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Genetic dyslexia risk variant is related to neural connectivity patterns underlying phonological awareness in children

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ABSTRACT

Phonological awareness is the best-validated predictor of reading and spelling skill and therefore highly relevant for developmental dyslexia. Prior imaging genetics studies link several dyslexia risk genes to either brain-functional or brain-structural factors of phonological deficits. However, coherent evidence for genetic associations with both functional and structural neural phenotypes underlying variation in phonological awareness has not yet been provided. Here we demonstrate that rs11100040, a reported modifier of SLC2A3, is related to the functional connectivity of left fronto-temporal phonological processing areas at resting state in a sample of 9- to 12-year-old children. Furthermore, we provide evidence that rs11100040 is related to the fractional anisotropy of the arcuate fasciculus, which forms the structural connection between these areas. This structural connectivity phenotype is associated with phonological awareness, which is in turn associated with the individual retrospective risk scores in an early dyslexia screening as well as to spelling. These results suggest a link between a dyslexia risk genotype and a functional as well as a structural neural phenotype, which is associated with a phonological awareness phenotype. The present study goes beyond previous work by integrating genetic, brain-functional and brain-structural aspects of phonological awareness within a single approach. These combined findings might be another step towards a multimodal biomarker for developmental dyslexia.

Introduction

Phonological awareness is the central precursor skill of reading and spelling and thus, the best predictor of the word recognition difficulties that characterize developmental dyslexia, one of the most common learning disorders (Gabrieli, 2009; Peterson and Pennington, 2012).

It is assumed that phonological deficits strongly depend on genetic factors, but a link between the best validated dyslexia risk genes, *KIAA0319*, *DCDC2* and *DYX1C1*, and phonological awareness, which takes intermediate neural phenotypes into account has not been established yet (Galaburda et al., 2006; Giraud and Ramus, 2013). In a recent genome-wide screening, however, two variants on chromosome 4 affecting expression levels of *SLC2A3* were found to be specifically associated with a late left-lateralized auditory mismatch negativity (MMN) component peaking around 300 to 600 ms (Roeske et al., 2011) that is considered a specific functional neural marker of phonological processing deficits (Korpilahti et al., 1995; Stoodley et al., 2006).

The available neuroimaging literature provides converging evidence that difficulties in phonological processing are characterized by a reduced hemodynamic reactivity and functional connectivity of left superior temporal, inferior parietal and inferior frontal cortices (Boets et al., 2013; Koyama et al., 2011; Raschle et al., 2012). These functional differences are corroborated by structural findings in the same regions indicating an altered gray matter morphometry (Silani et al., 2005) and white matter fractional anisotropy (FA) (Klingberg et al., 2000). Furthermore, the left arcuate fasciculus, which forms the connection of these cortical areas, was also linked to phonological processing (Vandermosten et al., 2012a; Saygin et al., 2013; Myers et al., 2014).

The picture emerging from the literature is that variation in phonological processing skills is based on brain-structural and brain-functional factors, which in turn depend on genetic factors (Peterson and Pennington, 2012). However, although a few studies revealed relations between subsets of these factors (Darki et al., 2012, 2014; Pinel et al., 2012), no experiment so far has succeeded in integrating all explanatory levels within a single approach. To provide an integrative and comprehensive analysis

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of normal variation in phonological awareness, we genotyped 34 children aged 9 to 12 years for two single nucleotide polymorphisms (SNPs) acting on the gene *SLC2A3*, which is known to regulate neural glucose transport (Maher et al., 1994; Maher and Simpson, 1994; McCall et al., 1994). Furthermore, they underwent functional and structural MRI and were tested behaviorally for their phonological awareness as well as reading and spelling skills. Additionally, results of a dyslexia screening conducted when the participants were 5 to 6 years of age were available. The children were separately grouped either as carriers or as non-carriers of the two dyslexia risk variants rs11100040 and rs4234898 decreasing the expression of *SLC2A3* in individuals that have at least one risk (T) allele. These two particular SNPs were selected because they are currently the only variants that were specifically linked to phonological processing at the genome wide level (Roeske et al., 2011).

It is considered highly informative to explore the influence of genetic risk variants on a normal population, but not dyslexics since the phenotype of interest, i.e. phonological awareness, can be reliably related to dyslexia as previously shown (Saygin et al., 2013; Myers et al., 2014). An imaging genetics approach was taken in the present study because the effect size of genetic variants on intermediate neural phenotypes is known to be higher compared to behavioral phenotypes. This increases the power to detect statistically significant effects in small samples (Meyer-Lindenberg and Weinberger, 2006; Mier et al., 2010). As a first step, we investigated if the children's genetic risk profiles were related to their resting state functional connectivity profiles. Second, we tested the hypothesis that there was an association between the children's genetic risk profile and their structural connectivity profiles. Third, we hypothesized that the individual functional and structural connectivity indices were correlated with the individual performance in the phonological awareness test. Finally, we expected associations between phonological awareness, the retrospective dyslexia screening and the reading and spelling tests. The results section is organized relative to the order of these hypotheses.

