

## Short Communication

A common variant of the *CNTNAP2* gene is associated with structural variation in the left superior occipital gyrusJulia Uddén<sup>a,b,\*</sup>, Tineke M. Snijders<sup>b,c,1</sup>, Simon E. Fisher<sup>a,b</sup>, Peter Hagoort<sup>a,b</sup><sup>a</sup> Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands<sup>b</sup> Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands<sup>c</sup> Centre for Language Studies, Radboud University, Nijmegen, The Netherlands

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## ABSTRACT

The *CNTNAP2* gene encodes a cell-adhesion molecule that influences the properties of neural networks and the morphology and density of neurons and glial cells. Previous studies have shown association of *CNTNAP2* variants with language-related phenotypes in health and disease. Here, we report associations of a common *CNTNAP2* polymorphism (rs7794745) with variation in grey matter in a region in the dorsal visual stream. We tried to replicate an earlier study on 314 subjects by Tan et al. (2010), but now in a substantially larger group of more than 1700 subjects. Carriers of the T allele showed reduced grey matter volume in left superior occipital gyrus, while we did not replicate associations with grey matter volume in other regions identified by Tan et al. (2010). Our work illustrates the importance of independent replication in neuroimaging genetic studies of language-related candidate genes.

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## 1. Introduction

Communicative and linguistic abilities depend on complex multifactorial genetic and environmental influences. Linkage mapping of families with rare mutations, as well as large-scale genetic association studies of common polymorphisms, have implicated a number of candidate genes in communicative and linguistic traits in health and disease (Graham & Fisher, 2013). The most well-studied of such genes is *FOXP2* (Fisher & Scharff, 2009; Hoogman et al., 2014), a transcription factor which regulates multiple downstream targets involved in neural connectivity and plasticity (Vernes et al., 2007). Among these targets, the gene encoding contactin-associated protein-like 2, *CNTNAP2*, is of particular interest (Vernes et al., 2008). Common polymorphisms of *CNTNAP2* have been associated with quantitative variation in language-related phenotypes in different neurodevelopmental disorders, including specific language impairment (Newbury et al., 2011; Vernes et al., 2008), autism spectrum disorder (ASD) (Alarcon et al., 2008; Chiocchetti et al., 2014) and dyslexia (Peter et al., 2011); but see (Newbury et al., 2011). Recent large-scale studies suggest that common *CNTNAP2* variants are not associated with a qualita-

tive diagnosis of ASD (i.e. whether or not a child is considered “affected”, Sampath et al., 2013). Thus such variants seem to be relevant for language-related variation (both within and between disorders) rather than for diagnostic status (although see Toma et al., 2013). Indeed, common *CNTNAP2* variants have also been associated with quantitative variation in early language development in the normal population (Whitehouse, Bishop, Ang, Pennell, & Fisher, 2011). Rare high-penetrance variants that disrupt *CNTNAP2* have been reported in children with severe neurodevelopmental syndromes; common themes found in multiple patients are intellectual disability, epileptic seizures, features of autism, and speech and language impairments (for a recent review see Rodenas-Cuadrado, Ho, & Vernes, 2014). Next-generation sequencing of *CNTNAP2* in large cohorts suggests that rare heterozygous variants do not account for a significant proportion of ASD cases (Murdoch et al., 2015). Nonetheless, in-depth phenotypic studies of children with unambiguous aetiological mutations clearly support the relevance of this gene for neurobiology of language and communication (Strauss et al., 2006). These children present with focal epilepsy and severe regression in language and communicative traits. Combined MRI and histological evidence suggest structural abnormalities, e.g. in morphology, orientation and density of neurons and glia, in particular in the temporal lobe (Strauss et al., 2006).

The investigation of genetic pathways, such as the *FOXP2*-*CNTNAP2* pathway, has started to inform the study of the

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neurobiology of language at the molecular level (Fisher & Vernes, 2015). *CNTNAP2* encodes a neurally-expressed transmembrane protein belonging to the Neurexin superfamily and has been implicated in mediating cell–cell interactions, synchronicity of neuronal firing, interneuron function and synaptic architecture (reviewed in Rodenas-Cuadrado et al., 2014). To help further bridge the gap between molecular mechanisms and behavioural/cognitive outcomes, researchers have used neuroimaging genetic approaches to study impacts of common *CNTNAP2* variation on structural and functional brain properties. There is however still little convergence in findings, most likely due to the limited power of standard structural and functional imaging genetic studies to observe small effects.

