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Imaging genetics of *FOXP2* in dyslexia

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Running title:

FOXP2 as a potential candidate gene for dyslexia

Imaging genetics of *FOXP2* in dyslexia

Abstract

Dyslexia is a developmental disorder characterised by extensive difficulties in the acquisition of reading or spelling. Genetic influence is estimated at 50-70%. However, the link between genetic variants and phenotypic deficits is largely unknown.

Our aim was to investigate a role of genetic variants of *FOXP2*, a prominent speech and language gene, in dyslexia using imaging genetics. This technique combines functional magnetic resonance imaging (fMRI) and genetics to investigate relevance of genetic variants on brain activation. To our knowledge, this represents the first usage of fMRI based imaging genetics in dyslexia.

In an initial case/control study (n=245) for prioritisation of *FOXP2* polymorphisms for later use in imaging genetics, nine SNPs were selected. A non-synonymously coding mutation involved in verbal dyspraxia was also investigated.

SNP rs12533005 showed nominally significant association with dyslexia (genotype GG odds ratio recessive model=2.1 [95% confidence interval 1.1-3.9], p=0.016). A correlated SNP was associated with altered expression of *FOXP2 in vivo* in human hippocampal tissue. Therefore, influence of the rs12533005-G risk variant on brain activity was studied. fMRI revealed a significant main effect for the factor “genetic risk” in a temporo-parietal area involved in phonological processing as well as a significant interaction effect between the factors “disorder” and “genetic risk” in activation of inferior frontal brain areas. Hence, our data may hint at a role of *FOXP2* genetic variants in dyslexia specific brain activation and demonstrate use of imaging genetics in dyslexia research.

Keywords: *dyslexia, imaging genetics, FOXP2, fMRI, genetics*

Introduction

The feasibility to combine functional imaging studies and genetics was demonstrated in several studies¹. Imaging genetics approaches investigate the role of certain genetic variants (e.g. single nucleotide polymorphisms - SNPs) on brain activation independently of indirect measurements like behavioural or neuropsychological testing. Promising results were already shown for other neurological disorders, e.g. in studies of anxiety², attentional processes³ or schizophrenia⁴. However, to our knowledge, no functional imaging genetics study has been published on dyslexia so far.

Dyslexia is a specific and severe disorder of reading and spelling, with approximately 5% affected schoolchildren in Germany⁵. About 50-70% of dyslexia can be explained by genetic influence⁶. However, as all so far identified disease genes account only for a small part of genetic risk, more dyslexia related genes need to be identified. *FOXP2* is a highly relevant candidate gene, situated on chromosome 7q31, close to 7q32, a genomic region in linkage with dyslexia⁷. *FOXP2* was discovered to be a central gene in language development⁸⁻¹⁰, not limited to humans¹¹⁻¹⁴. Its functional mechanism could be connected with a downregulation of *CNTNAP2*, a gene implicated in neuronal recognition and cell adhesion¹⁵. *FOXP2*'s role in language originally became apparent by the discovery of a missense mutation (R553H) leading to developmental verbal dyspraxia¹⁶. However, a mutation screen of *FOXP2* in six affected individuals⁷ found no specific mutations in dyslexia.

Several functional magnetic resonance imaging (fMRI) studies revealed specific differences between dyslexics and normal readers in reading related brain regions. Consistently, three left hemispheric areas (inferior frontal¹⁷, partly involving Broca's area, dorsal, and ventral temporal cortices, including Wernicke's area) showed significant activation differences in dyslexics in phonological tasks^{18, 19}. The dorsal and

ventral areas show, independently from language²⁰, less activation in dyslexics compared to normal readers. In older dyslexics, the inferior frontal area is often overactivated. However, this overactivation could also be interpreted as a compensational reading strategy¹⁹.

The functional effect of a FOXP2 mutation on inferior frontal and supramarginal brain areas has been demonstrated by Liégeois et al. (2003) for silent and spoken language processing¹⁰. Therefore our aim was to analyse the role of genetic variants of *FOXP2* in the processing of written language in dyslexia. Thus we combined genetics and fMRI using an imaging genetics approach. To select a possibly functionally relevant *FOXP2* variant, we initially applied a case/control (n=245) study. Relevance of the most promising variant was investigated in respect to potential functional consequences on brain activation in a reading related task in fMRI.

