FOXP2* and executive function warrants more detailed examination that was not possible in the WAFSS sample.

In conclusion, following a systematic literature review we found eight peer-reviewed published articles reporting significant associations between language phenotypes and *FOXP2* SNPs. None of the five independent SNPs reported in these previous studies were significantly associated with any of the five language measures in the WAFSS sample of schizophrenia patients, their relatives and healthy controls. Extending analysis of the *FOXP2* gene beyond the few previously reported SNPs revealed that the most strongly associated SNP in this study is in an intronic region not well tagged by the SNPs previously investigated. Future studies should adopt a broader approach to understand how *FOXP2* is implicated in severe mental illnesses such as schizophrenia, for instance by including more comprehensive genetic data, and investigating other measures of language and executive function.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.016.

References

Badcock, J.C., 2010. The cognitive neuropsychology of auditory hallucinations: a parallel auditory pathways framework. Schizophr. Bull. 36 (3), 576–584. https://doi.org/10.1093/schbul/sbn128.

Badcock, J.C., Dragovic, M., 2006. Schizotypal personality in mature adults. Pers. Individ. Dif. 40 (1), 77–85. https://doi.org/10.1016/j.paid.2005.06.015.

Benton, A., Hamsher, K., 1989. Multilingual Aphasia Examination. AJA Associates, Iowa City, IO.

Bleuler, E., 1950. Dementia Praecox or the Group of Schizophrenias. In: Zinkin, J. (Ed.), International Universities Press, New York, NY.

Browning, B.L., Browning, S.R., 2016. Genotype imputation with millions of reference samples. Am. J. Hum. Genet. 98 (1), 116–126. https://doi.org/10.1016/j.ajhg.2015.11.020.

Cannon, T.D., Huttunen, M.O., Lonnqvist, J., Tuulio-Henriksson, A., Pirkola, T., Glahn, D., . . . Koskenvuo, M., 2000. The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. Am. J. Hum. Genet. 67 (2), 369–382. https://doi.org/10.1086/303006.

Castle, D.J., Jablensky, A., McGrath, J.J., Carr, V., Morgan, V., Waterreus, A., . . ., Farmer, A., 2006. The diagnostic interview for psychoses (DIP): development, reliability and applications. Psychol. Med. 36 (1), 69–80. https://doi.org/10.1017/S0033291705005969.

Crespi, B., Read, S., Hurd, P., 2017. Segregating polymorphisms of FOXP2 are associated with measures of inner speech, speech fluency and strength of handedness in a healthy population. Brain Lang. 173, 33–40. https://doi.org/10.1016/j.bandl.2017.06.002.

Davies, G., Marioni, R.E., Liewald, D.C., Hill, W.D., Hagenaars, S.P., Harris, S.E., . . ., Deary, I.J., 2016. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N = 112 151). Mol. Psychiatry 21 (6), 758–767. https://doi.org/10.1038/mp.2016.45.

Deters, K.D., Nho, K., Risacher, S.L., Kim, S, Ramanan, V.K., Crane, P.K., . . ., Alzheimer's Disease Neuroimaging Initiative, 2017. Genome-wide association study of language performance in Alzheimer's disease. Brain Lang. 172, 22–29. https://doi.org/10.1016/j.bandl.2017.04.008.

Fisher, S.E., Vargha-Khadem, F., Watkins, K.E., Monaco, A.P., Pembrey, M.E., 1998. Localisation of a gene implicated in a severe speech and language disorder. Nat. Genet. 18 (2), 168–170. https://doi.org/10.1038/ng0298-168.

Fonseca-Pedrero, E., Debbane, M., Ortuno-Sierra, J., Chan, R.C.K., Cicero, D.C., Zhang, L.C., . . ., Jablensky, A., 2018. The structure of schizotypal personality traits: a cross-national study. Psychol. Med. 48 (3), 451–462. https://doi.org/10.1017/S0033291717001829.

Ford, J.M., Morris, S.E., Hoffman, R.E., Sommer, I., Waters, F., McCarthy-Jones, S., . . . Cuthbert, B.N., 2014. Studying hallucinations within the NIMH RDoC framework. Schizophr. Bull. 40, S295–S304. https://doi.org/10.1093/schbul/sbu011.

Gauderman, W., Morrison, J., 2006. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. http://hydra.usc.edu/gxe.

Graham, S.A., Fisher, S.E., 2013. Decoding the genetics of speech and language. Curr. Opin. Neurobiol. 23 (1), 43–51. https://doi.org/10.1016/j.conb.2012.11.006.

Hallmayer, J.F., Kalaydjieva, L., Badcock, J., Dragovic, M., Howell, S., Michie, P.T., . . ., Jablensky, A., 2005. Genetic evidence for a distinct subtype of schizophrenia characterized by pervasive cognitive deficit. Am. J. Hum. Genet. 77 (3), 468–476. https://doi.org/10.1086/432816.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12 (3), 426–445.

Jablensky, A., 2006. Subtyping schizophrenia: implications for genetic research. Mol. Psychiatry 11 (9), 815–836. https://doi.org/10.1038/sj.mp.4001857.

Lai, C.S.L., Fisher, S.E., Hurst, J.A., Vargha-Khadem, F., Monaco, A.P., 2001. A forkhead-domain gene is mutated in a severe speech and language disorder. Nature 413 (6855), 519–523. https://doi.org/10.1038/35097076.