The neuroimaging literature on *CNTNAP2* variants has so far focused primarily on two common single-nucleotide polymorphisms (SNPs) more than 1 Mb apart (rs7794745 and rs2710102), both located in introns. These SNPs received particular attention in neuroimaging studies due to their earlier reported associations with behavioural and cognitive phenotypes. The neuroimaging findings consist of a handful of associations with functional and structural phenotypes (Dennis et al., 2011; Folia, Forkstam, Ingvar, Hagoort, & Petersson, 2011; Kos et al., 2012; Scott-Van Zeeland et al., 2010; Tan et al., 2010; Whalley et al., 2011) and none have yet been independently replicated.

The T-allele of rs7794745 was initially associated with autism susceptibility as a qualitative trait (Arking et al., 2008) but this finding was not replicated in more recent reports (Sampath et al., 2013; Toma et al., 2013). In another recent study, investigating autistic-like traits in the general population, there was a nominally significant association which did not survive multiple comparison correction (Jonsson et al., 2014). This SNP maps within a region of *CNTNAP2* that has been connected to a range of disorders (reviewed in Rodenas-Cuadrado et al., 2014) and there is also evidence for a link with language-relevant functions in the healthy population. There is tentative evidence of rs7794745 being associated with orthographical word coding in families with dyslexia (Newbury et al., 2011). Positive associations have also been observed with language processing beyond the lexical level in healthy individuals, e.g. fMRI BOLD response during sentence processing (Whalley et al., 2011), behavioural and BOLD response during processing of artificial syntax (Folia et al., 2011), EEG response in reaction to a syntactic violation (Kos et al., 2012) and a compound score of intelligibility and fluency, syntax and coherence at 9 years of age, as well as sociability at 38 month of age (Steer, Golding, & Bolton, 2010). A neuroimaging genetics study (Tan et al., 2010) on the same SNP reported widespread association with white and grey matter volume as measured with voxel based morphometry (VBM), as well as fractional anisotropy (FA) as measured with diffusion tensor imaging (DTI). The overlap of these three phenotypes was observed in the cerebellum, occipital and right frontal lobes (Tan et al., 2010).

Associations between functional imaging phenotypes and rs7794745/rs2710102 have been observed in frontal and temporal lobes. The previously mentioned study of Whalley et al. (2011) found associations between rs7794745 (as well as rs2710102) and brain activity in right inferior frontal gyrus and right lateral temporal cortex during a sentence completion task, in healthy individuals. For rs7794745, individuals homozygous for the T allele demonstrated significantly increased activation in the right middle temporal gyrus compared to the remaining subjects. For rs2710102, T allele carriers showed increased activity in the left superior parietal lobe, while homozygotes for the C allele (a putative risk allele with respect to ASD and specific language impairment) showed increased activity in the right inferior frontal gyrus. Variation in structural and functional connectivity in healthy individuals has been associated with rs2710102 in three

separate studies, but with little spatial overlap between studies (Clemm von Hohenberg et al., 2013; Dennis et al., 2011; Scott-Van Zeeland et al., 2010).

In summary, multiple different associations have been reported between *CNTNAP2* variants and brain imaging endophenotypes of various kinds. However, the current literature provides no independent replications and thus needs to be considered with caution, especially since study sample sizes are small (50–350 subjects), which increases the risk of false-positive findings.