Materials and methods

Ethical approval

Our study was approved by the ethics committees of the Universities of Leipzig and Jena as well as the Saxon Ministry of Culture and Sports. Informed and written consent was obtained from subjects' parents.

Study group for genetic epidemiology

This group consisted of 61 dyslexics of German origin and 184 healthy, ethnically matched blood donors as controls. Dyslexics were recruited in the 3rd or 4th grade of special Saxonian dyslexia classes. Age was between 8.8 and 11.4 years, 63% were male. Cases were ascertained in a two-stage approach: First, schools with special dyslexia classes were contacted. Children in those classes were already tested thoroughly at the end of 2nd grade by the local school board with a variety of psychometric tests,

including non-verbal intelligence, letter knowledge, phoneme mergence, spelling, memory, mathematical skills as well as reading and listening comprehension. Pursuant to the admission criteria for the special dyslexia classes only children without memory or math problems and a discrepancy between IQ and reading performance of at least 1.25 standard deviations get access to those classes.

Second, additional tests²¹⁻²³ described elsewhere in detail²⁴ were applied to assess reading performance, minimise inclusion of children with attention deficit/hyperactivity (ADD/ADHD) and ensure an IQ \geq 85. No siblings or twins were included.

SNP selection

We identified tagging SNPs covering common variants with a minor allele frequency (MAF) \geq 0.1 (HapMap data release 24, Haploview-Version 4.0 beta 13 and HapMap Public Release #21a,) applying aggressive tagging, an r^2 threshold of 0.8, and a logarithm of the odds (LOD) threshold for multi-marker tests of 3.0. Nine SNPs were selected: rs12533005, rs10228350, rs10268637, rs4727799, rs17137124, rs7782412, rs12670585, rs936146, and rs10953766. They covered 87% of all common HapMap SNPs located in *FOXP2* with a mean r^2 of 0.97. Furthermore, we genotyped the mutation R553H, previously found in a large Pakistani family with severe speech and language disorder⁸.

Genotyping and genetic analysis

DNA extraction and PCR primer design were done as described²⁴. Primers for single base extension (SBE) including photo-cleavable sites²⁵ were designed using CalcDalton²⁶. SNPs were genotyped applying the method “GenoSNIP” as described previously^{25,27}. Primer sequences are shown in Supplemental table I.

Genetic statistics

We analysed SNPs for association with dyslexia applying χ^2 statistics in allelic, dominant, and recessive models as well as applying the Cochran-Armitage test. SNPs of cases and of controls were in Hardy-Weinberg Equilibrium (HWE) ($p > 0.05$) indicating no major genotyping errors. Average genotyping rate was 98% for cases and controls. *Haploview 4.1* was used to test for haplotype association for haplotypes with a frequency $> 5\%$. P-values are shown without correction for multiple testing. The study was powered to detect a difference of at least 15% in the minor allele frequencies (MAF) of selected SNPs between cases and controls translating to a minimum detectable odds ratio (OR) of 1.85²⁸.

Prediction of altered gene expression in silico

MatInspector and Genomatix software suite (NCBI 37, EIDorado 12-2010), were used to investigate possible loss/gain of transcription factor (TF) binding sites as described elsewhere²⁹. Minimum core similarity (score of the highest conserved positions of a matrix match) was 1.00.

