The effect of a schizophrenia polygenic risk score on brain function during verbal fluency in health, schizophrenia and bipolar disorder

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Abstract — Psychotic disorders, including schizophrenia (SCZ) and bipolar disorder (BD), are highly heritable illnesses with a polygenic and overlapping architecture. To better characterize and validate the effect of previously identified genetic risk variants on psychosis susceptibility, their impact on the intermediate phenotypes of these disorders warrants examination. I investigated for the first time the effect of a polygenic risk score (PRS) for SCZ, which gathers the cumulative impact of several genetic risk factors, on brain activation and connectivity during verbal fluency (VF), an intermediate phenotype showing impairment in SCZ and in BD. For this, functional magnetic resonance images (fMRI) were collected from a group of SCZ (n=10) and BD (n=25) patients, their healthy relatives (n=27) and healthy controls (n=39). The fMRI data were analyzed using statistical parametric mapping, in order to identify the effects of PRS on brain activation and on task-modulated connectivity, taking into account the effects of diagnosis as well as a number of nuisance covariates. I found a negative association trend (uncorrected for multiple comparisons) between the SCZ-PRS and the activation of the language-related left inferior frontal gyrus and right insula, effects that were more pronounced in the control group. I also found a positive trend between the SCZ-PRS and the task-modulated connectivity of the left inferior frontal gyrus with the left angular gyrus (in BD) and left thalamus and right lingual gyrus (in relatives), areas also implicated in language. These findings suggest that SCZ-PRSs may preclude changes in brain function during VF – and now warrant independent validation.

Keywords — polygenic risk score, schizophrenia, verbal fluency, fMRI, endophenotype, imaging genetics

I. Introduction

Psychotic disorders, including schizophrenia (SCZ) and bipolar disorder (BD), are a group of heterogeneous, polygenic and multifactorial illnesses, with shared symptomatology and etiology (American Psychiatric Association [APA], 2013). Both disorders are highly heritable and seem to result as a combination of genetic, environmental and neurodevelopmental risk factors (van Os & Kapur, 2009). Psychotic disorders have a lifetime prevalence of around 3% (Perälä et al., 2007) and represent one of the greatest causes of economic and humanistic burden worldwide (Millier et al., 2014). In the last 100 years, a great effort has been made to understand the etiological factors that contribute to the onset these disorders, such as genetic risk factors. However, the underlying pathways that contribute to these disorders are still unknown, compromising the prediction of their development and an early intervention on individuals at risk mental state.

Genome-wide association studies (GWAS) have discovered several risk variants, by identifying new single-nucleotide polymorphisms (SNPs) significantly associated with the risk of developing SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium [SWGPGC], 2014) and/or BD (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), but the impact of these variations on functional mechanisms that lead to psychosis is still not clear.

Imaging genetics is a research method that aids in the comprehension of the unknown disease pathways, by integrating genetics and brain function and structure phenotypes to discover the risk variants that underlie a relevant biological process associated with a target phenotype or to characterize the neural circuits affected by certain genetic variants. It uses imaging-based phenotypes, called intermediate phenotypes or endophenotypes that lie closer to the disease pathophysiology than a heterogeneous clinical phenotype, contributing to an increased power to detect the effects of genetic risk variants. Cognitive dysfunction, particularly verbal fluency (VF) impairment, is considered a promising endophenotype for psychotic disorders due to its manifestation on patients and their unaffected relatives, in a smaller extent on the latter (Kim et al., 2015).

A polygenic risk score (PRS) can be used to analyze the cumulative effect of hundreds or thousands of disease-related

risk variants found in GWAS studies, as an alternative to study their individual effect on brain function. A PRS is calculated for each individual as the weighted sum of multiple risk variants for a particular disease, explaining thus a larger fraction of heritability when compared to individual SNPs.

Several studies have analyzed the association between PRSs, calculated based on the presence of susceptibility risk variants for SCZ and brain activation (SCZ-PRS). On working memory paradigms, SCZ-PRSs were found to be positively associated with the left dorsolateral prefrontal cortex (DLPFC) and the left inferior frontal gyrus (pars triangularis) (Walton et al., 2012), positively associated with a region including the left DLPFC and ventrolateral prefrontal cortex (VLPFC) and other including the left frontal medial cortex (comprising the anterior cingulate cortex) (Walton et al., 2013) and negatively associated with the activation of the right inferior frontal gyrus, middle and superior prefrontal cortex and right middle temporal gyrus (Kauppi et al., 2014).

The initial hypothesis of this work is that an increasing PRS is associated with an inefficient performance during the task. It was expected that a larger PRS would be associated with increased activation of areas that are associated with the VF task, associated with SCZ dysfunction, or with the regions previously linked to functional imaging studies where a PRS was applied. This hypothesis is in concordance with the two studies of Walton et al. listed before (Walton et al., 2012; Walton et al., 2013).

My work will focus on the investigation of the relationship between a SCZ-PRS and the functional phenotypes (of brain activation and connectivity) exhibited during a VF paradigm, in a group of participants comprising SCZ patients, BD patients, SCZ and BD relatives and healthy controls. I will also analyze if the effect of PRS on these endophenotypes depends on the diagnostic groups and if it is associated with the paradigm and in concordance with the previous findings. This is the first study to analyze the effect of PRS on endophenotypes revealed by a verbal fluency paradigm and to study the effects of PRS on task-modulated connectivity on psychotic subjects.