Materials and methods

Participants

Data from 34 right-handed 9- to 12-year-old children, randomly selected from the cohort of the German Language Development Study (Friedrich and Friederici, 2004), were included in the final analyses (Table 1). Initially, the sample consisted of 36 children but datasets from two children were disregarded because they did not meet our MR data quality criterion (see below for details). All parents completed a questionnaire revealing that no participant had a history of neurologically relevant diseases. None of the children has been diagnosed with developmental dyslexia. Based on an informative briefing regarding study aims and methodology, parents were asked to give written informed consent while children gave documented verbal assent to participate in the study. All experimental procedures were approved by the University of Leipzig Ethical Review Board.

Genotyping

Genotyping and DNA extraction were carried out on saliva using standard procedures (Quinque et al., 2006) or using Oragene DNA Genotek saliva kits (Kanata, Ontario, Canada). The alternative protocol was performed according to the user-developed protocol of the DNeasy® Blood & Tissue Kit (Purification of total DNA from animal saliva using the DNeasy® Blood & Tissue Kit). The following changes were applied: Centrifugation in steps 6, 7 and 8 was executed with 15,700 times gravity. The final elution was completed with 100 µl AE buffer. At least 0.75 ml of saliva was collected per subject. Two SNPs, rs11100040 (T) and rs4234898 (T) (Roeske et al., 2011) (risk alleles in parentheses) were genotyped by the mass spectrometry based technique GenoSNIP (Bruker Daltonics, Bremen, Germany) as described elsewhere (Kirsten et al., 2007) with minor modifications. PCR primers (MWG-Biotech AG, Ebersberg, Germany) were:

5′-ACGTTGGATGAACAGTAAGGAAAATGACAGT-3′
and 5′-ACGTTGGATGGATGAAACACAGTTGTTTACA-3′
as well as 5′-ACGTTGGATGTGGATCCTACACCTACACA-3′
and 5′-ACGTTGGATGGTTTTCAGATTCTGCCAT-3′, respectively.
The sequence of the single base extension primer (Biotez, Berlin, Germany) was
5′-bioAACGTTTACATTTLATCACACTTTCTTA-3′
and 5′-bioTGTGTTCLCTGGCCTCTGGA-3′,
respectively, where “bio” is a biotin residue and L is a photo cleavable linker (Wenzel et al., 2003). Additionally, the variant rs11100040 was verified by genotyping using iPlex (Sequenom, Hamburg, Germany). No inconsistencies were found. Both SNPs had a call rate of 100% and did not violate Hardy–Weinberg equilibrium among all 34 individuals. Genetic risk was assigned according to the number of risk

Table 1
Demographic information, genotyping results and psychometric assessment results.

	Measure	N	Mean	Range
Demographic information	Age		10 years 05 months	09 years 00 months to 12 years 02 months
	Gender	20 male, 14 female		
Genotyping results	rs11100040	17 non-risk, 17 risk		
	rs4234898	26 non-risk, 8 risk		
Psychometric assessment results	IQ		111.22	86–126
	Speech therapy	23 not treated, 11 treated		
	Musical instrument instruction	18 not instructed, 16 instructed		
	Attention deficit disorder	29 without suspicion, 5 diagnosed		
	Phonological awareness test	22 non-risk, 12 risk[a]	49.47[c]	19–71[c]
	Reading test* (acquired at ages 11 to 14)	23 non-risk, 11 risk[a]	50.8[c,d]	27–79[c,d]
			48.15[c,e]	38–80[c,e]
	Spelling test	25 non-risk, 9 risk[a]	47.28[f]	3–99[f]
	Preschool dyslexia screening (acquired at ages 5 to 6)	23 non-risk, 11 risk[b]	2.59[g]	0–6[g]

[a] 25th percentile rank.
[b] 15th percentile rank.
[*] Available for 24/34 children.
[c] *T* values.
[d] Accuracy.
[e] Speed (number of words read in a time interval of 4 min).
[f] Percentile ranks.
[g] Risk score (1 risk point assigned when performance in a subtest below 15th percentile rank; 10 subtests; 4 or more risk points indicate at risk status).

alleles per SNP with '0' for 0 risk alleles and '1' for 1 or 2 risk alleles (Lewis, 2002) (Table 1).

MR data acquisition

MRI was conducted on a 3.0-T Siemens TIM Trio (Siemens AG) whole-body magnetic resonance scanner using a 12-radiofrequency-channel head coil.

For anatomical localization, T1-weighted three-dimensional magnetization-prepared rapid-acquisition gradient echo (MP2RAGE) pulse sequences with TR = 5.000 ms, TE = 2.82 ms, TI_1 = 700 ms, TI_2 = 2.500 ms, FOV = 256 × 240, matrix size = 250 × 219 × 144 and voxel size = 1.3 × 1.3 × 1.3 mm^3 were acquired.

For resting state fMRI, a T2*-weighted gradient-echo echo-planar imaging (EPI) sequence comprising 100 volumes was applied to the participants (closed eyes, no active stimulation) using 28 slices with TR = 2 s, TE = 30 ms, FOV = 192 mm, matrix size = 64 × 64 voxels and voxel size 3.0 × 3.0 × 3.0 mm^3.

