Lack of Evidence for Regional Brain Volume or Cortical Thickness Abnormalities in Youths at Clinical High Risk for Psychosis

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Lack of evidence for regional brain volume or cortical thickness abnormalities in youths at clinical high risk for psychosis: findings from the Longitudinal Youth at Risk Study (LYRIKS)

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	ARMS Subjects (SD)	Healthy Controls (SD)	Difference (*p* value)
Count	69	32	
Age	21.52 (3.49)	22.97 (3.94)	0.07
Gender			0.15
Male %	68	53	
Female %	32	47	
Handedness			0.64
Right-handed %	84	91	
Left-handed %	7	3	
Ambidextrous %	9	6	
Ethnicity			0.13
Chinese %	67	56	
Malay %	23	16	
Indian %	6	19	
Other %	4	9	
Education			
PSLE	196.3 (47.75)	206.1 (31.34)	0.48
Baseline clinical scores			
CAARMS positive	16.33 (7.35)	-	
GRD %	30	-	
APS %	81	-	
BLIPS %	7	-	
CDSS	5.42 (4.61)	-	
BAI	20.74 (11.16)	-	
Comorbidities			
Depression and/or anxiety %	48	0	
Past history SUD			
Alcohol %	6	3	0.56
Illicit drug %	3	0	0.33
Brain volumes			
VBM - ICV (ml)	1502.18 (141.05)	1448.24 (118.67)	0.59
SBM - ICV (ml)	1465.61 (146.64)	1410.48 (152.81)	0.31
SBM - Total GM (ml)	685.71 (55.49)	663.55 (47.09)	0.79
SBM - Total WM (ml)	470.84 (52.12)	460.79 (47.80)	0.38
Hippocampi (ml)	8.73 (0.76)	8.72 (0.61)	0.09
Ventricles (ml)	14.91 (6.88)	12.60 (5.54)	0.22

Title

Lack of evidence for regional brain volume or cortical thickness abnormalities in youths at clinical high risk for psychosis: findings from the Longitudinal Youth at Risk Study (LYRIKS).

Running title

Volume and surface analysis in risk-for-psychosis

Authors

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Abstract

There is cumulative evidence that young people in an "at risk mental state" (ARMS) for psychosis show structural brain abnormalities in fronto-limbic areas, comparable to, but less extensive than those reported in established schizophrenia. However, most available data come from ARMS samples from Australia, Europe and North America while large studies from other populations are missing. We conducted a structural brain magnetic resonance imaging (MRI) study from a relatively large sample of 69 ARMS individuals and 32 matched healthy controls recruited from Singapore as part of the Longitudinal Youth At-Risk Study (LYRIKS). We used two complementary approaches: a voxel-based morphometry (VBM) and a surface-based morphometry (SBM) analysis to extract regional gray and white matter volumes (GMV and WMV) and cortical thickness (CT). At the whole brain-level, we did not find any statistically significant difference between ARMS and healthy controls (HC) groups concerning total GMV and WMV or regional GMV, WMV and CT. The additional comparison of two regions of interest, hippocampal and ventricular volumes, did not return any significant difference either. Several characteristics of the LYRIKS sample like Asian origins or the absence of current illicit drug use could explain, alone or in conjunction the negative findings and suggest that there may be no dramatic volumetric or cortical thickness abnormalities in ARMS.

Keywords

magnetic resonance imaging

voxel-based morphometry

surface-based morphometry

early psychosis

schizophrenia

Introduction

Adolescents and young adults in the putative prodrome of psychotic illness – variously labeled as being at "ultra high risk" (UHR), "clinical high risk" (CHR), or in an "at risk mental state" (ARMS) – experience distressing sub-threshold psychotic symptoms and have a 30-43% risk of transition to psychosis over a 36 month-period [1]. These individuals are typically identified through clinical assessment of help-seeking individuals who present (i) attenuated or (ii) brief and intermittent psychotic symptoms, or (iii) a decrease in global functioning combined with a genetic risk for psychosis [2,3].

Structural MRI brain studies have featured prominently in attempts to identify biomarkers of ARMS. In general, this work has shown baseline grey matter volume (GMV) reductions in frontal, temporal and limbic areas of ARMS individuals [4-10]. Though the results of ARMS MRI research, typically obtained in small samples, are heterogeneous and contradictory [11,12], many of the identified brain changes are similar to those seen in patients with established schizophrenia [13,14]. Some GMV reductions, particularly in fronto-limbic areas, have been confirmed to be statistically robust through meta-analysis [15] and multi-centre investigations [16].

In parallel to GMV findings, only four whole-brain studies compared cortical thickness between ARMS individuals and controls and their results were divergent. One study reported cortical thinning in several brain regions, including frontal, temporal and limbic areas [17] while three studies did not report any cortical thinning significant at the whole-brain level in a larger sample of ARMS individuals when compared at baseline with healthy controls (HC) [18-20].

Fewer studies have investigated alterations of white matter volume (WMV) in ARMS but their findings are consistent with what has been reported for GMV. They reported smaller WMV in fronto-temporo-limbic areas [5,6,21] as well as a global reduction of WM growth over time [22] in ARMS compared to HC.