II. Materials and methods

1) Participants

In this project, a total number of 134 participants were studied. 101 participants were selected as they provided suitable fMRI data and genetic information. From these 101 subjects, 39 were unrelated healthy controls, 25 were BD patients, type I (22 with psychotic symptoms), 10 were SCZ patients and 27 were healthy relatives of subjects with BD or SCZ (20 are relatives of BD patients and 7 are relatives of SCZ patients). All participants were native English-speakers and their diagnosis was confirmed using a structured clinical interview for the Diagnostic and Statistical Manual of Mental

Disorders, 4th edition (DSM-4) (APA, 1994). This project was approved by the National Health Service South East London Research Ethics Committee, UK (Project Mental Health Genetics and Psychosis) reference number 047/04 and all volunteers gave written informed consent at the time of participation.

2) Demographics

Intelligence quotient (IQ) scores were obtained using the Wechsler Abbreviated Intelligence Scale-III (WAIS-III) (Wechsler, 1997), the Wechsler Adult Intelligence Scale–Revised (WAIS-R) (Wechsler, 1981), the Wechsler Abbreviated Scale of Intelligence – Full Scale IQ (WASI-FSIQ-4) (Wechsler, 2008) or the National Adult Reading Test (NART) (Nelson & Willison, 1991). The values were standardized to Z-scores to permit comparison between different tests.

Analysis of differences between the sample characteristics was performed using Microsoft Word Excel (2013 version) and MATLAB R2014a (Table 1). I have used chi-square (χ^2) tests for categorical variables and one-way ANOVA tests for continuous variables. There were no significant differences between diagnostic groups in age, IQ (z-scores), Years of Education (YE), gender or handedness. The groups differed in terms of PRS (F=4.11; p=0.009). The PRS was statistically different between controls and SCZ (p=0.027 corrected for multiple comparions using the Bonferroni method).

Table 1 – Sample characteristics per diagnostic group. Age, IQ (z-scores), Years of Education and PRS are presented as mean (standard deviation).

	Controls (n=39)	BD (n=25)	SCZ (n=10)	Relatives (n=27)	Statistic, df, p-value
Age (years)	40.14 (14.49)	41.72 (11.91)	40.93 (12.53)	39.65 (11.39)	χ^2= 0.56, df=3, p=0.91
IQ (z-scores)	0.10 (0.96)	-0.33 (1.74)	-0.69 (1.36)	0.28 (0.94)	χ^2= 4.5, df=3, p=0.21
YE	14.70 (2.77)	14.72 (3.39)	13.70 (2.79)	15.41 (2.80)	χ^2= 3.6, df=3, p=0.31
Gender (M/F)	18/21	10/15	2/8	15/12	χ^2= 4.01, df=3, p=0.26
Handedness (R/L/M)	33/6/0	22/1/2	9/1/0	27/0/0	χ^2= 11.94, df=6, p=0.06
PRS	-0.43 (1.01)	0.10 (0.79)	0.54 (0.68)	0.16 (1.04)	χ^2=11, df=3, p=0.011

3) DNA extraction and genotyping

DNA was obtained from the participants using blood or buccal swabs. All participants were genotyped at the South London and Maudsley Trust/ King's College London BRC Genomics Laboratory on the Illumina HumanCore Exome BeadChip. The GenomeStudio Analysis software 2011.1 (Illumina Inc., San Diego, California) was used to process the genotypes. Quality control was performed using PLINK 1.9 (https://www.cog-genomics.org/plink2) (Chang et al., 2015). A detailed description of the genetic samples processing can be find elsewhere (Vassos et al., 2016).

4) Polygenic risk scores

The PRSs were generated with the PRSice software (http://prsice.info/) (Euesden, Lewis, & O'Reilly, 2014), using the most recent PGC schizophrenia meta-analysis as a discovery sample (SWGPGC, 2014).

A logistic regression was used to analyze the association between the calculated PRSs and the disease trait (Vassos, 2016). The PRSs were calculated for a set of thresholds (p_T = 0.00000005; 0.00001; 0.0001; 0.001; 0.01; 0.05; 0.1; 0.2; 0.5; 1). The PRSs used on the analysis were obtained at a threshold of 0.1, as it has reached the greatest proportion of variance between PRS and the case-control status, 9,3% (Nagelkerker's pseudo-R^2).

5) Paradigm

The task required the generation of a word that started with a visually presented letter, shown at a rate of one letter every 4 seconds. An "easy" and a "hard" set of letters were showed alternately to the subjects. The "easy" set of letters were: T, L, B, R, S or T, C, B, P, S and the "hard" set of letters were O, A, N, E, G or I, F, N, E, G. The task was contrasted with a "repetition" condition, in which the subjects were presented visually with the word "rest" and were asked to say "rest" also within a period of 4 seconds (Figure 1).

Each participant performed two different runs: one easy and one hard. The same letter was presented in consecutive seven trials between each "rest" condition, lasting 28s. Five blocks of each condition were presented and contrasted with five blocks of "rest" conditions.