Diffusion-weighted MR images were collected as a twice-refocused spin EPI sequence (Reese et al., 2003) with TE = 83 ms, TR = 8000 ms, matrix size = 100 × 100 voxels, voxel size = 1.9 × 1.9 × 1.9 mm^3, 66 axial slices covering the whole brain. We used 60 isotropically distributed diffusion-encoding gradient directions with a b-value = 1000 s/mm^2. Seven anatomical reference b0 images without diffusion weighting were acquired at the beginning of the sequence and after each block of 10 diffusion-weighted images for off-line motion correction. Fat saturation was applied together with 6/8 partial Fourier imaging and generalized auto-calibrating partially parallel acquisitions (GRAPPA) with an acceleration factor of 2 (Griswold et al., 2002). Random noise was reduced by averaging two acquisitions. All images were visually checked for motion artifacts (signal losses). As mentioned above, 2 out of 36 participants were removed from the study because they exceeded the cut-off quality criterion of maximum 5 head-motion-corrupted image directions in the entire dataset (Brauer et al., 2013). The final sample therefore consisted of 34 participants.

Resting state fMRI data analysis

The functional resting state images were slice-time-corrected, realigned, motion-corrected, normalized to a group-specific template, and spatially smoothed with a 4 mm FWHM Gaussian kernel using the DPARSF 2.3 software package (http://www.restfmri.net/forum/DPARSF). Framewise displacement was less than 0.5 mm in all participants. To control for head motion and nuisance, realignment parameters, global signals, white matter signals and cerebrospinal fluid signals were entered as regressors into the first-level model. Time courses of hemodynamic gray matter signals within a low-frequency range of 0.01 to 0.1 Hz were extracted from three seed regions (r = 6 mm) in MNI space including the left inferior frontal gyrus (IFG) (−51, 10, 10), left posterior superior temporal gyrus (pSTG) (−53, −31, 9), and left temporo-parietal junction (TPJ) (−59, −45, 15) using DPARSF 2.3 (http://rfmri.org/DPARSF). These areas were chosen as regions of interest (ROIs) as they have been found to support phonological processes during word reading in previous meta-analyses of event-related fMRI studies and as they have been used in a previous resting-state fMRI study (Koyama et al., 2011). Finally, individual Pearson's correlation coefficients of the BOLD time courses (mean $r = 0.41$, $SD = 0.2$, range: −0.11 to 0.77) were computed and then entered separately in 3 one-way ANOVAs (for rs11100040, equal group sizes) and 3 Mann–Whitney U tests (for rs4234898, unequal group sizes) to compare all combinations of ROI pairs. These statistical tests were carried out using PASW 18 (http://www.spss.com.hk/statistics/).

All ROI-wise seed based correlation analyses were adjusted for the effects of age, gender, IQ, speech therapy, musical instrument instruction, and attention deficit disorder (ADD) entering these variables as covariates into the models. The rationale for controlling for speech therapy and musical instrument instruction in these analyses was to avoid possible confounds introduced by these two environmental factors with respect to their potential to induce compensatory mechanisms altering brain structure and function and performance (Goswami et al., 2011). Attention deficits, a comorbidity of developmental dyslexia, were covaried out to account for possible indirect effects of attention on phonological processing, reading and writing (Kibby et al., 2009).

All parametric and nonparametric tests resulting in a $P < 0.0083$ which equals a Bonferroni corrected $P < 0.05$ (divided by 6 for the three ROI pairs analyzed for each of the two SNPs) were considered significant. The Pearson correlation coefficients were normally distributed in each of the two rs11100040 risk groups (0 risk alleles vs. 1 or 2 risk alleles) and the three corresponding ROI pairs according to Kolmogorov–Smirnov and Shapiro–Wilk tests (no risk: IFG-pSTG: *P = 0.2/0.923; IFG-TPJ: P = 0.2/0.183; pSTG-TPJ: P = 0.23/0.272*; risk: *P = 0.2/0.768; IFG-TPJ: P = 0.2/0.314; pSTG-TPJ: P = 0.2/0.684*). For the rs4234898 risk groups it was not necessary to assess these distributions since the data were passed to non-parametric Mann–Whitney U tests. These statistical tests were carried out using PASW 18 (http://www.spss.com.hk/statistics/).

DTI data analysis

Motion correction parameters for the diffusion-weighted images were combined with a global rigid-body registration (Jenkinson et al., 2002) to the individual skull-stripped T1-weighted structural image using the FSL linear image registration tool (flirt, http://www.fmrib.ox.ac.uk/fsl). The gradient direction for each volume was corrected with the rotation parameters (Leemans and Jones, 2009). In the registration process, the images were interpolated to an isotropic voxel resolution of 1 mm before the FA was computed. Note that the registration to the T1 anatomical image with 1 mm isotropic resolution was preferred over an analysis in the diffusion space to reduce smoothing artifacts introduced by several interpolation steps included in standard procedures and to reduce the smoothing bias to the different directions by registration to an independent image.