### 1.1. Rationale of the study

The current study investigates the influence of a common genetic polymorphism in *CNTNAP2* on individual differences in grey matter volume, with a focus on testing whether earlier results (Tan et al., 2010) on 314 subjects are replicable in a substantially larger sample of more than 1700 subjects. The prior study reported reduced grey matter volumes in the bilateral cerebellum, fusiform gyri, occipital and right frontal cortices for TT carriers (Tan et al., 2010). Like Tan et al. (2010), we limited our analysis to rs7794745 in order to minimize multiple testing issues and since this SNP had been directly genotyped on a large sample in our database (Franke et al., 2010). In Tan et al. (2010) grey and white matter volumes as well as FA were investigated separately. Considerable variation in heritability estimates of FA has been reported across five large family cohorts (Kochunov et al., 2014). Furthermore, compared to grey and white matter volumes, FA was available in a much smaller sample in our database. Thus, we chose to focus on testing whether associations between grey matter volume and rs7794745 would replicate in our data set. Across the brain, varying degrees of heritability ( $h^2 = 0-0.8$ ) have been demonstrated for grey matter (Winkler et al., 2010). Grey matter volume is highly correlated with cortical thickness and surface area (Winkler et al., 2010) in the regions where rs7794745 was proposed to influence grey matter volume in Tan et al. (2010). These areas show  $h^2$  estimates of 0.3–0.8 (Winkler et al., 2010). In order to further reduce multiple testing and maximize power, we focused our replication study on grey matter volume only. In this context, we note that there is virtually no genetic correlation between cortical surface area and thickness (Winkler et al., 2010). Grey matter volume is in some sense a combination of these two measures, although more closely related to surface area (Winkler et al., 2010). It can thus be considered a limitation of the original study that grey matter volume was used, rather than e.g. surface area (and perhaps cortical thickness as an additional measure which might reveal separate genetic associations).

In the Tan et al. (2010) paper, AT and AA-carriers were grouped in one analysis, and compared to results grouping AT and TT-carriers. The significant associations with grey matter volume were observed by grouping AT and AA-carriers, comparing those subjects to a relatively small sample of TT-carriers (~10% of 314 subjects). However, it should be noted, as in a recent review (Rodenas-Cuadrado et al., 2014), that most of the prior literature associating this SNP with behavioural/cognitive deficits is consistent with a dominant effect for the T allele.

Our approach in the present study was to test each of the nine clusters reported by Tan et al. (2010), by forming 10 mm spherical regions of interest (ROIs) around the nine peak voxels. We did not consider the present literature to provide strong enough converging evidence to form a hypothesis on which of these nine peaks might or might not replicate. Association of *CNTNAP2* alleles with functional and structural variation in right frontal cortices (Tan et al., 2010; Whalley et al., 2011) could be considered as convergent evidence, but these are as yet non-replicated findings concerning different phenotypes measured with separate techniques, more often than not producing disparate spatial foci of association.

We formed allele groups in the same way as the original Tan et al. (2010) study, in a staged approach, first grouping AA and AT-carriers, then grouping AT and TT-carriers. Finally, we performed a whole brain analysis to investigate whether there were additional loci of association in our large sample. Our alpha-level throughout the study was 0.05.

## 2. Results

### 2.1. Genotyping

Of the 1717 (995 female, 722 male) genotyped subjects, 752 (311 males, 441 females) were homozygous for the A allele (AA group), 750 (327 males, 423 females) were heterozygous (AT group) and 215 (84 males, 131 females) were homozygous for the T allele (TT group), with no deviation from Hardy–Weinberg equilibrium ( $p = .19$ ). There were no differences in allele frequencies across protocols (see methods section,  $p = 0.49$ ).

### 2.2. VBM analysis

For all analyses, we compared groups with unidirectional tests, testing the directed hypothesis that the T allele would be associated with reduction in grey matter volume. In a first stage, following Tan et al. (2010), we grouped AT and AA-carriers. None of the nine loci reported in Tan et al. (2010) showed any suprathreshold voxels (with a threshold of  $p < 0.001$ , uncorrected, see Table 1).

In a second stage, again following Tan et al. (2010), we grouped AT and TT carriers and compared against AA carriers (see Table 1). With this genetic model, in the left superior occipital gyrus (LSOG), extending along the intraparietal sulcus, there were significant cluster level (raw  $p_{\text{FWE-corr}} = 0.005$ , Bonferroni corrected  $p_{\text{FWE-corr}} = 0.045$ ) and voxel level effects (peak voxel,  $T(1712) = 4.03$ , raw  $p_{\text{FWE-corr}} = 0.001$ , Bonferroni corrected  $p_{\text{FWE-corr}} = 0.009$ , Cohen's  $d = 0.196$ ) surviving multiple comparison correction. The corresponding test survived the multiple comparison corrections when performed on the Caucasian subset ( $N = 1571$ ) of the sample, at least at the voxel level (peak voxel,  $T(1566) = 4.03$ , raw  $p_{\text{FWE-corr}} = 0.005$ , Bonferroni corrected  $p_{\text{FWE-corr}} = 0.045$ , Cohen's  $d = 0.184$ ). The cluster level statistics on the Caucasian subset were nominally significant and close to the significance threshold with Bonferroni correction (cluster level raw  $p_{\text{FWE-corr}} = 0.008$ , Bonferroni corrected  $p_{\text{FWE-corr}} = 0.072$ ). In a third stage, we performed a whole brain level analysis, which did not show any significant results, with either grouping.