While baseline comparisons between ARMS and HC are useful for identifying putative biomarkers of young people in need of care, the majority of ARMS individuals do not transition to

frank psychosis (ARMS-NT), spurring attempts to identify ARMS individuals at incipient risk of psychosis onset (ARMS-T). At the whole-brain level, gray matter differences associated with transition to psychosis have been localized in the same fronto-temporo-limbic regions that also distinguish the overall ARMS group (regardless of transition) from HC [4,6,23,24]. More precisely, baseline GMV reductions in ARMS-T when compared to ARMS-NT were especially consistent in the fronto-insular and superior temporal regions [15].

All these studies recruited ARMS samples from North America, Europe and Australia. There are few structural brain MRI studies performed in ARMS samples from Asia and all were conducted in small cohorts [17,25,26]. Nevertheless, establishing consistency across different ethnic groups represents a critical step in the development of any putative biomarkers.

An additional advantage of such research in Asian countries is the very low prevalence of cannabis and other drug use [27]. Substance use is more frequent in patients with psychotic disorders in Western countries [28] and could be a problematic confound for ARMS research in Western populations [29,30]. Substance use, and cannabis in particular, have been associated with structural changes in at-risk populations [31-34].

We used both voxel-based (VBM) and surface-based (SBM) morphometry analyses to run a comprehensive and not regionally biased whole-brain investigation of baseline GMV, WMV, and cortical thickness (CT) alterations in a relatively large sample of 69 ARMS individuals with minimum antipsychotics or substance use recruited from Singapore as part of the Longitudinal Youth At-Risk Study (LYRIKS) [35]. Given the good statistical power offered by our large sample size, we hypothesized that we should reproduce some of the grey and white matter volume and cortical thickness alterations in the frontal and temporal lobes as reported by previous whole-brain studies.

Methods and Materials

Participants

Our sample comprised 75 ARMS subjects and 40 HC between 14 and 29 years old, matched for age, gender, handedness and educational level. The participants were part of the Longitudinal Youth At-Risk Study (LYRIKS), in Singapore. ARMS subjects were recruited from programs targeted at identifying individuals at-risk for developing psychosis run by the Institute of Mental Health, and from various community mental health agencies. Details of the recruitment strategy were previously reported [36]. In brief, we adopted an active approach of recruiting individuals from various psychiatric clinics and community mental health agencies, and a passive approach of self-referrals from print and social media advertisements. ARMS subjects met inclusion criteria for the prodromal state of schizophrenia in accordance to the comprehensive assessment of at-risk mental states (CAARMS) [3]. CAARMS assessments were performed by experienced psychometricians that were trained at ORYGEN in Melbourne. Inter-rater reliability was established and monthly supervisions were conducted throughout the study period to guarantee diagnostic validity. At-risk participants had no history of psychiatric, neurological or serious medical disorders, or mental retardation; and were not on antipsychotic medications. We excluded anyone with a current substance abuse as defined by the DSM-IV. Six AMRS subjects and 1 HC had a past history of substance use disorder (Table 1). 6 ARMS subjects and 8 HC were excluded from the original sample due to the use of a different T1-weighted structural MRI sequence (n=10) or the presence of gross structural abnormalities or movement artifacts (n=4). The demographics and clinical information of the remaining 69 ARMS and 32 HC are detailed in Table 1. Out of 69 ARMS subjects, 33 had a concomitant diagnostic of depression and/or anxiety and 37 were medicated with antidepressants, mostly selective serotonin reuptake inhibitor (SSRI, n=28), but also non-SSRI (n=7) or both SSRI and non-SSRI in association (n=2). During 28-month follow-up, 7 ARMS subjects converted to psychosis and 13 withdrew from the study, leaving a final sample of 56 ARMS-NT and 7 ARMS-T at baseline.

Additional exclusion criteria for controls were: (i) history of severe head injury, (ii) personal history of psychotic disorder, (iii) and personal history of other neuropsychiatric disorder. Controls did not have any family history of neuropsychiatric disorders, except, three controls had a first-degree relative with a history of depression, two had a second-degree relative with history of schizophrenia (n=1) or depression (n=1). In both the ARMS and HC groups, Primary School Leaving Examination (PSLE) scores, which are the result of a standardized multidisciplinary test of scholastic achievement, were used as a measure of educational level. Written informed consent was provided by all participants aged 21 and above or from a legally acceptable representative for participants under 21 with participant's assent. Ethics approval for this study was provided by the National Healthcare Group's Domain Specific Review Board.

Image acquisition

T1-weighted structural MRI data were obtained from a 3T Siemens Trio Tim scanner (Siemens, Erlangen, Germany) at the Center for Cognitive Neuroscience, Duke-NUS Graduate Medical School, Singapore. The principal sequence relevant to this study was a T1-weighted 3-D magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence optimized for grey-white matter contrast. It was identical to that used by the Alzheimer's Disease Neuroimaging Initiative ADNI consortium [37]. Parameters were as follows: TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, flip angle = 9°, BW = 240 Hz / pixel, FOV = 256 ×240 mm, Matrix = 256 × 240; resulting voxel dimensions: 1.0 × 1.0 × 1.0 mm, acquisition time 5 min 03 sec. Parallel imaging was used to improve the signal-to-noise ratio instead of shortening the scan time. We obtained a single high-quality image instead of averaging two or more rapidly acquired images. Images were inspected for motion artifact at the time of acquisition and scanning was repeated as necessary. Images were reviewed for any gross pathological findings.