All single-subject FA images were then mutually aligned on each other by nonlinear registration to determine the anatomically most typical template image and all individual FA images were registered to this target image. Subsequently, all FA images averaged and skeletonized using the FSL tract based spatial statistics (TBSS) (Smith et al., 2006) toolbox. The skeleton was masked with an FA threshold of 0.25 which is slightly higher than the commonly used default threshold (0.2). It represented the best trade-off between reducing as much cross-subject variability as possible by disregarding peripheral branches of the skeleton while at the same time keeping as much information as possible.

Finally, the mean FA skeletons entered a voxel-wise one-way analysis of variance (ANOVA) separately for each SNP including age, gender, IQ, speech therapy, musical instrument instruction, and attention deficit disorder (ADD) as covariates for the reasons provided in the Resting State fMRI Data Analysis section. Cross-subject variance was estimated separately for each genetic risk group to account for unbalanced distributions of risk alleles across the sample potentially resulting in unequal variances. These analyses were carried out using FSL FEAT (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT).

We corrected for multiple comparisons within the entire FA skeleton on a cluster level based on the Gaussian random field theory (GRFT). In order to meet the assumptions of the GRFT with respect to the two-dimensionality of the white matter skeleton we used a threshold of $P < 0.01$ which delivers an optimal approximation to a quadratic representation of the cluster (Hagler et al., 2006). The minimum number of contiguous voxels showing a statistically significant difference at $P < 0.01$ was simulated by a Monte Carlo simulation taking into account the voxel resolution of 1 × 1 × 1 mm^3 and the intrinsic smoothness of

the data which was x = 1.37 mm, y = 1.78 mm and z = 1.59 mm. Clusters were considered significant if they exceeded a threshold of k = 17 controlling for the chance of ever reporting a false-positive finding to be less than 0.0025 which equals a Bonferroni corrected $P < 0.005$ (divided by 2 for the two single nucleotide polymorphisms under investigation).

Individual FA values were extracted from the cluster revealed by the analysis described in the previous passage (k = 36, MNI coordinates: −34, −16, 34). According to the ICBM-DTI-81 white matter labels atlas (Mori et al., 2008), 76% of this cluster was located in a subregion of the left superior longitudinal fasciculus which we visually identified as the arcuate fasciculus based on previous literature (Makris et al., 2005).

Psychometric assessment

We used the BAKO test (test for basal competences for reading and spelling abilities) to assess the children's phonological awareness (Stock et al., 2003). The BAKO comprises valid measures for phonological processing skills both at the phoneme level (phoneme categorization, phoneme deletion, phoneme permutation, vowel length assignment, and vowel replacement) and the word level (word inversion and pseudoword segmentation). Additionally, we tested the children's spelling skills using running text-dictations from the DERET (German spelling test) (Stock and Schneider, 2008). Retrospective data of an early dyslexia screening (BISC—Bielefeld screening for early recognition of reading and spelling deficits) (Jansen et al., 1999) acquired 10 months before school enrollment (5 to 6 years of age) were available for all participants. In an additional assessment 2 years later (age of the children: 11 to 14 years), reading performance was assessed using a reading accuracy and speed test (Schneider et al., 2007). *T* scores were used for further statistical analyses with the exception of the spelling test, for which only percentile ranks were available, and the dyslexia screening, for which only risk scores were available. Note that higher scores in the dyslexia screening indicate worse performance.

IQ scores were determined using the German version of the Kaufman Assessment Battery for Children (K-ABC) (Melchers and Preuss, 2009). Missing IQ values for seven children (four children at risk, three non-risk children) were imputed using multiple imputation as implemented in PASW 18 (http://www.spss.com.hk/statistics/). Based on a parental questionnaire, we assessed if the children had undergone speech therapy, if they had learned how to play a musical instrument, and if they had a medically diagnosed ADD (Table 2).

In order to relate the individual behavioral performance measures (see Table 1, Psychometric assessment results) to each other and to the brain-structural and brain-functional measures, respectively, we computed non-parametric partial Spearman's rho correlations removing the effects of age, gender, IQ, speech therapy, musical instrument instruction, and ADD for the reasons provided in the Resting State fMRI Data Analysis section. All *P* values for the direct and indirect correlation analyses between FA, the functional connectivity indices and phonological awareness were Bonferroni corrected for the four statistical tests conducted. Correlation coefficients were directly compared running Meng's *z* test (Meng et al., 1992).

Results

rs11100040 is associated with fronto-temporal functional connectivity at resting state

We compared pair-wise temporal correlations of low-frequency BOLD signal fluctuations in three ROIs, the left inferior frontal gyrus (IFG), the left posterior superior temporal gyrus (pSTG), and the left temporo-parietal junction (TPJ), which are known to support phonological processes during word reading (Koyama et al., 2011).