The verbal responses were recorded using a MRI-compatible microphone. Incorrect responses were considered situations where the subject did not generate any response or generated proper names, repetitions, grammatical variations of the previous word and "pass" responses.

6) Image acquisition

Seventy-four T_2*-weighted functional images were acquired on a 1.5T GE LX System (General Electric, Milwaukee, USA), with echo planar imaging capability, at the Maudsley Hospital, London, UK. Twelve non-contiguous axial planes (with 7mm thickness, 1mm slice gap and 64x64 matrix size) parallel to the anterior commissure-posterior commissure line were collected over 1100ms (echo-time (TE) =40ms, flip angle=70°, and voxel size = 3.75×3.75×8.00mm^3). During one repetition time (TR, 4000ms), a letter (or the "rest" word) was presented during a period of 750ms and an overt verbal response could be made during a period of 2900ms, followed by image acquisition over 1100ms (Figure 1).

The T_1-weighted structural images were acquired on the same equipment, using a spoiled gradient-echo sequence, in axial (33 participants) and coronal slicing acquisition (68 participants). All images had similar parameters (1.5mm slice thickness, no slice gap, flip angle=20° and 256×256 matrix size), varying in echo time, repetition time and field of view. The voxel size for the images acquired in the axial plane is 0.9375×0.9375×1.500mm^3 and for the ones acquired in the coronal plane is 0.8594×0.8594×1.500mm^3.

7) Image preprocessing

The preprocessing of the functional volumes was made using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK), running under MATLAB 8.1.0.604. The preprocessing involved five steps: realignment, coregistration, segmentation, normalization and smoothing (Figure 2). The images were realigned using the first image as a reference to remove movement-related artifacts, normalized to the
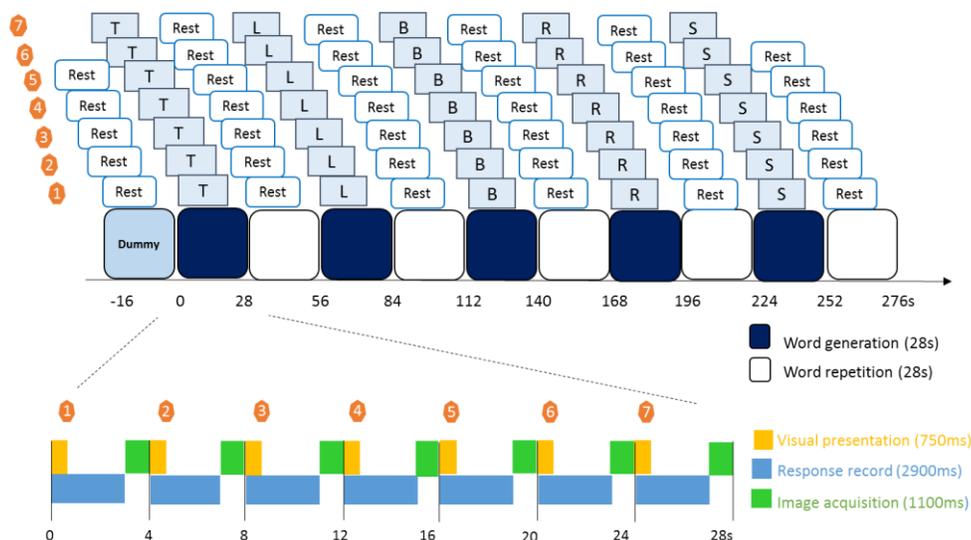

Figure 1 – Schematic representation of the verbal fluency paradigm, arranged in a block design. The first word generation trial is zoomed to exemplify the acquisition timeline, which is similar in the other blocks. A run consisted of 69 trials, with 35 word generation blocks and 34 repetition condition blocks.
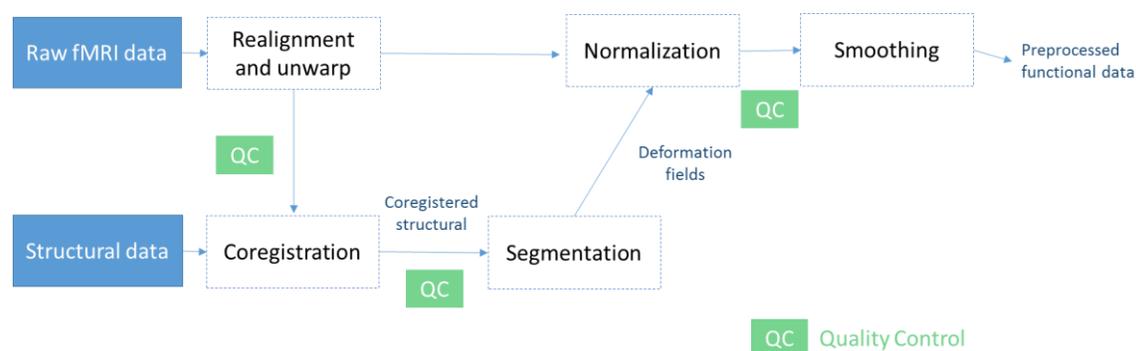
Figure 2 - Image preprocessing stream. It involves five steps: Realignment and unwarp, Coregistration, Segmentation, Normalization and Smoothing. Functional scans were realigned, spatially normalized and smoothed. Appropriate quality control steps were made at different time points.