As can be seen in Fig. 1, grey matter volume in left superior occipital gyrus reduces with increasing number of T alleles. However, note that we, following Tan et al. (2010) did not analyse an additive model (AA compared to AT compared to TT) in addition, in order to keep the total number of test to a minimum. To make assessment of spatial specificity easier, see Fig. 2 which displays the continuous variation in the genetic effects (when grouping AT and TT carriers against AA carriers) through a T-map displayed at a very low statistical threshold ( $p < 0.1$ , uncorrected). This figure is also intended for aiding future meta-analysis.

## 3. Discussion

Reductions in grey matter volume associated with the T allele in this *CNTNAP2* rs7794745 SNP were identified in the left superior occipital gyrus (LSOG), extending along the intraparietal sulcus, in a large group of subjects of over 1700 healthy individuals. The prior study by Tan et al. (2010) compared grey matter volumes of subjects homozygous for the T allele (TT group) to carriers of at least one A allele (AT/AA group) in the rs7794745 SNP. Eight

of the nine loci with reduced grey matter volumes reported for the TT group, across brain regions including the cerebellum, fusiform gyri, occipital and frontal cortices (Tan et al., 2010), were not replicated in the current study. The findings of Tan et al. (2010) were significant only when A-carriers were grouped (AT/AA) instead of T-carriers, although one of the groups (TT) was small (~10% of a total of 314 subjects). With our substantially larger sample of 1717 subjects, the association with the T allele in LSOG survived Bonferroni correction only when grouping T-carriers (AA compared to AT/TT). When these alternative genetic models were compared in Tan et al. (2010), the stronger associations were observed with the dominant A-allele model (AA/AT compared to TT). Thus, we replicate the association between the T-allele and reduced grey matter volume in the LSOG, but the results concerning which genetic model best captures the variance in the association differ across the two studies. In both studies however, grey matter volume in LSOG decreased when groups with greater numbers of T alleles were compared to groups with fewer numbers of T alleles. We chose not to add analyses of an additive model (AA compared to AT compared to TT), in order to keep the total number of tests to a minimum. Note that in the Arking et al. (2008) report that initially suggested association of rs7794745 with risk of autism, the effect was only identified in the context of maternal transmission. In our heterozygote group (just as in Tan et al., 2010) we could not distinguish between paternally and maternally inherited T alleles, since parental DNA samples were not collected, and thus we were not able to test for maternal-specific effects.