Children without any risk allele at rs11100040 ($n = 17$) showed significantly stronger temporal correlations of the BOLD signals induced at rest in the left IFG and left pSTG than children carrying at least one risk allele ($n = 17$) ($F_{1, 33} = 2.81$, $P < 0.05$, Bonferroni corrected) (Fig. 1). However, no significant effects could be detected between the left IFG and the left TPJ ($F_{1, 33} = 0.9$, $P = 0.524$) or the left pSTG and the left TPJ ($F_{1, 33} = 0.69$, $P = 0.682$). Children with a risk allele at rs4234898 ($n = 8$) did not differ significantly from children carrying at least one risk allele ($n = 26$) in all three pairs of ROIs (IFG-pSTG: $U = 67$, $P = 0.133$; IFG-TPJ: $U = 91$, $P = 0.618$; pSTG-TPJ: $U = 103$, $P = 0.968$).

rs11100040 is associated with the fractional anisotropy of the arcuate fasciculus

Given that rs11100040 was related to the functional resting state connectivity of fronto-temporal cortices involved in phonological processing, we hypothesized that the observed brain functional effect should be reflected in the fractional anisotropy of their structural white matter fiber connection via the arcuate fasciculus.

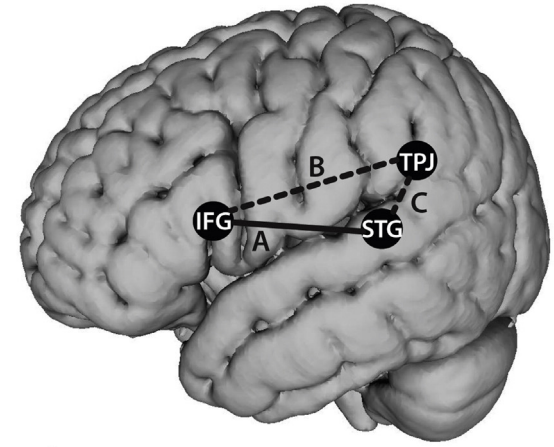
Tract-based spatial statistics revealed that children who carried at least one risk allele at the *SLC2A3* modifier rs11100040 had significantly reduced FA values in a cluster located in the left arcuate fasciculus

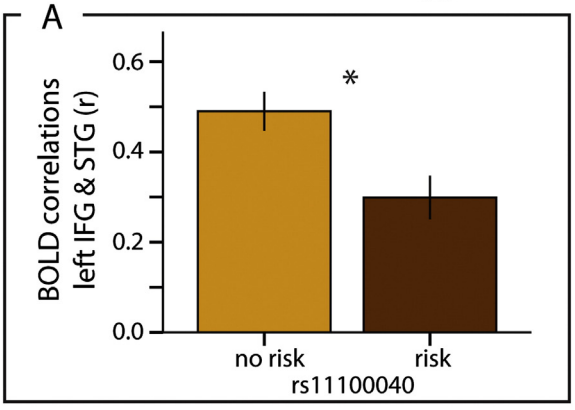
Table 2
Effects of potential confounders on analyzed variables.

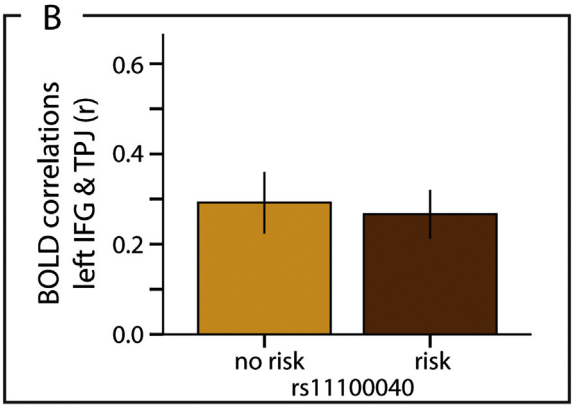
Variable	Potential confounder	*P*	Effect size (SE)	R^2
FA values of the arcuate fasciculus	Age	0.549	0.105 (0.173)	0.011
	Gender	0.964	0.008 (0.174)	0.000
	IQ	0.221	0.249 (0.198)	0.057
	Speech therapy	0.163▪	0.241 (0.169)	0.058
	ADD	0.579	−0.097 (0.173)	0.009
	Musical instrument	0.415	0.142 (0.172)	0.020
Temporal correlation coefficients left IFG and pSTG	Age	0.21	−0.217 (0.17)	0.047
	Gender	0.263	−0.195 (0.171)	0.038
	IQ	0.955	0.01 (0.178)	0.000
	Speech therapy	0.029*	0.369 (0.162)	0.136
	ADD	0.041*	0.347 (0.163)	0.120
	Musical instrument	0.993	−0.002 (0.174)	0.000
Phonological awareness test score	Age	0.006**	0.458 (0.155)	0.210
	Gender	0.865	−0.03 (0.174)	0.001
	IQ	0.055▪	0.383 (0.19)	0.135
	Speech therapy	0.032*	−0.364 (0.162)	0.132
	ADD	0.042*	−0.345 (0.163)	0.119
	Musical instrument	0.651	0.079 (0.174)	0.006