Montreal Neurological Institute (MNI) template with a voxel size of $2 \times 2 \times 2mm^3$, using the deformation fields estimated on the segmentation step, and smoothed through the convolution with 8-mm full-width at half maximum Gaussian kernel.

8) Statistical analysis

Statistical analysis of fMRI data on SPM uses a mass univariate data analysis based on General Linear Models (GLMs). Statistical analysis involves the specification of the GLM design matrix, the estimation of the GLM parameters using a classical method and hypothesis testing through the generation of contrast vectors. The GLM can be expressed as in matrix notation as:

$$Y = X\beta + \varepsilon, \varepsilon \sim \mathcal{N}(0, \sigma^2 I) \qquad (1)$$

Where Y is the image data (scans × voxels), where the scans are acquired along a time period and all voxels constitute a three-dimensional image, X is the design matrix (scans × design variables), β are the parameters to be estimated (design variables × voxels) and ε is an error matrix. It is assumed that the error is normally distributed, such that ε has a mean of 0, a variance of σ^2 and any two elements of the error term are uncorrelated.

After preprocessing, I performed the statistical analysis for each subject independently (first-level analysis). Five different conditions (easy word generation, word repetition in easy runs, hard word generation, word repetition in hard runs and incorrect trials) were defined and six regressors (the individual movement parameters estimated in the realignment) were added to the model and considered covariates of no interest. Each experimental condition was convolved with a canonical HRF. The images were concatenated and filtered using a high pass filter with 128s cutoff, to remove low-frequency trends.

Subjects with more than 3mm translation or more than 3° rotation parameters were selected for graphical inspection of their estimated time-series translation and rotation. In 4 subjects, specific volumes (in which there was an abrupt displacement) were removed from the analysis, by adding a new binary regressor into their first-level analysis (with a 1 in the position of the volume being excluded and a 0 otherwise).

For each subject, a contrast image "easy+hard>rest" was computed using *t*-tests, excluding the wrong responses. These images were entered on a full factorial ANOVA, to generate group-level statistical maps. The ANOVA model was composed by one factor (diagnosis), divided into four levels: controls, SCZ, BD and relatives. Five covariates were also included, the covariate of interest (PRS) and four covariates of no interest (age, IQ (z-scores), gender and YE). The covariate of interest was entered as a single regressor per group.

I inspected the statistical maps at a p<0.001 (uncorrected) and considered regions statistically significant at a voxel-wise of p<0.05 corrected using family-wise error (FWE) rate for multiple comparisons across the whole brain. The clusters are labeled using the SPM toolbox Automated Anatomical Labeling 2 (AAL2) (Rolls, Joliot, & Tzourio-Mazoyer, 2015) and the labels were confirmed on an anatomical atlas (Netter, 2006).

9) Regional brain activation analysis

The ANOVA design allowed the localization of brain structures activated by word generation trials relatively to repetition trials, the study of the effect of diagnosis and the study of the effect of PRS (main effect, diagnosis-specific effect and its interaction with each diagnosis group) during word generation relatively to repetition.

10) Connectivity analysis

A psychophysiological interaction (PPI) analysis was conducted to study the task-dependent changes between activity in different brain structures and a seed region. The seed region was defined individually for each subject, by searching for each participant local maxima on a sphere of 6mm radius around (-42, 10, 26), a voxel located on the left inferior frontal gyrus. These coordinates correspond to the global maximum of the contrast image of the main effect of task.

In a new first-level analysis, I added the task "easy+hard>rest" time course, the frontal region time course and the PPI interaction term, created by multiplying the deconvolved physiological time-course with the psychological vector (Gitelman, Penny, Ashburner, & Friston, 2003), along with the six individual movement parameters. A contrast image of the interaction term against baseline was created for each subject and entered on a new full factorial ANOVA, with the same covariates of the previous group-level design.

III. Results

1) Main effect of VF task on brain activation

The paradigm was associated with activation of the left inferior frontal gyrus (both pars opercularis and triangularis), bilateral insula, right caudate nucleus and left middle temporal gyrus. Moreover, contrasting word repetition with word generation revealed an increased activation on a network of areas comprising the bilateral precuneus, angular gyrus and insula, right rolandic operculum and right putamen.

2) Main effect of VF task on brain connectivity

I did not find any FWE-corrected regions on this analysis. At an uncorrected threshold of 0.001, the connectivity between the left inferior frontal gyrus (seed region) and the right caudate nucleus and left temporal gyrus (Figure 3) was greater during word generation trials than during word repetition trials (Figure 4). To understand if there is any group driving the effect of the cluster found in the left superior temporal gyrus, a plot of the contrast estimates was extracted at (-56,14,0). The plot shows that in all groups there is a slight positive correlation of this area with the seed that is greater during word generation than repetition and this effect is larger in the SCZ group.
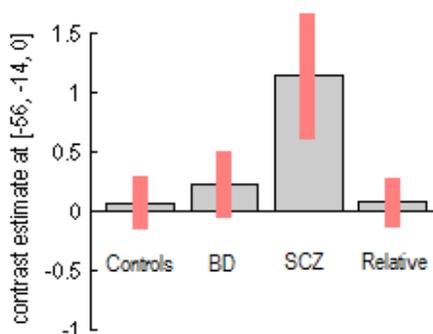

Figure 3 - Plot of the contrast estimates on the left superior temporal gyrus. There is a slight positive correlation of this area with the interaction term in all diagnostic groups whereas SCZ shows a strong correlation with the interaction term.