Voxel-based morphometry

Every scan was visually checked to exclude the presence of artifacts or gross anatomical abnormalities that could impact image pre-processing. Voxelwise analyses of brain GMV and WMV differences were conducted using the DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) procedure [38] implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) running under MATLAB 2009b (http://www.mathworks.com.au/products/matlab/). Briefly, each participant's T1-weighted anatomical scan was segmented into distinct tissue compartments (i.e. GMV and WMV) and spatially normalized via a non-linear algorithm using a unified procedure [38]. A study-specific template was then generated by normalizing each participant's segmented grey or white matter image to a common space. Native-space grey or white matter images were then spatially normalized to this template. Jacobian modulation of voxel intensities was employed to preserve grey or white matter volumes. The images were smoothed with an 8 mm full-width-half-maximum Gaussian kernel prior to statistical analysis.

The General Linear Model (GLM) was used to test for group differences in volume at each voxel, as implemented in Randomise (http://fsl.fmrib.ox.ac.uk). All results were corrected for multiple comparison type I error with a non-parametric cluster-size based procedure [39,40]. A voxel-wise threshold was initially set to 0.001 to compromise between sensitivity to spatially extended vs. focal and intense differences. Then, a cluster-size threshold was calculated via permutation testing (10,000 permutations). We compared baseline GMV and WMV between ARMS group and HC group, while covarying for age, gender, intracranial volume (ICV), handedness and ethnicity.

Surface-based morphometry

The semi-automated cortical thickness measurements were performed using FreeSurfer v5.1.0 (http://surfer.nmr.mgh.harvard.edu/; Martinos Imaging Centre, Charlestown MA), as described by Dale, Fishl and colleagues [41,42].

The white (i.e., gray-white matter boundary) and pial (gray-cerebrospinal fluid boundary) surfaces were visually inspected and edited, where necessary, using standard procedures (http://surfer.nmr.mgh.harvard.edu/fswiki/Edits), blind to diagnostic status. Surfaces for each participant were registered to a study specific template and smoothed using a Gaussian kernel of 25 mm prior to group analysis.

We used a GLM implemented in Freesurfer to estimate group differences in cortical thickness at each vertex of the cerebral surface while controlling for the effect of age, gender, handedness, and ethnicity. Right and left hemispheres were tested separately. False Discovery Rate (FDR) $p < 0.05$ was used for multiple comparison correction.

Volume-of-interests measurements

We derived five volume-of-interests measurements from the Freesurfer analysis: total intracranial volume (ICV), total GMV, total WMV, hippocampal volume, and ventricular volume. ICV was calculated using a validated method described elsewhere [43]. Total ventricular volume was defined as the total volume of lateral ventricles, third ventricle, fourth ventricle, and fifth ventricle.

Statistical analyses were performed with the Statistical Package for the Social Sciences, version 21 (SPSS 21.0, IBM Corp. Armonk, NY, USA). Differences in cerebral volume were tested using one-way analysis of covariance (ANCOVA) with age, gender, handedness, ethnicity, and ICV as covariates.

Results

Demographics and volume-of-interest differences

There was no group difference in sociodemographics (age, gender, handedness, ethnicity, and educational level) or past history of substance use disorder (Table 1). No group difference in intracranial volume (ICV), total GMV, total WMV, hippocampal volume or ventricular volume between ARMS and HC was observed (Table 1).

GMV and WMV differences between ARMS subjects and healthy controls

We found no regional GMV or WMV differences between ARMS and HC (i.e., voxel-wise clustering-forming threshold of $p<0.001$ and $p < 0.05$ corrected at the cluster level). Lowering the initial voxel-wise cluster-forming threshold to $p < 0.01$ did not return significant group differences either ($p < 0.05$ corrected at the cluster level).

At a voxel-wise threshold of $p < 0.001$ and $k > 10$ voxels (uncorrected at the cluster level), we found one cluster of increased GMV on the right precentral gyrus (k = 88 voxels, t peak = 3.64, MNI = 4, 9, 44) and a second cluster of decreased GMV on the right frontal inferior gyrus (k = 17 voxels, t peak = 3.58, MNI = 46, 15, 21) in ARMS when compared to HC.

Cortical thickness differences between ARMS subjects and healthy controls

We found no regional cortical thickness differences between ARMS and HC at $p < 0.05$ (FDR corrected). At a voxel-wise cluster-forming threshold of $p < 0.001$ (uncorrected at the cluster level) we found one cluster of increased cortical thickness on the right frontal pole in ARMS when compared to HC (k = 230 vertices, t peak = 3.78, MNI = 21, 69, -2).

Conversion to psychosis

We found no significant difference between HC and ARMS-T, or between ARMS-T and ARMS-NT concerning GMV, WMV, cortical thickness or VOI analyses based on the same set of

thresholds. For the VBM analysis, lowering the initial voxel-wise cluster-forming threshold to $p < 0.01$ ($p < 0.05$ corrected at the cluster level) did not return significant group differences either.

Comorbid depression and anxiety disorders.

To investigate structural differences that could be related to anxio-depressive disorders and that affect a large proportion of AMRS individuals, we compared GMV, WMV, CT and VOI between ARMS with a concomitant diagnostic of depression and/or anxiety (n = 33) and ARMS without (n = 36). We found no significant differences. An additional comparison of GMV, WMV, CT, and VOI between ARMS individuals with antidepressant (n = 37) and those without (n = 32) found no significant difference either.