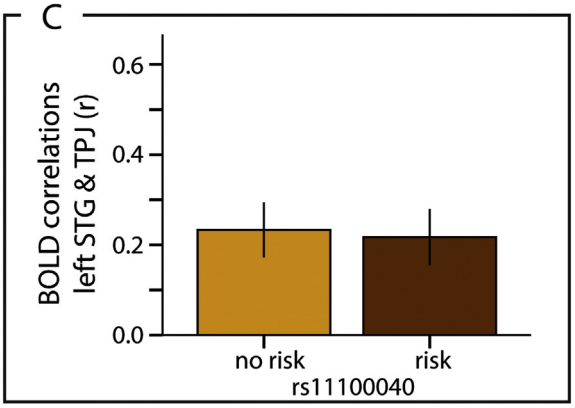
Effect size (SE) = effect size beta in a linear regression model; SE = standard error of effect size; R^2 = variance of the variable explained by the potential confounder.
**$P < 0.01$; *$P < 0.05$; ▪$P < 0.2$.

compared to non-carrier children ($k = 36$, MNI coordinates: −34, −16, 34, $P < 0.01$, cluster size Bonferroni corrected to $P < 0.01$) (Fig. 2). We did not find any effects on the white matter skeletons for the other SNP. The reported cluster was the only one withstanding multiple comparison correction at $P < 0.01$ and $k > 17$. The individual FA values were correlated with the individual functional connectivity indices (partial $r_s = 0.6$, $P < 0.005$).









Relations between the neural phenotypes and phonological awareness

The individual FA values of the cluster detected in the arcuate fasciculus were related to the performance of the children in the phonological awareness tasks (partial $r_s = 0.44$, $P < 0.05$, Bonferroni corrected) when controlling for the influence of age, gender, IQ, and of external factors including speech therapy, musical instrument instruction, and ADD (Fig. 3 and Table 2). The strength of this correlation decreased after inclusion of the functional connectivity indices as a covariate into this model (partial $r_s = 0.31$, $P = 0.181$). This indicates that the correlation between FA and phonological awareness was partly explained by functional connectivity. Phonological awareness was not significantly associated with the resting state functional connectivity indices (partial $r_s = 0.09$, $P = 0.6537$).

Relations between phonological awareness and other dyslexia-relevant behavioral phenotypes

Given that phonological awareness was related to the FA of the arcuate fasciculus which in turn was related to rs11100040 we finally aimed to confirm that phonological awareness was also related to other dyslexia-relevant behavioral measures. The individual phonological awareness scores at ages 9 to 12 were predicted retrospectively by the individual risk scores in an early screening for DD (BISC) at ages 5 to 6 (partial $r_s = -0.42$, $p = 0.049$) when controlling for the influence of age, gender, IQ, and of external factors including speech therapy, musical instrument instruction, and ADD. Additionally, the individual phonological awareness at ages 9 to 12 was associated with the individual spelling performance at the same age (partial $r_s = -0.38$, $p = 0.03$). However, it did neither predict the individual reading accuracy (partial $r_s = 0.47$, $p = 0.15$) nor reading speed (partial $r_s = 0.41$, $p = 0.218$) at ages 11 to 14.

Discussion

Numerous task-based fMRI and PET studies have previously identified altered functional responses in left inferior frontal and temporo-parietal cortices as playing a central role for the phonological deficit of dyslexic individuals (Hoeft et al., 2007; McCrory et al., 2005; Paulesu et al., 2001; Raschle et al., 2012). Rather than providing a replication of these results, we followed the hypothesis that a differential hemodynamic interplay between these cortices can already be detected at resting state in the default language network in the absence of any linguistic stimulation (Lohmann et al., 2010). Our main finding was that the temporal correlations of low frequency BOLD signal fluctuations between inferior frontal and superior temporal cortices were significantly reduced in children carrying risk alleles at rs11100040. As this gene variant was found to be associated with phonological deficits in a genome-wide association study (Roeske et al., 2011), our finding extends recent studies linking

Fig. 1. Group differences between pair-wise temporal correlations of low frequency BOLD signals at resting state in three seed regions (left inferior frontal gyrus (IFG): −51, 10, 10, left posterior superior temporal gyrus (pSTG): −53, −31, 9, and left temporo-parietal junction (TPJ): −59, −45, 15) known to support phonological processing. (A) Children without any risk allele at the *SLC2A3* modifier rs11100040 showed significantly higher correlations between the left IFG and the left pSTG compared to children with a genetic risk (solid line) ($F_{1, 33} = 2.81$, $P < 0.05$, Bonferroni corrected). (B and C) The groups did not differ significantly from each other in the two other seed pairs (dashed lines) (left IFG and left TPJ: $F_{1, 33} = 0.9$, $P = 0.524$; left pSTG and left TPJ: $F_{1, 33} = 0.69$, $P = 0.682$). Children without a risk allele at rs4234898 did not differ significantly from children carrying risk alleles in all three pairs of ROIs.

the functional resting state connectivity of these regions to reading phenotypes in children and adults (Koyama et al., 2010, 2011). We introduced rs11100040 as a genetic variant potentially playing a role for the development of fronto-temporal functional connectivity profiles. This observation is supported by a considerable body of literature providing evidence that *SLC2A3* is a glucose transporter strongly expressed in neurons of the cortical gray matter (Maher et al., 1994; Maher and Simpson, 1994; McCall et al., 1994).