Conversely, the connectivity between the seed and the right thalamus, left cuneus and left superior occipital cortex was greater during word repetition trials than during word generation trials (Figure 4).

3) Main effect of PRS on brain activation and connectivity

There were only uncorrected results for the main effect of PRS on brain activation. The effects of PRS on brain activation were only pointing out in the negative direction. Specifically, an increasing PRS was correlated with decreased activation of a network of regions including the left inferior frontal gyrus, the left middle temporal gyrus, the bilateral insula, the right putamen, right thalamus and right caudate nucleus (Figure 5).

I did not find an effect of PRS on the connectivity of the left inferior frontal gyrus with the brain regions.

4) Diagnosis-specific effect of PRS on brain activation

At a corrected level, two clusters were found for the negative association of PRS in controls, in regions outside the parcellation areas defined by AAL2 (Rolls et al., 2015) – on the white matter.

Other negative effects of PRS were found at an uncorrected threshold for all diagnostic groups (Figure 6). In SCZ patients, an increasing PRS was correlated with decreased activation of the right calcarine fissure and surrounding cortex and the left middle temporal gyrus. In BD patients, there was a negative effect of PRS on the activation of a cluster incorporating right inferior frontal gyrus (pars orbitalis and triangularis). In controls, increasing PRS was correlated with a decreased activation of areas outside the anatomical volumes of interest defined by AAL2 (Rolls et al., 2015), regions that also incorporated the left inferior frontal gyrus, right cingulate and paracingulate gyri and left insula. The clusters found in relatives reside in areas outside the regions identified by AAL2 (Rolls et al., 2015).

5) Diagnosis-specific effect of PRS on brain connectivity

At an uncorrected level, PRS had a negative effect on the correlation of the cerebellar vermis and the right occipital gyrus with the interaction term in SCZ. In BD, PRS was positively associated with the correlation between the left angular gyrus and the interaction term. In relatives group, the PRS had a positive effect on the correlation between the left thalamus, right lingual gyrus and right calcarine, and the interaction term (Figure 7). There were no effects of PRS on the task-modulated connectivity in the control group.
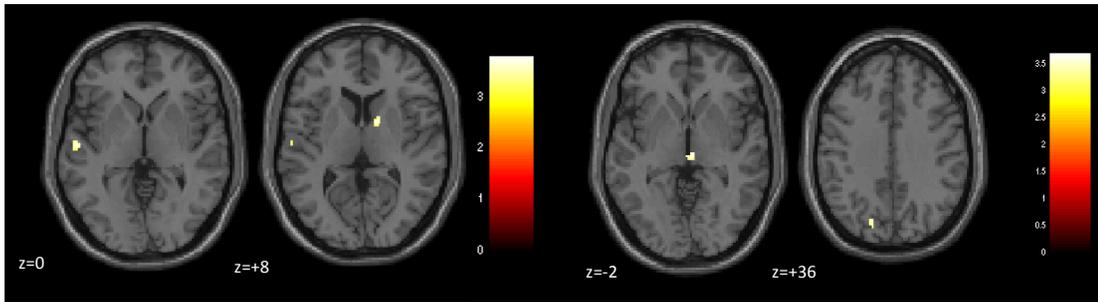
Figure 4 - Axial slices obtained at p<0.001 uncorrected, depicting the regions found on the main effect of task on the task-modulated connectivity with the left inferior frontal gyrus. The connectivity of the left inferior frontal gyrus with the left temporal gyrus (z=0) and right caudate (z=8) is greater during word generation than word repetition (left figures). The connectivity of the left inferior frontal gyrus with the right thalamus (z=-2) and the left cuneus and left superior occipital cortex (z=36) is greater during word repetition than word generation (right figures). The color bars represent T-values.
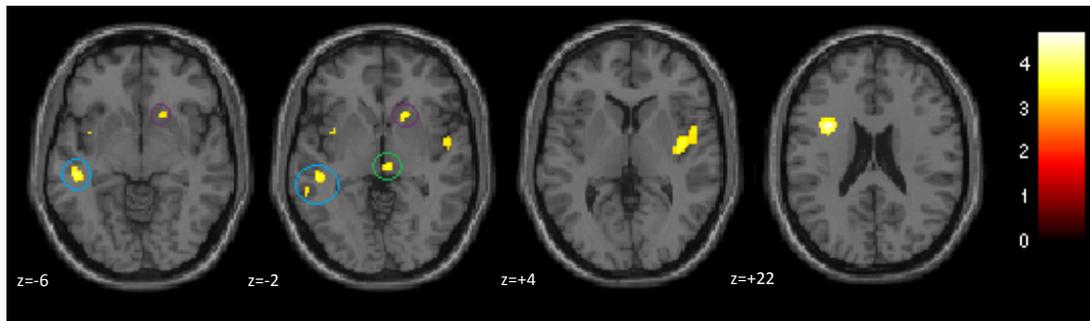

Figure 5 - Axial slices obtained at p<0.001 uncorrected, where a main effect of PRS on brain activation was found. PRS was negatively associated with activation of the left middle temporal gyrus (z=-6 and z=-2, blue circle), right putamen (z=-6 and z=-2, purple circle), right thalamus (z=-2, green circle), right insula (z=+4) and left inferior frontal gyrus (z=+22). The color bars represent T-values.