Discussion

Although there is evidence for the involvement of frontal, temporal and limbic areas in ARMS for psychosis, the sample size of previous studies is often modest and findings mainly concern ARMS samples from Western countries. In this study, we examined brain structural changes in a large sample of 69 ARMS subjects recruited in Singapore, and for which potential biases introduced by drug use, including antipsychotics and cannabis, were well controlled. Comparison of regional GMV, WMV, and CT as well as ventricular and hippocampal volumes between ARMS individuals and HC revealed no significant differences. The further analysis of the same structures between ARMS-T and ARMS-NT as well as between ARMS-T and HC did not return any positive result either.

Regional reductions of GMV in ARMS subjects are the most common findings in whole-brain VBM studies [15,44]. Only 3 whole-brain VBM studies reported negative findings but their ARMS sample was either unusually young (12-18 years old) [20,22] or small (n=14) [26]. Concerning CT, only one previous study [18] used the same preprocessing technique (Freesurfer), while three others [17,19,20] used a different algorithm: CLASP [45] or voxel-based cortical thickness [46]. Their findings were divergent, reporting either extended [17] or no CT differences at the whole-brain level [18-20] in ARMS subjects when compared to HC at baseline. Our results are consistent with the absence of cross-sectional difference between ARMS subject and HC at the whole-brain level reported by the three largest studies [18,19,22]. Additional comparison of hippocampal volumes between ARMS and HC showed no significant difference as well. Reduced hippocampal volume is a frequent finding from region-of-interest studies in ARMS samples [47-51] and has been shown to be statistically significant at the whole-brain level in one VBM study [4], although some inconsistences have also been reported [52,53]. The higher sensitivity of manual tracing methods to detect volumetric changes in medial temporal structures could explain our inability to replicate hippocampal volume reduction often reported by manually traced region-of-interest studies in ARMS samples. However,

Freesurfer automated segmentation performance has been shown to produce volumetric data that were very close to those obtained with the "gold standard" manual tracing method [54].

The sensitivity of our analyses did not improve when specifically comparing ARMS-T with HC or ARMS-NT. However, these additional group comparisons were clearly underpowered due to the small number of subject in the ARMS-T group (n = 7). A recent well powered study has also reported the absence of structural abnormalities in ARMS-T when compared to ARMS-NT at the whole brain level [19]. There was lack of evidence on the structural differences between ARMS-T and ARMS-NT or HC at the baseline.

The absence of relationship between clinical high-risk status (regardless of later transition or non-transition to psychosis) and brain structure might be attributed to unique characteristics of LYRIKS. Understanding the local pathways to care for the ARMS subjects is an important area of work, and efforts are currently underway. In a previous publication, we found that LYRIKS sample, was comparable to other samples from the UK or Australia concerning social and clinical profiles [35]. Accordingly, clinical characteristics reported in Table 1 (i.e., CAARMS ratings, grouping and comorbidities) are also comparable to those from OASIS and PACE samples [55], although the rate of conversion to psychosis (i.e., 10% at 28-month) is probably among the lowest reported [1]. However, ethnicity differences might be contributing to the negative findings as most participants in the LYRIKS sample have Asian origins. Another interesting difference could be the relative lack of drug use, including cannabis and/or antipsychotics in our sample. Half the ARMS individuals were pharmacologically treated for depression and/or anxiety and both the medication and the affective disorder could potentially impact brain structure. Last, the relatively conservative whole-brain approach could explain divergences with other region-of-interest studies. These four points are developed below.

Ethnicity

It is widely recognized that the expression of psychotic symptoms varies among ethnic groups [56,57]. Although these disparities seem more related to psychosocial inequalities than to ancestry differences [58], it raised the idea that ethnical differences could be instructive regarding the pathogenesis of schizophrenia [59]. Accordingly, a structural MRI study reported an effect of ethnicity on gray-matter findings following a first episode of psychosis [60]. These neuroimaging findings should be interpreted with caution regarding the modest sample size and the abundance of possible confounds, nevertheless, they suggest that some neuroanatomical features of psychosis could be specific to the ethnic group under investigation. In general, it is not very likely that our negative findings are attributable to the ethnical characteristics of our sample alone. Nevertheless, a different genetic background may modify the susceptibility of the brain to different etiological factors [61] and could impact the neuroanatomical correlates of the pathophysiological process.

Drugs

Singapore has the second lowest annual prevalence of cannabis-use worldwide (0.005 in 2006) [62] and no participant in our sample reported current illicit drug use. While most neuroimaging studies in ARMS excluded subjects with current and/or past substance abuse and/or dependence regarding the DSM or the International Classification of Diseases (ICD), they possibly included cannabis users as long as they did not fulfill the criteria for abuse of dependence. Only few studies specified the proportion of cannabis users in their sample but the reported rate can be as high as 35 % for current use [9,63] and up to 70% for a history of cannabis use [10]. In these previous studies, the prevalence of cannabis use did not statistically differ between ARMS subjects and controls, suggesting that neuroimaging findings were not driven by cannabis use only. Nevertheless, this does not exclude the possibility that cannabis use could act as a risk-modifying factor by interacting with other risk factors like genetics and have more dramatic consequences in the group of ARMS than in healthy controls [64,65]. Accordingly, three recent studies in early psychosis have shown that the amount of grey matter loss in the cingulate cortex was either positively correlated with

cannabis-use [34,66] or restricted to cannabis-users only [67]. Moreover, the hippocampus is rich in endocannabinoid receptors and hippocampal volume reduction has been strongly associated with cannabis use in a recent meta-analysis [68], suggesting that the absence of hippocampal atrophy in our sample may be partly related to the relative lack of cannabis use.