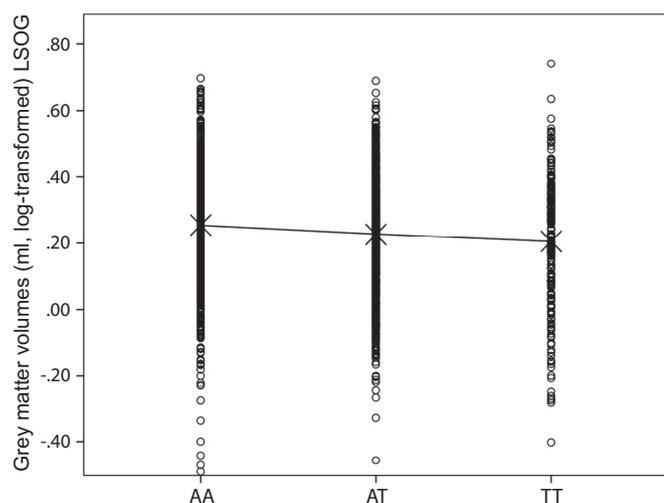
The LSOG and intraparietal sulcus are parts of the dorsal extrastriate cortex implicated in higher level visual association processes, as a part of the visual dorsal stream. Behaviourally, this stream has been implicated in a number of developmental disorders (Braddick, Atkinson, & Wattam-Bell, 2003), including those previously associated with *CNTNAP2* variations such as dyslexia and autism. In autistic children, it has been hypothesized that problems with co-ordination, gait, balance and posture depend on dysfunctions in motion detection and visually guided action processing in the visual dorsal stream (Grinter, Maybery, & Badcock, 2010). Developmental coordination problems are common in dyslexia and also show comorbidity with specific language impairment (Zwicker, Missiuna, & Boyd, 2009). Since *CNTNAP2* plays roles in brain development (Graham & Fisher, 2013; Rodenas-Cuadrado et al., 2014), it is interesting to note that the dorsal stream has been observed as particularly susceptible to damage during early development (Braddick et al., 2003; Grinter et al., 2010) and that genetic influences are greatest on brain regions which mature early, exemplified by the occipital lobe (Brun et al., 2009). Brain lateralization similarly begins early in development (Francks, 2015), and our lateralized finding in LSOG might add to discussions about the neurogenetics of language lateralization (Ocklenburg, Beste, Arning, Peterburs, & Gunturkun, 2014). There are a number of lateralized functional accounts of the dorsal visual stream (including LSOG and the left intraparietal sulcus) in relation to aspects of language processing. For instance, the left intraparietal sulcus has been implicated in verbal short term memory (Majerus et al., 2006). In addition, functional recruitment of the left dorsal visual stream has recently been shown to change as a function of acquired literacy in healthy adult subjects (Eisner et al., 2015). *CNTNAP2* has been broadly related to the ways that neurons migrate, connect, and communicate (Rodenas-Cuadrado et al., 2014), which might imply that alterations of this gene should have wide-ranging impacts. Indeed, most neurodevelopmental genes have diverse effects through actions in multiple neuronal subpopulations and/or at different developmental stages. The reuse of the same gene in different contexts allows a limited group of molecular factors, working together in a combinatorial manner, to help guide the assembly of a highly intricate nervous system (Graham & Fisher, 2015). Even

**Table 1**

*CNTNAP2* rs7794745 genotype effects on grey matter volume. Replication of ROI from Tan et al. (2010). Information on the clusters and peaks we observe in our larger sample, with  $p < 0.001$  as cluster forming threshold for reporting significant findings, and  $p < 0.5$ , which corresponds to positive  $T$ -values, as cluster forming threshold for reporting non-significant findings. Corresponding clusters and peaks from Tan et al. (2010) in parenthesis (their results were obtained comparing AA/AT > TT, with  $p < 0.001$  as cluster forming threshold).

Region (ROI) SVC 10 mm sphere	Current peak			Cluster Size	Cluster $p_{FWE-corr}$	Voxel $p_{FWE-corr}$	Voxel Z-score	Voxel Cohen's $d$
	x	y	z					
<b>AA &gt; AT/TT, <math>p &lt; 0.001</math> unc.</b>								
LSOG	-21(-21)	-94(-94)	29(24)	271(19)	<b>0.005*(0.01)</b>	<b>0.001*(0.006)</b>	4.02(5.12)	0.20
<b>AA &gt; AT/TT, <math>p &lt; 0.5</math> unc.</b>								
Right fusiform	35(29)	-75(-71)	-11(-16)			0.36*(3.01E-08)	1.58(7.22)	
Left fusiform	42(-41)	-39(-45)	-30(-23)			0.41*(6.55E-05)	1.43(6.00)	
RSOG	24(26)	-93(-97)	16(15)			0.32*(0.016)	1.68(4.91)	
Left superior cerebellum	-26(-23)	-87(-78)	-17(-18)			0.59*(1.90E-04)	0.82(5.80)	
	-24(-23)	-72(-78)	-11(-18)			0.66*(1.90E-04)	0.33(5.80)	
Cerebellar vermis	-8(-2)	-66(-61)	-36(-42)			0.61*(0.006)	0.73(5.14)	
	5(-2)	-66(-61)	-50(-42)			0.66*(0.006)	0.37(5.14)	
Right posterior cerebellum	15(15)	-84(-83)	-29(-33)			0.16*(0.014)	0.99(4.94)	
Left posterior cerebellum	-8(-10)	-64(-72)	-35(-33)			0.61*(0.020)	0.75(4.86)	
Right frontal pole	18(15)	69(71)	-9(-12)			0.10*(0.033)	2.44(4.75)	
<b>AA/AT &gt; TT, <math>p &lt; 0.5</math> unc.</b>								
Right fusiform	24(29)	-63(-71)	-12(-16)			0.23*(3.01E-08)	1.94(7.22)	
Left fusiform	-39(-41)	-39(-45)	-27(-23)			0.28*(6.55E-05)	1.79(6.00)	
LSOG	-21(-21)	-96(-94)	27(24)			0.054*(0.006)	2.73(5.12)	
RSOG	17(26)	-93(-97)	22(15)			0.28*(0.016)	1.81(4.91)	
Left superior cerebellum	-18(-23)	-73(-78)	-11(-18)			0.40*(1.90E-04)	1.44(5.80)	
Cerebellar vermis	-3(-2)	-70(-61)	-44(-42)			0.62*(0.006)	0.65(5.14)	
Right posterior cerebellum	9(15)	-78(-83)	-27(-33)			0.60*(0.014)	0.77(4.94)	
Left posterior cerebellum	0(-10)	-70(-72)	-33(-33)			0.64*(0.020)	0.62(4.86)	
Right frontal pole	18(15)	69(71)	-9(-12)			0.32*(0.033)	1.70(4.75)	