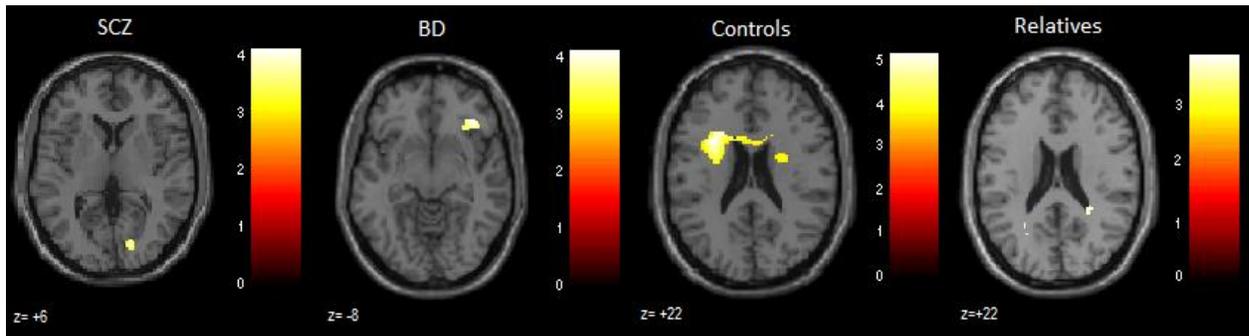**Figure 6** - Axial slices obtained at p<0.001 uncorrected, depicting the global maximum of the diagnosis-specific effect of PRS on brain activation. In SCZ the global maximum is on the right calcarine (z=+6), in BD is on the right inferior frontal gyrus (z=-8) and in controls and relatives, tested separately, it covers a portion of the white matter (z=+22). The color bars represent T-values.
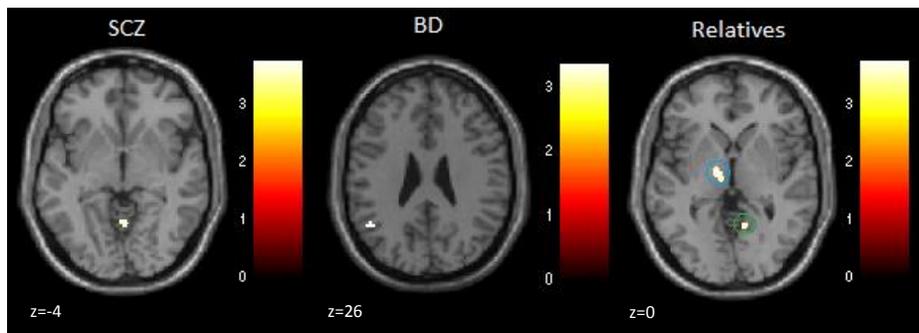

Figure 7 - Axial slices obtained at p<0.001 uncorrected, depicting the global maximum of the diagnosis-specific effect of PRS on brain connectivity. PRS has a negative effect on the correlation of the interaction term with the cerebellar vermis (left figure, z=-4) in SCZ, a positive effect on the correlation of the interaction term with the left angular gyrus (middle figure, z=+26) in BD, and in relatives (right figure, z=0), a positive effect on the correlation of the left thalamus (blue circle) and the right lingual gyrus (green circle) with the interaction term. The color bars represent T-values.

IV. DISCUSSION

The main effect of the verbal fluency paradigm on brain activation revealed that the task activated a brain network comprising areas involved in language such as the left inferior frontal gyrus (both pars opercularis and triangularis), bilateral insula, right caudate nucleus and left middle temporal gyrus. All these areas have previously associated with verbal fluency tasks (Baldo, Schwartz, Wilkins, & Dronkers, 2006; Birn et al., 2010; Meinzer et al., 2009; Phelps, Hyder, Blamire, & Shulman, 1997). The areas more engaged during word repetition than generation were the bilateral precuneus, angular gyrus and insula, right rolandic operculum and right putamen. Both precuneus and angular gyrus belong to a network of areas that are activated and intrinsically correlated when the human brain is at rest and not focused on any particular task (Utevsky, Smith, & Huettel, 2014). This brain network is the so called default mode network and it has been showed to be usually less activated during the task (Prata et al., 2012).

Furthermore, in the PPI analysis, the main effect of task was associated with correlation of the left inferior frontal gyrus (seed region) with the right caudate and left temporal gyrus, an effect that is greater during word generation trials than during word repetition trials.

On a sentence completion task, a study from Li et al. found that the connectivity between the left superior temporal and frontal regions is greater between sentence completion and pseudo-sentence completion trials, using also a PPI analysis (Li et al., 2017). However, in another study that used a silent phonemic verbal fluency task, the left inferior frontal gyrus did not show task-modulated connectivity with the left temporal gyrus (La et al., 2016). Although the connectivity between frontal and temporal regions during VF tasks is still not clear, both regions are consistently found to be associated with this task and seem to belong to a network that is highly connected during the task. Lesions on both inferior and temporal regions have been found to be associated with language dysfunctions (Baldo et al., 2006). Particularly in SCZ, the connectivity between the left inferior frontal gyrus and left superior temporal gyrus is dysfunctional, i.e., weaker when compared to healthy controls, a finding that is not consistent with this work but reveals that the interaction between these two regions is abnormal in SCZ (Jeong, Wible, Hashimoto, & Kubicki, 2009).