Antipsychotics are another potential confounding factor because they have been shown to alter GMV in schizophrenia after both continued [69] and short-term treatment [70] administration. In this study, we can exclude the potential influence of antipsychotic treatment on our results as only 3 subjects received a very small dose (< 15mg week of haloperidol equivalent). However, the absence of antipsychotic use is unlikely to explain our negative findings, given the results of a recent meta-analysis indicating an effect of antipsychotics on GMV in the opposite direction (i.e., antipsychotics reverse the GMV reductions associated with a greater risk of transition to psychosis) [15].

Affective comorbidity

Approximately half of ARMS individuals in our sample had a comorbid depressive and/or anxious disorder, a proportion that is comparable with other ARMS samples [55]. Disentangling emerging psychosis with concomitant mood disturbances from depression or anxiety with attenuated psychotic symptoms is challenging from both a clinical and neuroanatomical point of view. Similarly to psychosis, affective disorders may also show neuroanatomical features within medial prefrontal and medial temporal structures [71] and this could represent an important source of confound for neurostructural findings in ARMS. Accordingly, a recent study showed that comorbid depression and anxiety may contribute to GMV reduction in the anterior cingulate cortex in ARMS [72]. In our sample, we did not find any effect of comorbid depression and/or anxiety or antidepressant treatment on regional GMV, WMV, CT or VOI. However, we cannot exclude that antidepressant treatment may have interfered with the natural course of ARMS individuals [73,74].

Whole-brain analysis

We made the initial choice of a whole-brain analysis because it is a common and well accepted statistical approach for both VBM and SBM analyses. Moreover, in the context of an excess of significance in the neuroimaging literature [75,76], the whole-brain approach limits the risk of publication bias toward positive findings that is thought to be partially responsible for the lack of reliable biomarkers in psychiatry despite intense research in neuroimaging [77]. Indeed, region-of-interest studies are directed towards regions that can be easily anatomically delimited or regions of theoretical importance, which intrinsically depend on results from previous studies, thereby inflating the risk of confirmation bias. We completed the initial whole-brain approach with the individual analysis of two VOIs (i.e. ventricles and hippocampus) that are commonly implicated among structural findings in psychosis but are the best assessed individually, using volumetric information from the subcortical segmentation in Freesurfer. Instead of running additional region-of-interest analyses in the hypothesized fronto-temporal and limbic regions, we examined the group difference using $p < 0.001$ uncorrected, at the voxel or the vertex level for both the VBM and SBM analyses respectively. In the context of the literature, neither the direction of the trend (i.e., increased GMV or CT), nor the location of the clusters (i.e. precentral gyrus, frontal pole) advocate in favor of true differences between ARMS and HC. For inclusion of these data in a meta-analysis, GMV, WMV or CT for a specific region are available on request to the corresponding author (J.Z).

Our results might also be limited by the cross-sectional design of the study. Cannon and colleagues have recently reported greater GM loss over time in several frontal areas of ARMS-T when compared to ARMS-NT or HC, although they observed no CT differences between all 3 groups when compared cross-sectionally at baseline [18].

Last, our analysis was limited to anatomical changes in gray and white matter segments. Two functional MRI studies in the same ARMS sample have previously reported alterations in task-based activations [78] as well as abnormalities in functional-connectivity at rest [79] when compared to HC. This suggests that, in our sample, (1) there might be very little structural change in ARMS or (2) VBM and SBM analyses may not be sensitive to detect subtle structural differences. Functional

or diffusion MRI studies might reveal more insights on the pathophysiology changes in youths at high clinical risk for psychosis.

Conclusion

Taken together, this comprehensive cross-sectional analysis of regional volumes and cortical thickness was conducted in a relatively large sample of ARMS subjects, mainly free of possibly important confounds including antipsychotic medication and substance abuse. Only few whole brain studies have examined brain structural changes in an ARMS sample of comparable size, particularly in Asian populations [80]. We found no evidence of regional GMV, WMV or CT differences between ARMS and HC, ARMS-T and HC or ARMS-T and ARMS-NT at baseline. The small number of ARMS transitioning to psychosis and the absence of longitudinal analysis of brain changes over-time are clear limitations, especially in light of recent findings suggesting progressive structural changes in ARMS despite the absence of baseline differences with HC [18]. Nevertheless, our negative findings suggest that there may be no dramatic alterations of regional brain volumes or cortical thickness in ARMS when the incidence of possible confounds is limited.