\* Raw  $p$ -values. These  $p$ -values should be compared to a Bonferroni-corrected significance threshold of  $0.05/9 = 0.0056$  ( $p$ -values that meet the corrected threshold are indicated in bold).



**Fig. 1.** Log-transformed ( $\ln x$ ) grey matter volume (in ml) in left superior occipital gyrus (LSOG, spherical 10 mm ROI around  $x = -21$ ,  $y = -94$ ,  $z = 29$ ) reduces with increasing number of T alleles. Every circle gives the volume for one participant. Crosses represent the mean of each genotype group. The AA group consisted of 752 participants, AT group 750 participants and the TT group 215 participants.

for a widely expressed gene, genetic variations can have differential effects on distinct brain networks, depending on interactions with the other genes expressed in the relevant brain regions, and because different neuronal subpopulations can have differential sensitivity to altered gene dosage (Fisher, 2006). Nevertheless, the observation that rs7794745 only shows replicated association for LSOG does not indicate that effects of this variant must be exclusive to just one region of the brain. The polymorphism could have biological effects elsewhere in the brain that our study design was not able to detect; to formally test for specificity of association would require even larger samples than those used here. Moreover,

although the region where we identified our effect (LSOG) is related to the extended language network (Ferstl, Neumann, Bogler, & von Cramon, 2008), we do not make any claims about how exactly these structural findings may relate to variations in linguistic performance, as opposed to other aspects of cognitive function, because we do not have such behavioural data available for the present sample.

In conclusion, in our investigation of nine brain loci associated with a key *CNTNAP2* polymorphism from a prior study, we identified significant effects for one region, the LSOG. Our work illustrates the importance of independent replication in neuroimaging genetic studies of language-related candidate genes. The findings implicate a *CNTNAP2* variant in individual differences in the structural neuronal infrastructure of the visual dorsal stream, highlighting the complexity of relationships between genes, neurons, circuits and cognitive processing.