As for the right caudate nucleus, on previous studies of VF, it was not correlated with the left inferior frontal, although it has been reported to be activated during VF paradigms, suggesting that the basal ganglia structures are important for the VF performance (Fu et al., 2002; Fu et al., 2005; Thames et al., 2012).

A trend was also found in the connectivity between the left inferior frontal gyrus and right thalamus, left cuneus and left superior occipital gyrus, which is greater during word repetition trials than word generation trials, across all participants. This may suggest that these areas show an impaired connectivity with the frontal gyrus that is overactivated during the task to compensate for the decreased connectivity with these regions, which were often showed to be associated with the VF task (Li et al., 2017; Prata, 2008).

The results for the main effect of PRS on regional brain activation were exclusively pointing on the negative direction, in contrast to the hypothesis defined initially. In fact, the PRS is negatively associated with activation of a network of regions including the left inferior frontal gyrus, the left middle temporal gyrus, the bilateral insula, the right putamen, right thalamus and right caudate nucleus, areas that have all been previously associated with VF paradigms and showed to have a robust association with language processes (Baldo et al., 2006; Birn et al., 2010; Meinzer et al., 2009).

This finding suggests that PRS has an effect on task-modulated networks rather than have an effect on single regions not related with the task, as showed in Dima et al. (Dima et al., 2016). Moreover, the negative direction of the effect of PRS on regional brain activation might indicate that the subjects with increased risk for developing SCZ show decreased flexibility in the recruitment of neuronal resources during the word generation process.

The right insula and left inferior frontal gyrus, depicted on the two right slices of Figure 6, not only have been previously linked to VF paradigms, as they were additional found to be strongly associated with the task during this study. The insula, particularly its anterior region, is important for articulatory coordination of the speech (Dronkers, 1996). The left inferior frontal gyrus is associated with word recognition and processing on both semantic and phonemic verbal fluency tasks, as it is involved in processes related to the sound of words and its meaning (Fiez, 1997). Both areas have also been showed to be dysfunctional in schizophrenia (Mubarik & Tohid, 2016; Wylie & Tregellas, 2010).

It is also important to emphasize that a reasonable proportion of brain regions activated by this contrast were localized on the white matter. Although it is widely known that fMRI signal can capture considerably better the BOLD signal on grey matter than on white matter, due to its increased cerebral blood volume and flow, there is not a clear evidence against regional activation on white matter (Gawryluk, Mazerolle, & D'Arcy, 2014). Additionally, the number of studies reporting white matter activation keeps rising. However, as this effect is still controversial and there are no reports of white matter

activation during VF paradigms, it will be regarded as an artifactual result.

The studies that analyzed the effect of SCZ-PRSs on brain activation, from Walton et al., 2012, Walton et al., 2013 and Kauppi et al., 2014, indicate that PRS might be associated with hypo- or hyperactivation of frontal regions and that the direction of the association does not depend on the task, as working memory paradigms were always used. Moreover, frontal regions (such as inferior frontal gyrus and prefrontal cortex) seem to have a strong relationship with the SCZ-PRSs, highlighting the importance of frontal structures and its impairment on SCZ.

The PPI analysis revealed that no voxels that survived the significance threshold of p=0.001 uncorrected for multiple comparisons, on the main effect of PRS. Possibly, this was due to a lack of power of PRS to produce effects on the connectivity between the left inferior frontal gyrus and other regions in the brain and lack of power of the PPI analysis. The PRSs only explained a very small proportion of the variance of the disease trait, 9,3% and only take into account common variants, excluding the rare variants with large effect. Second, the lack of power of the PPI analysis is associated with the fact that the interaction term and the time-series of the seed are two correlated vectors, as the interaction term is obtained by the multiplication of the seed time-series with a task vector. Therefore, if the two vectors share some variance, which is likely to happen, the GLM model won't assign the variance to either, resulting in a small power to detect PPI effects (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012).

As for the diagnosis-specific effect of PRS on brain activation, there were only negative effects at an uncorrected level, across all groups. The results found are against the initial hypothesis, as a greater effect of PRS was expected on the diseased groups and particularly on SCZ, due to the use of a PRS specific for this disorder. The predominance of PRS effects on controls, is noticeable by the presence of larger cluster sizes and the existence of larger T-values, when compared to the other groups (Figure 6). These results might be explained by the increased size of this group (n=39 participants versus n=10 for SCZ, n= 25 for BD and n=27 for relatives), which is associated with a greater power to detect effects.

Second, another key point is that the brain regions where a diagnosis-specific effect of PRS was found have been previously linked to SCZ. This might suggest that the SCZ dysfunctions, particularly the ones on the insula and left inferior frontal gyrus are heritable and present in individuals with increased risk, rather than being a secondary effect of the illness. This may explain the greater effect of PRS in controls than in SCZ and/or BD (Whalley et al., 2014).