Lai, C.S.L., Gerrelli, D., Monaco, A.P., Fisher, S.E., Copp, A.J., 2003. FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. Brain 126, 2455–2462. https://doi.org/10.1093/brain/awg247.

McCarthy-Jones, S., Green, M.J., Scott, R.J., Tooney, P.A., Cairns, M.J., Wu, J.Q., . . ., Carr, V., 2014. Preliminary evidence of an interaction between the FOXP2 gene and childhood emotional abuse predicting likelihood of auditory verbal hallucinations in schizophrenia. J. Psychiatr. Res. 50, 66–72. https://doi.org/10.1016/j.jpsychires.2013.11.012.

McCarthy, N.S., Melton, P.E., Ward, S.V., Allan, S.M., Dragovic, M., Clark, M.L., . . ., Jablensky, A., 2017. Exome array analysis suggests an increased variant burden in families with schizophrenia. Schizophr. Res. 185, 9–16. https://doi.org/10.1016/j.schres.2016.12.007.

Mozzi, A., Riva, V., Forni, D., Sironi, M., Marino, C., Molteni, M., . . ., Mascheretti, S., 2017. A common genetic variant in FOXP2 is associated with language-based learning (dis)abilities: Evidence from two Italian independent samples. Am. J. Med. Genet. B. https://doi.org/10.1002/ajmg.b.32546.

Mueller, K.L., Murray, J.C., Michaelson, J.J., Christiansen, M.H., Reilly, S., Tomblin, J.B., 2016. Common Genetic Variants in FOXP2 Are Not Associated with Individual Differences in Language Development. PLOS One 11 (4), e0152576. https://doi.org/10.1371/journal.pone.0152576.

Nudel, R., Newbury, D.F., 2013. Foxp2. Wiley Interdiscip. Rev. 4 (5), 547–560. https://doi.org/10.1002/wcs.1247.

Ocklenburg, S., Arning, L., Gerding, W.M., Epplen, J.T., Güntürkün, O., Beste, C., 2013. FOXP2 variation modulates functional hemispheric asymmetries for speech perception. Brain Lang. 126 (3), 279–284. https://doi.org/10.1016/j.bandl.2013.07.001. SepEpub 2013 Aug 2.

Padovani, A., Cosseddu, M., Premi, E., Archetti, S., Papetti, A., Agosti, C., . . ., Borroni, B., 2010. The speech and language FOXP2 gene modulates the phenotype of frontotemporal lobar degeneration. J. Alzheimers Dis. 22 (3), 923–931. https://doi.org/10.3233/JAD-2010-101206.

Palmer, C., Pe'er, I., 2017. Statistical correction of the Winner's Curse explains replication variability in quantitative trait genome-wide association studies. PLOS Genet. 13 (7) doi:ARTN e1006916. 10.1371/journal.pgen.1006916.

Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Willer, C.J., 2010. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 26 (18), 2336–2337. https://doi.org/10.1093/bioinformatics/btq419.

R Development Core Team, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org.

Raine, A., 1991. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophr. Bull. 17 (4), 555–564.

Rossell, S., Tan, E., Bozaoglu, K., Neill, E., Sumner, P., Carruthers, S., . . ., Gurvich, C., 2017. T44 - Is language Impairment in Schizophrenia related to Language Genes. Eur. Neuropsychopharmacol. 27 (Supplement 3), S459–S460. https://doi.org/10.1016/j.euroneuro.2016.09.532.

Sanjuan, J., Tolosa, A., Gonzalez, J.C., Aguilar, E.J., Molto, M.D., Najera, C., de Frutos, R., 2005. FOXP2 polymorphisms in patients with schizophrenia. Schizophr. Res. 73 (2-3), 253–256. https://doi.org/10.1016/j.schres.2004.05.012.

Sanjuan, J., Tolosa, A., Gonzalez, J.C., Aguilar, E.J., Perez-Tur, J., Najera, C., . . . de Frutos, R., 2006. Association between FOXP2 polymorphisms and schizophrenia with auditory hallucinations. Psychiatr. Genet. 16 (2), 67–72. https://doi.org/10.1097/01.ypg.0000185029.35558.bb. DOI.

Schreiweis, C., Bornschein, U., Burguiere, E., Kerimoglu, C., Schreiter, S., Dannemann, M., . . ., Graybiel, A.M., 2014. Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance. PNAS 111 (39),

14253–14258. https://doi.org/10.1073/pnas.1414542111.
Spaniel, F., Horacek, J., Tintera, J., Ibrahim, I., Novak, T., Cermak, J., . . ., Hoschl, C., 2011. Genetic variation in FOXP2 alters grey matter concentrations in schizophrenia patients. Neurosci. Lett. 493 (3), 131–135. https://doi.org/10.1016/j.neulet.2011.02.024.
Tolosa, A., Sanjuan, J., Dagnall, A.M., Molto, M.D., Herrero, N., de Frutos, R., 2010. FOXP2 gene and language impairment in schizophrenia: association and epigenetic studies. BMC Med. Genet. 11, 114. https://doi.org/10.1186/1471-2350-11-114.
Vernes, S.C., Nicod, J., Elahi, F.M., Coventry, J.A., Kenny, N., Coupe, A.M., . . ., Fisher, S.E., 2006. Functional genetic analysis of mutations implicated in a human speech and language disorder. Hum. Mol. Genet. 15 (21), 3154–3167. https://doi.org/10.1093/hmg/ddl392.
Zhao, JH, 2007. Gap: genetic analysis package. J. Statist. Software 23 (8), 1–18.