Analysis of differential allelic gene expression in human hippocampus

Biopsy samples (n=142) were obtained from patients with chronic pharmaco-resistant temporal lobe epilepsy. After quality control, 138 individuals were included in subsequent analyses (63 male, mean age 31.72 years, standard deviation SD=16.27, age range newborn to 64 years). Fresh frozen human hippocampal segments were prepared as tissue-slices under cryostat-conditions (Bonn tissue bank). Total DNA and RNA were isolated using the AllPrep DNA/RNA Micro Kit (Qiagen, Hilden, Germany). Quality of total RNA of all samples was checked for degradation (RNA integrity number RIN >7.9) via BioAnalyzer measurements (Agilent Technologies, Waldbronn,

Germany). 50 ng of total RNA were amplified (Illumina TotalPrep 96-RNA Amplification Kit, Ambion/Applied Biosystems, Darmstadt, Germany). Labelled cRNA was hybridised to Illumina human HT-12 Expression v3 BeadChips (Illumina, San Diego, USA). All expression profiles were extracted using GenomeStudio software (Illumina, San Diego, USA). For genome-wide SNP-genotyping 200 ng of DNA was hybridised to the Illumina Human660W-Quad v1 DNA Analysis BeadChip (Infinium® HD Assay Super manual, Illumina). Individuals were excluded if their position differed more than 6 standard deviations from the mean position on at least one of the first two axes of the multi-dimensional scaling analysis on the identical by state matrix of genotyped SNPs. Quality thresholds were: HWE p-value $\geq 1 \times 10^{-5}$, MAF $\geq 1\%$, individual callrate $\geq 98\%$, SNP callrate $\geq 98\%$ and a false discovery rate of 1% for autosomal heterozygosity. Average genotyping rate was 98%. The sequences of expression probes were re-aligned to UCSC version 18 (hg18) allowing only perfect matches. Probes containing either intrinsic polymorphisms or matching to multiple positions in the human genome were excluded from downstream analysis. Remaining probes were normalised using the vsn2 option implemented in the package “VSN” for R. For quantitative trait analysis linear regression of an additive allelic model was performed using the GenABEL package for R. Covariates included in the model were gender and age at sampling. To take population stratification into account, we also included the first five components resulting from multidimensional scaling analysis.

For the investigation of the SNP's potential influence on *FOXP2* expression, we had to use proxy SNPs since rs12533005 was not included on the Illumina Human660-W Quad array. We identified proxy SNPs by filtering variants correlated with rs12533005 with $r^2 > 0.5$ according to HapMap release 22 (International HapMap Project

<http://www.hapmap.org>), and an additionally effect on gene expression of *FOXP2*-probes with a nominal p-value ≤ 0.05 . Out of five resulting SNPs, we report data of the variant with strongest effect size (rs10249531).

Study group for fMRI investigation

In an additional, independent sample we investigated children with fMRI. The sample consisted of 19 dyslexics (12 male, mean age 11.46 years, standard deviation SD=1.04) and 14 controls (11 male, mean age 11.56, SD=0.73). Dyslexics and controls were recruited in Thuringia, a German federal state adjacent to Saxony. Inclusion criteria were a IQ ≥ 85 (nonverbal part of HAWIK-III³⁰), right handedness³¹, no attention deficit disorder^{21,31}), and no history of neurological or psychiatric disorders. Diagnosis of dyslexia was based upon two criteria: (a) discrepancy between nonverbal IQ and reading³²/spelling³³⁻³⁶ ≥ 1.5 standard deviations and (b) reading/spelling performance (transformed to IQ-scale) < 85 . Normal performance of controls was controlled by reading/spelling performance (transformed to IQ-scale) > 85 . Controls were matched to dyslexics according to nonverbal IQ and age. Both groups did not differ significantly in IQ and age. In accordance with the additive model applied in differential allelic gene expression analysis, carriers of allele rs12533005-G were classified as subjects with genetic risk, individuals homozygous for rs12533005-C as subjects without genetic risk.

fMRI paradigm

fMRI data were acquired in a Siemens Magnetom Vision 1.5 Tesla MRI Scanner (Erlangen, Germany) using a T2-weighted epi-sequence (TE=60 ms, TR=0.6 ms, TA=3496 ms, FOV=192 mm, FA=90°, 64x64 matrix) with 32 slices (4 mm slice thickness).

To operationalise phonological as well as low level visual processing we used a paradigm comprising (1) phonological stimuli (“rhyming”: do the names of two letters rhyme; e. g. “G” and “T”) and (2) visual control stimuli (“slashes”: are two slashes inclined in the same direction; e. g. “/” vs. “\”).