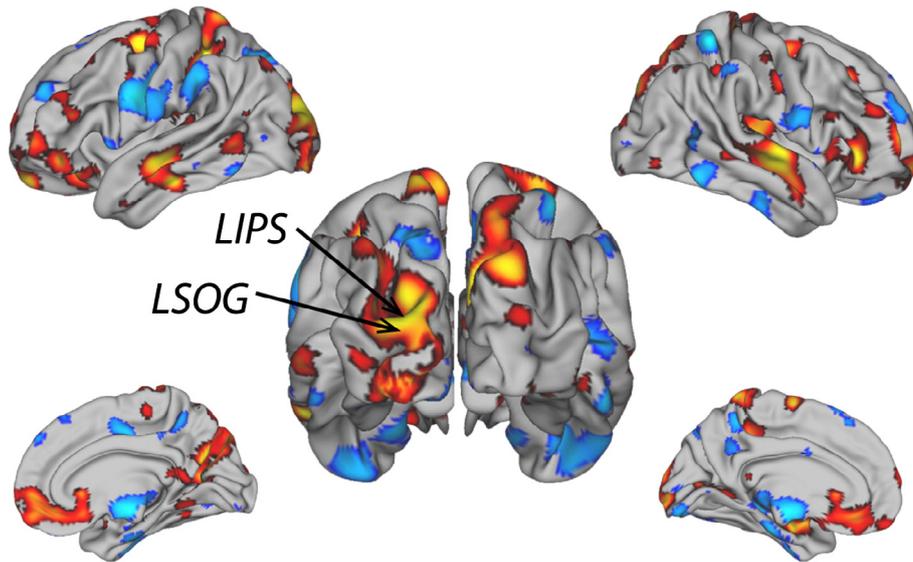
## 4. Methods

### 4.1. Participants

Structural MRI was acquired from healthy adult subjects taking part in the Brain Imaging Genetics (BIG) initiative in Nijmegen (Franke et al., 2010). Data from 1717 BIG subjects (1571 European Caucasian) were available for the present study (mean age 24.3 with a standard deviation of 6.50). The subjects were either scanned at a 1.5 Tesla (878 subjects) or a 3 Tesla scanner (839 subjects). Subjects had no self-reported neurological or psychiatric history and mainly a high level of education (bachelor student level or higher). The study was approved by the local ethics committee (CMO Region Arnhem-Nijmegen, The Netherlands).

### 4.2. Data analysis and statistical inference

The structural MRI data acquisition and VBM processing pipeline have been described elsewhere (Hoogman et al., 2014). A sub-



**Fig. 2.** Whole-brain spm T-map thresholded at  $p_{\text{uncorr}} < 0.1$  for the purpose of assessing spatial specificity (and to aid future meta-analysis). Larger grey matter volume for AA compared to AT/TT genotype groups is shown in orange-red, larger grey matter volume for AT/TT compared to AA is shown in light blue-dark blue. The upper row shows the left and right lateral surface. The middle picture shows the posterior view of the left and right lateral surface, indicating the only area with significant results (at a FWE and Bonferroni corrected level of  $p < 0.05$ ): the left superior occipital gyrus (LSOG), extending into the left intraparietal sulcus (LIPS). The lower row shows the left and right medial surface.

set of the participants were genotyped by taking data from genome-wide SNP-chips, as described in Hoogman et al. (2014) and a subset were directly typed, as described in (Kos et al., 2012). Statistical analysis was performed using a GLM approach in SPM8. Full-factorial ANCOVAs were applied using genotype as a factor. The age, sex, total brain volume, and scanner field strength of each participant were added to the models as covariates. When investigating regionally specific differences in tissue composition across e.g. genetic groups, global brain volume should be corrected for, to remove any variance which can be accounted for by global differences in tissue composition across those groups (Mechelli, Price, Friston, & Ashburner, 2005). Tan et al. (2010) included a global volume measure as a covariate in the statistical model. We used an increasingly common method (endorsed e.g. by the VBM8 and FSL-VBM packages) to adjust for global brain volume. This method separates the linear vs non-linear part of the transformations estimated during normalization and only applies the non-linear part. These methods are however considered to produce highly similar, if not equivalent (Malone et al., 2015) results.

T-tests were performed assessing the difference between AA, AT and TT-carriers, by grouping them in stages, as described in the result section. Statistical inference was based on the cluster level and voxel level statistics ( $p_{\text{FWE-corr}} < 0.05$ ) as implemented in SPM8 and the VBM5 toolbox. To form clusters, voxels were thresholded at  $p < 0.001$  (uncorrected). The cluster forming threshold and the final multiple comparison correction method (and threshold) used where the same in the present and the original study. When replicating the associations from Tan et al. (2010), we performed a small volume correction analysis within 10 mm spherical region of interests (ROIs) around the nine peaks reported in Tan et al. (2010). Significance of the cluster level statistics was corrected for multiple comparisons using random field theory (Worsley et al., 1996) and corrected for non-stationarity using the VBM5 toolbox (Hayasaka, Phan, Liberzon, Worsley, & Nichols, 2004).

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