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References

1. Fusar-Poli P, Bonoldi I, Yung AR et al. Predicting psychosis: meta-analysis of transition outcomes in individuals at high clinical risk. *Arch Gen Psychiatry* 2012; 69:220-229.
2. Miller TJ, McGlashan TH, Rosen JL et al. Prodromal assessment with the structured interview for prodromal syndromes and the scale of prodromal symptoms: predictive validity, interrater reliability, and training to reliability. *Schizophr Bull* 2003; 29:703-715.
3. Yung AR, Yuen HP, McGorry PD et al. Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. *Aust N Z J Psychiatry* 2005; 39:964-971.
4. Borgwardt SJ, Riecher-Rossler A, Dazzan P et al. Regional gray matter volume abnormalities in the at risk mental state. *Biol Psychiatry* 2007; 61:1148-1156.
5. Meisenzahl EM, Koutsouleris N, Gaser C et al. Structural brain alterations in subjects at high-risk of psychosis: a voxel-based morphometric study. *Schizophr Res* 2008; 102:150-162.
6. Koutsouleris N, Schmitt GJ, Gaser C et al. Neuroanatomical correlates of different vulnerability states for psychosis and their clinical outcomes. *Br J Psychiatry* 2009; 195:218-226.
7. Fusar-Poli P, Broome MR, Woolley JB et al. Altered brain function directly related to structural abnormalities in people at ultra high risk of psychosis: longitudinal VBM-fMRI study. *J Psychiatr Res* 2011; 45:190-198.
8. Fusar-Poli P, Crossley N, Woolley J et al. Gray matter alterations related to P300 abnormalities in subjects at high risk for psychosis: longitudinal MRI-EEG study. *Neuroimage* 2011; 55:320-328.
9. Smieskova R, Allen P, Simon A et al. Different duration of at-risk mental state associated with neurofunctional abnormalities. A multimodal imaging study. *Hum Brain Mapp* 2012; 33:2281-2294.
10. Stone JM, Day F, Tsagaraki H et al. Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume. *Biol Psychiatry* 2009; 66:533-

539.

11. Wood SJ, Pantelis C, Velakoulis D, Yucel M, Fornito A, & McGorry PD. Progressive changes in the development toward schizophrenia: studies in subjects at increased symptomatic risk. *Schizophr Bull* 2008; 34:322-329.

12. Pantelis C, Yucel M, Bora E et al. Neurobiological markers of illness onset in psychosis and schizophrenia: The search for a moving target. *Neuropsychol Rev* 2009; 19:385-398.

13. Ellison-Wright I, Glahn DC, Laird AR, Thelen SM, & Bullmore E. The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. *Am J Psychiatry* 2008; 165:1015-1023.

14. Fornito A, Yucel M, Patti J, Wood SJ, & Pantelis C. Mapping grey matter reductions in schizophrenia: an anatomical likelihood estimation analysis of voxel-based morphometry studies. *Schizophr Res* 2009; 108:104-113.

15. Fusar-Poli P, Borgwardt S, Crescini A et al. Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. *Neurosci Biobehav Rev* 2011; 35:1175-1185.

16. Mechelli A, Riecher-Rossler A, Meisenzahl EM et al. Neuroanatomical abnormalities that predate the onset of psychosis: a multicenter study. *Arch Gen Psychiatry* 2011; 68:489-495.

17. Jung WH, Kim JS, Jang JH et al. Cortical thickness reduction in individuals at ultra-high-risk for psychosis. *Schizophr Bull* 2011; 37:839-849.

18. Cannon TD, Chung Y, He G et al. Progressive Reduction in Cortical Thickness as Psychosis Develops: A Multisite Longitudinal Neuroimaging Study of Youth at Elevated Clinical Risk. *Biol Psychiatry* 2015; 77:147-157.

19. Tognin S, Riecher-Rossler A, Meisenzahl EM et al. Reduced parahippocampal cortical thickness in subjects at ultra-high risk for psychosis. *Psychol Med* 2014; 44:489-498.

20. Ziermans TB, Durston S, Sprong M et al. No evidence for structural brain changes in young adolescents at ultra high risk for psychosis. *Schizophr Res* 2009; 112:1-6.

21. Koutsouleris N, Gaser C, Patschurek-Kliche K et al. Multivariate patterns of brain-cognition

associations relating to vulnerability and clinical outcome in the at-risk mental states for psychosis. *Hum Brain Mapp* 2012; 33:2104-2124.

22. Ziermans TB, Schothorst PF, Schnack HG et al. Progressive structural brain changes during development of psychosis. *Schizophr Bull* 2012; 38:519-530.

23. Dazzan P, Soulsby B, Mechelli A et al. Volumetric Abnormalities Predating the Onset of Schizophrenia and Affective Psychoses: An MRI Study in Subjects at Ultrahigh Risk of Psychosis. *Schizophr Bull* 2012; 38:1083-1091.

24. Pantelis C, Velakoulis D, McGorry PD et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet* 2003; 361:281-288.

25. Jung WH, Jang JH, Shin NY et al. Regional Brain Atrophy and Functional Disconnection in Broca's Area in Individuals at Ultra-High Risk for Psychosis and Schizophrenia. *PLoS One* 2012; 7:e51975.

26. Nakamura K, Takahashi T, Nemoto K et al. Gray Matter Changes in Subjects at High Risk for Developing Psychosis and First-Episode Schizophrenia: A Voxel-Based Structural MRI Study. *Front Psychiatry* 2013; 4:16.

27. Dargan PI, & Wood DM. Recreational drug use in the Asia Pacific region: improvement in our understanding of the problem through the UNODC programmes. *J Med Toxicol* 2012; 8:295-299.