In order to link the brain-functional differences to brain-structural differences, we analyzed the relation between rs11100040 and FA following a whole brain white matter approach in order not to limit our analyses a priori only to a subset of the various candidate tracts discussed in the literature (Vandermosten et al., 2012b). Using this approach we made the observation that rs11100040 is associated with the FA of the arcuate fasciculus, a long-distance white matter fiber tract essential for language processing (Friederici, 2011). Since this result is strongly supported by recent studies reporting a link between the arcuate fasciculus and phonological processing (Vandermosten et al., 2012a; Boets et al., 2013; Saygin et al., 2013; Myers et al., 2014), our study extends these findings by suggesting a possible contribution of rs11100040 to the development of this fiber tract. Currently, the actual neurobiological foundation of the FA is a matter of ongoing scientific debate. On the one hand, FA provides several microstructural characteristics of the white matter related to its maturity including the overall number of fibers within a tract, their degree of myelination (Friederici, 2012), their axonal caliber (Paus, 2010), and the amount of surrounding glia cells (Wandell and Yeatman, 2013). On the other hand, FA is also influenced by the orientation of fibers and by fiber crossings. It is likely that both microstructure and coherence of the fibers have an influence on the observed FA differences. Future studies will have to clarify this issue.

The present study goes beyond the results of previous studies by detecting a strong correlation between fronto-temporal functional connectivity and arcuate fasciculus FA suggesting a tight explanatory relation between both neural markers. Still, given that a recent study did not find a correlation between an effective, i.e. task-based, functional connectivity measure and the FA of the arcuate fasciculus (Boets et al., 2013), further research is needed to corroborate the link between functional and structural connectivity indices of the phonological processing network.

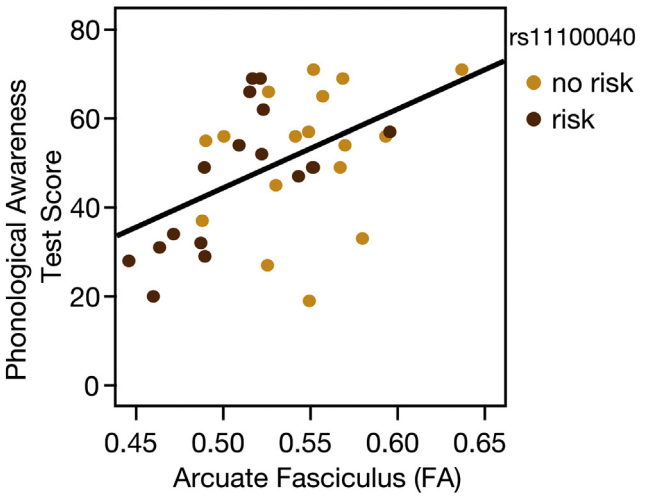


Fig. 3. FA in the left arcuate fasciculus was correlated with phonological processing performance. Individual FA values in a central sub-portion of the left arcuate fasciculus were significantly associated with the individual phonological awareness scores (partial $r_s = 0.44$, $P < 0.05$, Bonferroni corrected).

We were able to link brain structural variance to behavior by showing that the FA of the left arcuate fasciculus was associated with phonological awareness. This extends results from previous studies demonstrating that FA is a sensitive neuroanatomical correlate of phonological processing abilities in the arcuate fasciculus (Vandermosten et al., 2012a; Saygin et al., 2013; Myers et al., 2014). Individual phonological awareness, however, and individual functional connectivity were not significantly related when directly correlated. This observation is in line with data from a recent resting state fMRI study on dyslexia, in which brain–behavior correlations were only found in fronto-parietal attention areas but not