Third, the lack of power of the diseased groups might be associated with the heterogeneous manifestation of SCZ and BD. The etiology, symptoms and underlying mechanisms of these disorders may vary between individuals, and the genetic vulnerability explained by the score itself might not capture the functional alterations across all patients.

The results for the diagnosis-specific effect of PRS on task-modulated connectivity are heterogeneous and difficult to interpret, as there are no studies on the literature analyzing this effect. The findings suggest that an increasing PRS is associated with greater connectivity of the left inferior frontal gyrus with the left angular gyrus (in BD) and the left thalamus, right lingual gyrus and right calcarine (in relatives) and the opposite effect is present in SCZ on the cerebellar vermis and the right occipital gyrus. The lingual gyrus, thalamus and cerebellum (De Smet, Paquier, Verhoeven, & Mariën, 2013) have been linked to language processes while the calcarine fissure seems to have no association with language processes or psychosis. The occipital structures in SCZ seem to be altered in terms of volume, grey and white matter, but the variations don't include the area of the calcarine fissure and surrounding cortex (Tohid, Faizan, & Faizan, 2015). In particular, the lingual gyrus is a structure that belongs to the visual cortex and seems to be associated with the identification of letters and words, contributing to language comprehension (Ghosh, Basu, Kumaran, & Khushu, 2010).

V. CONCLUSIONS

At an uncorrected level, PRS has a negative effect on brain activation between word generation and repetition on areas associated with the task, such as the left inferior frontal gyrus, middle temporal gyrus, the bilateral insula, the right putamen, right thalamus and right caudate nucleus. The negative association between PRS and brain activation was also found individually on all diagnostic groups. Particularly on healthy controls the effect of PRS was more pronounced, suggesting that PRS effect captures dysfunctions associated with genetic vulnerability, instead of dysfunctions associated with symptomatic phenotypes, but also possibly explained by the lower sample size in the patient groups.

The effect of PRS on task-modulated connectivity was also analyzed; although there are no main effects of PRS at an uncorrected level on the connectivity of the left inferior frontal gyrus (seed) with brain regions, trends were found for the diagnosis-specific effect of PRS on brain connectivity. The findings suggest that an increasing PRS is associated with a more robust connectivity during task blocks than repetition blocks, of the seed with areas associated with the VF paradigm, namely the left angular gyrus (in BD), the left thalamus and the right lingual gyrus and right calcarine (in relatives). Also, an increasing PRS in SCZ is associated with reduced connectivity

during task blocks than repetition blocks of the seed with the cerebellar vermis and the right occipital gyrus. Most of these structures have been linked to language processes and/or psychosis dysfunctions.

The results suggest that PRSs calculated using several risk-variations for SCZ affect brain function and in particular regional brain activation during a VF paradigm, and that this effect more robust on healthy controls than on psychiatric patients.

VI. Limitations and Future works

Several limitations might be pointed out in this study. First, functional images were acquired using a field strength of 1.5T, which is nowadays considered a reduced value. Thus, at this field strength, the fMRI images have an intrinsic low signal-to-noise ratio (SNR) (Edelstein, Glover, Hardy & Redington, 1986), which is associated with a reduced power to detect activation effects (Welvaert & Rosseel, 2013).

Also, the large voxel size (3.75×3.75×8 mm^3) combined with a large smoothing kernel (8mm full-width at half maximum Gaussian) might lead to partial volume effects (PVE), where the boundaries of different structures are averaged on a single voxel, resulting on an inaccurate signal intensity and a worse spatial resolution, compromising the spatial location of effects on detailed structures. Additionally, the signal of a particular voxel might be spread across neighboring voxels – in particular, the grey matter structures signal can reach the white matter.

Moreover, several preprocessing steps may affect the anatomical localization of several structures, mainly coregistration and spatial normalization – although quality control procedures have been applied to verify the accuracy of these methods, only images from 3 randomly selected participants were visually analyzed. An additional analysis without the coregistration of the functional images to the structural space could have been done to understand the effects of this step on regional brain activation.

In terms of power, the sample size in the SCZ patient group is less than the recommended number of 20 participants for fMRI analysis (Thirion et al., 2007). Therefore, any comparisons involving this group might have resulted in false positives due to sampling bias, or false negatives due to lack of power to detect a true effect, explaining the lack of significant effects of PRS on brain activation and connectivity on this group.

Other factors with impact on brain activation and connectivity are the environmental and neurodevelopmental factors, as they contribute to the onset of the psychotic disorders and their cognitive functioning. High-genetic-risk participants might not show cognitive dysfunction due to the absence of other non-genetic risk factors. Thus, the interaction between the genetic and environmental factors should be assessed and included in the model.

The PRSs used were obtained at a pT <0.1, as it was associated with the greatest proportion of variance. In the future, the PRSs obtained at different thresholds should also be used to understand if, as assumed, the larger proportion of variance is reflected in more significant results and to analyze the influence of the different thresholds on the final results.

The results reported here can only be validated after replication on an independent sample. Therefore, future imaging genetic studies with larger samples should be conducted to disentangle the complex relationship between the psychiatric genetic risk and the endophenotypes revealed by the participants during cognitive tasks. This would be essential to understand the link between genetic risk factors and the neurobiological mechanisms associated with the development of disorders of the psychosis spectrum.

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