28. Koskinen J, Lohonen J, Koponen H, Isohanni M, & Miettunen J. Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophr Bull* 2010; 36:1115-1130.

29. Malchow B, Hasan A, Fusar-Poli P, Schmitt A, Falkai P, & Wobrock T. Cannabis abuse and brain morphology in schizophrenia: a review of the available evidence. *Eur Arch Psychiatry Clin Neurosci* 2013; 263:3-13.

30. Rapp C, Bugra H, Riecher-Rossler A, Tamagni C, & Borgwardt S. Effects of cannabis use on

human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. *Curr Pharm Des* 2012; 18:5070-5080.

31. Habets P, Marcelis M, Gronenschild E, Drukker M, & van Os J. Reduced cortical thickness as an outcome of differential sensitivity to environmental risks in schizophrenia. *Biol Psychiatry* 2011; 69:487-494.
32. Welch KA, McIntosh AM, Job DE et al. The impact of substance use on brain structure in people at high risk of developing schizophrenia. *Schizophr Bull* 2011; 37:1066-1076.
33. Welch KA, Stanfield AC, McIntosh AM et al. Impact of cannabis use on thalamic volume in people at familial high risk of schizophrenia. *Br J Psychiatry* 2011; 199:386-390.
34. Rapp C, Walter A, Studerus E et al. Cannabis use and brain structural alterations of the cingulate cortex in early psychosis. *Psychiatry Res* 2013;
35. Lee J, Rekhi G, Mitter N et al. The Longitudinal Youth at Risk Study (LYRIKS)--an Asian UHR perspective. *Schizophr Res* 2013; 151:279-283.
36. Mitter N, Nah GQ, Bong YL, Lee J, & Chong SA. Longitudinal Youth-At-Risk Study (LYRIKS): outreach strategies based on a community-engaged framework. *Early Interv Psychiatry* 2014; 8:298-303.
37. Jack CR, Bernstein MA, Fox NC et al. The Alzheimer's disease neuroimaging initiative (ADNI): MRI methods. *Journal of Magnetic Resonance Imaging* 2008; 27:685-691.
38. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage* 2007; 38:95-113.
39. Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, & Evans AC. Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping Hum. Brain Mapp.* 1993; 1:210-220.
40. Nichols TE, & Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* 2002; 15:1-25.
41. Dale AM, Fischl B, & Sereno MI. Cortical surface-based analysis. I. Segmentation and

surface reconstruction. *Neuroimage* 1999; 9:179-194.

42. Fischl B, Sereno MI, & Dale AM. Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage* 1999; 9:195-207.

43. Buckner RL, Head D, Parker J et al. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage* 2004; 23:724-738.

44. Fusar-Poli P, Radua J, McGuire P, & Borgwardt S. Neuroanatomical Maps of Psychosis Onset: Voxel-wise Meta-Analysis of Antipsychotic-Naive VBM Studies. *Schizophr Bull* 2012; 38:1297-1307.

45. Lee JK, Lee JM, Kim JS, Kim IY, Evans AC, & Kim SI. A novel quantitative cross-validation of different cortical surface reconstruction algorithms using MRI phantom. *Neuroimage* 2006; 31:572-584.

46. Hutton C, De Vita E, Ashburner J, Deichmann R, & Turner R. Voxel-based cortical thickness measurements in MRI. *Neuroimage* 2008; 40:1701-1710.

47. Hurlemann R, Jessen F, Wagner M et al. Interrelated neuropsychological and anatomical evidence of hippocampal pathology in the at-risk mental state. *Psychol Med* 2008; 38:843-851.

48. Phillips LJ, Velakoulis D, Pantelis C et al. Non-reduction in hippocampal volume is associated with higher risk of psychosis. *Schizophr Res* 2002; 58:145-158.

49. Witthaus H, Mendes U, Brune M et al. Hippocampal subdivision and amygdalar volumes in patients in an at-risk mental state for schizophrenia. *J Psychiatry Neurosci* 2010; 35:33-40.

50. Wood SJ, Yucel M, Velakoulis D et al. Hippocampal and anterior cingulate morphology in subjects at ultra-high-risk for psychosis: the role of family history of psychotic illness. *Schizophr Res* 2005; 75:295-301.

51. Wood SJ, Kennedy D, Phillips LJ et al. Hippocampal pathology in individuals at ultra-high

risk for psychosis: a multi-modal magnetic resonance study. *Neuroimage* 2010; 52:62-68.

52. Buehlmann E, Berger GE, Aston J et al. Hippocampus abnormalities in at risk mental states for psychosis? A cross-sectional high resolution region of interest magnetic resonance imaging study. *J Psychiatr Res* 2010; 44:447-453.

53. Velakoulis D, Wood SJ, Wong MT et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch Gen Psychiatry* 2006; 63:139-149.

54. Morey RA, Petty CM, Xu Y et al. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage* 2009; 45:855-866.

55. Fusar-Poli P, Nelson B, Valmaggia L, Yung AR, & McGuire PK. Comorbid Depressive and Anxiety Disorders in 509 Individuals With an At-Risk Mental State: Impact on Psychopathology and Transition to Psychosis. *Schizophr Bull* 2014; 40:120-131.

56. Bresnahan M, Begg MD, Brown A et al. Race and risk of schizophrenia in a US birth cohort: another example of health disparity? *Int J Epidemiol* 2007; 36:751-758.