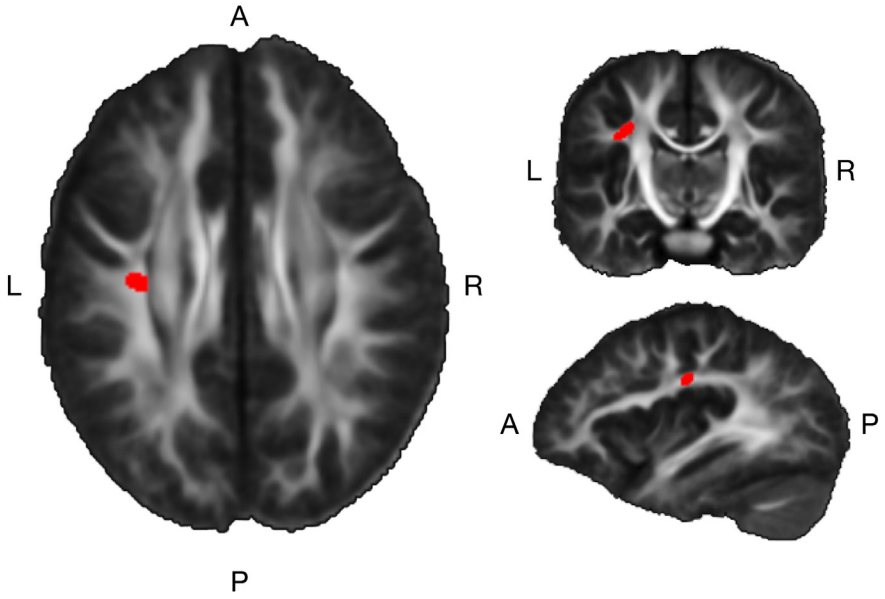


Fig. 2. Fractional anisotropy (FA) differences between children either with or without risk alleles at rs11100040. Children who did not carry any risk allele had significantly higher FA values in a cluster located in the left arcuate fasciculus ($k = 36$, MNI coordinates: −34, −16, 34, $P < 0.01$, cluster size Bonferroni corrected to $P < 0.01$). Depicted are axial, coronal, and sagittal views of the cluster within the group average white matter skeleton (A: anterior, P: posterior, L: left, R: right). No significant FA difference was observed between risk carriers and non-risk carriers at rs4234898.

in the temporo-parietal cortices selectively supporting phonological processing (Koyama et al., 2013). Thus, it remains to be demonstrated whether resting state functional connectivity can function as a direct measure for the individual phonological awareness, or whether brain structural measures are the more sensitive measure.

Phonological awareness at ages 9 to 12 was predicted significantly by retrospective behavioral risk scores in an early dyslexia screening administered at ages 5 to 6 and it was also significantly related to spelling performance measured at the same age. These findings corroborate numerous studies indicating that phonological awareness is a central correlate of literacy skills across development (Ziegler et al., 2010; Peterson and Pennington, 2012). The observation that phonological awareness was not significantly associated with reading performance might be specific to German as a language with an orthography that is relatively transparent with respect to its high grapheme–phoneme correspondence (Moll et al., 2014). Due to this high correspondence, reading acquisition is less dependent on phonological skills in German compared to other languages. Several previous investigations suggest that the impact of phonological awareness on reading is stronger in less transparent orthographies such as English or French (Ziegler et al., 2010; Peterson and Pennington, 2012; Moll et al., 2014).

We did not detect a significant difference between the genetic risk groups with respect to phonological awareness as determined by a one-way ANOVA adjusted for the confounders ($F_{1, 33} = 0.987$, $P = 0.33$). The most likely reason for this effect might be that the group difference did not reach significance due to the small effect size of the SNP on behavioral data. Therefore, this study focused on brain functional and brain structural endophenotypes where genetic effects can be much higher as we see in the present study. Our results are similar to those of other genetic imaging studies, where no correlation could be reported with the behavioral phenotype but a significant association was found with the brain-functional or the brain-morphological endophenotype (e.g. Mier et al., 2010; Darki et al., 2012).

The validity of our findings is bolstered by additional facts. First, it is known that effect sizes of a genetic variant in functional neuroimaging data are higher compared to other brain-functional measures like EEG (Mier et al., 2010). Second, it has been shown that FA variance in the arcuate fasciculus has one of the highest heritability rates of all white matter structures (Jahanshad et al., 2013), thereby strongly suggesting effects of genetic modulators. Third, all analyses were controlled for possible compensatory mechanisms induced by speech therapy and musical instrument instruction (Goswami et al., 2011) as well as for attention deficits as a relevant comorbidity (Kibby et al., 2009). The latter may potentially interact with the fronto-parietal phonological processing system via the fronto-parietal attention system located in the immediate vicinity (Koyama et al., 2013).

In principle, we cannot exclude a minor contribution of the other SNP analyzed in our experiment (rs4234898) to the analyzed structural and functional measures, particularly since Roeske et al. (2011) reported an association of this SNP with the auditory MMN by itself. We tested for effects of SNP rs4234898 on both neural measures but did not obtain any significant results. Therefore, we assume that if there was an effect of rs4234898 it should be considerably smaller than the effect of rs11100040. In this work, we did not recruit a replication sample to reproduce the observed effects. Accordingly, follow-up studies are necessary to disentangle the specific contributions of rs11100040, rs4234898 and the haplotype including both SNPs, to both electrophysiological and neuroimaging measures.

The challenge for future imaging genetics studies on phonological awareness will be to collect data from larger samples in order to validate all variables internally and to detect further direct linkage between the individual genome, neural markers, and the core behavioral phenotypes characterizing developmental dyslexia. This will not only augment our understanding of developmental neural plasticity, but may also allow a more reliable and earlier diagnosis of phonological deficits, potentially paving the way for a more effective treatment.

Conclusions

The present study integrates genetic, brain-functional, brain-structural and behavioral measures suggesting a complex association between a dyslexia risk genotype and two closely linked neural phenotypes, the fractional anisotropy of the left arcuate fasciculus, and the functional resting state connectivity of its termination areas in the inferior frontal and posterior superior temporal cortex. The fractional anisotropy of the left arcuate fasciculus is related to a phonological awareness phenotype in 9- to 12-year-old children which in turn is related to prereading dyslexia risk scores as well as to spelling skills. These findings call for a need to combine biomarkers from genetic and neural domains to optimize potential diagnostic tools for developmental dyslexia. Future work will have to show if such a multimodal neurogenetic biomarker can be applied to predict the risk to be affected by dyslexia before school entry so that existing preschool intervention tools can be used more efficiently.

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