57. Fearon P, Kirkbride JB, Morgan C et al. Incidence of schizophrenia and other psychoses in ethnic minority groups: results from the MRC AESOP Study. *Psychol Med* 2006; 36:1541-1550.

58. Hjern A, Wicks S, & Dalman C. Social adversity contributes to high morbidity in psychoses in immigrants--a national cohort study in two generations of Swedish residents. *Psychol Med* 2004; 34:1025-1033.

59. Cohen CI, & Marino L. Racial and ethnic differences in the prevalence of psychotic symptoms in the general population. *Psychiatr Serv* 2013; 64:1103-1109.

60. Morgan KD, Dazzan P, Morgan C et al. Differing patterns of brain structural abnormalities between black and white patients with their first episode of psychosis. *Psychol Med* 2010; 40:1137-1147.

61. Ajnakina O, Borges S, Di Forti M et al. Role of Environmental Confounding in the Association between FKBP5 and First-Episode Psychosis. *Front Psychiatry* 2014; 5:84.

62. UNODC. World Drug Report 2008. 2008;

63. Smieskova R, Fusar-Poli P, Aston J et al. Insular volume abnormalities associated with different transition probabilities to psychosis. *Psychol Med* 2012; 42:1613-1625.

64. Caspi A, Moffitt TE, Cannon M et al. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biol Psychiatry* 2005; 57:1117-1127.

65. Di Forti M, Iyegbe C, Sallis H et al. Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biol Psychiatry* 2012; 72:811-816.

66. Rais M, van Haren NE, Cahn W et al. Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. *Eur Neuropsychopharmacol* 2010; 20:855-865.

67. Szeszko PR, Robinson DG, Sevy S et al. Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. *Br J Psychiatry* 2007; 190:230-236.

68. Rocchetti M, Crescini A, Borgwardt S et al. Is cannabis neurotoxic for the healthy brain? A meta-analytical review of structural brain alterations in non-psychotic users. *Psychiatry Clin Neurosci* 2013; 67:483-492.

69. Ho BC, Andreasen NC, Ziebell S, Pierson R, & Magnotta V. Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Arch Gen Psychiatry* 2011; 68:128-137.

70. Girgis RR, Diwadkar VA, Nutche JJ, Sweeney JA, Keshavan MS, & Hardan AY. Risperidone in first-episode psychosis: a longitudinal, exploratory voxel-based morphometric study. *Schizophr Res* 2006; 82:89-94.

71. Klauser P, Fornito A, Lorenzetti V et al. Cortico-limbic network abnormalities in individuals

with current and past major depressive disorder. *Journal of Affective Disorders* 2015; 173:45-52.

72. Modinos G, Allen P, Frascarelli M et al. Are we really mapping psychosis risk? Neuroanatomical signature of affective disorders in subjects at ultra high risk. *Psychol Med* 2014; 1-11.

73. Fusar-Poli P, Valmaggia L, & McGuire P. Can antidepressants prevent psychosis? *Lancet* 2007; 370:1746-1748.

74. Cornblatt BA, Lencz T, Smith CW et al. Can antidepressants be used to treat the schizophrenia prodrome? Results of a prospective, naturalistic treatment study of adolescents. *J Clin Psychiatry* 2007; 68:546-557.

75. Ioannidis JP. Excess significance bias in the literature on brain volume abnormalities. *Arch Gen Psychiatry* 2011; 68:773-780.

76. Fusar-Poli P, Radua J, Frascarelli M et al. Evidence of reporting biases in voxel-based morphometry (VBM) studies of psychiatric and neurological disorders. *Hum Brain Mapp* 2014; 35:3052-3065.

77. Borgwardt S, Radua J, Mechelli A, & Fusar-Poli P. Why are psychiatric imaging methods clinically unreliable? Conclusions and practical guidelines for authors, editors and reviewers. *Behav Brain Funct* 2012; 8:46.

78. Yaakub SN, Dorairaj K, Poh JS et al. Preserved working memory and altered brain activation in persons at risk for psychosis. *Am J Psychiatry* 2013; 170:1297-1307.

79. Dandash O, Fornito A, Lee J et al. Altered Striatal Functional Connectivity in Subjects With an At-Risk Mental State for Psychosis. *Schizophr Bull* 2014; 40:904-913.

80. Jung WH, Borgwardt S, Fusar-Poli P, & Kwon JS. Gray matter volumetric abnormalities associated with the onset of psychosis. *Front Psychiatry* 2012; 3:101.

Figure legends

Table 1. Demographic, clinical and anatomical characteristics of participants

APS, attenuated psychotic symptoms; BAI, Beck anxiety inventory; BLIPS, brief limited intermittent psychotic symptoms; CAARMS, comprehensive assessment of at-risk mental states; CDSS, Calgary depression scale for schizophrenia; GRD, genetic risk and deterioration syndrome; GM, grey matter; ICV, intracranial volume; PSLE, primary school leaving examination; SBM, surface-based morphometry; SUD, substance use disorder; VBM, voxel-based morphometry; WM, white matter; Percentages were rounded to the nearest integer. All ARMS and control subjects belong to the three major ethnicities in Singapore (Chinese, Malay and Indian), except two ARMS (Javanese and Eurasian) and two controls (Javanese and Israeli).