The experiment was conducted in a block design. Four scans were acquired per block. For each stimulus type, 11 blocks were acquired, totalling 44 scans per task. Each stimulus block was followed by a baseline-task-block (“fixation”: fixation cross for 13984 ms, 21 baseline blocks in total with 4 scans per block). The block sequence was kept the same between subjects.

Subjects had to indicate their decision via button press, allowing the acquisition of reaction time and error rate (Presentation, Neurobehavioral Systems, <http://www.neurobs.com/presentation>). The trial setup was fixed to a duration of 3000 ms followed by a 450 ms inter stimulus interval. During each trial the stimuli were presented for a maximum of 3000 ms. The stimulus disappeared after the button press, leaving a blank screen until the end of the 3000 ms interval.

fMRI data analysis

fMRI data were analysed with the SPM8 package (Institute of Neurology, London, UK, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>). Spatial pre-processing included: (a) realignment of all functional images to the session’s first image and computation of a mean image out of realigned images, (b) normalisation of realigned functional images to the EPI-MNI-template (International Consortium for Brain Mapping template, Montreal Neurological Institute, Montreal, Canada) by estimation of normalisation parameters for the mean image and their application to functional images and (c) smoothing of images with a Gaussian kernel of 9 mm FWHM.

First-level analyses were computed for each subject using a voxel-by-voxel-t-test for the contrasts of (A) rhyming vs. fixation as well as (B) rhyming vs. slashes. Second-level analyses for the contrasts (A) and (B) were computed via 2 x 2 analysis of variance (ANOVA) with factors “disorder” (dyslexic or normal reader) and “genetic risk” (risk variant rs12533005-G or no risk variant) using a full factorial design. Post-hoc t-tests were done to test intergroup-effects for (1) controls vs. dyslexics, (2) dyslexics vs. controls, (3) no-risk subjects vs. risk subjects, (4) risk subjects vs. no-risk subjects. fMRI results are reported on an uncorrected p-level of 0.001 and a cluster level of 10.

Results

Selection of genetic variants for imaging analysis

The following SNPs in *FOXP2* were investigated for association with dyslexia: rs12533005 and rs10228350 (intron 1), rs10268637 and rs4727799 (intron 2), rs17137124 (intron 3), rs7782412 and rs12670585 (intron 9), rs936146 (intron 11), rs10953766 (intron 17) and their resulting haplotypes. We also studied the R553H mutation described by Lai et al.⁸ A summary including the linkage disequilibrium (LD) structure of *FOXP2* in our population is shown in Supplemental figure I.

SNP rs12533005 showed nominal significant association (odds ratio genotype G/G, recessive model: 2.1 [95% confidence interval 1.1-3.9] p=0.016; Cochran-Armitage test p=0.049, for more details see Supplemental table II). No effect of age on allele frequency as described elsewhere³⁷ was found. Other tested SNPs and haplotypes did not show significant frequency differences between cases and controls (Supplemental tables II and III). None of the haplotypes showed stronger association than single marker rs12533005. No sex specific differences were observed. Haplotype structure in our cohort was comparable to that in HapMap CEU panel. Mutation R553H was not

present in any cases or controls. Hence, we selected rs12533005 for further imaging genetic analyses.

Prediction of altered gene expression in silico

Three TF-binding sites for regulators ATBF1 (=ZFHX3), LEF1, and MEL1 (=PRDM16) are predicted in silico to be present for carriers of the risk variant rs12533005-G, but not for rs12533005-C. Matrix similarity (score of the complete matrix match) for ATBF1, LEF1, and MEL1 was 0.80, 0.86, and 1.00, respectively.

Analysis of differential allelic gene expression in human hippocampus

As rs12533005 was not originally included in the analysis of differential allelic expression in human hippocampus, we identified rs10249531 located 41 kb upstream of rs12533005 as most relevant proxy ($r^2=0.6$, $D'=1.0$). SNP rs10249531-C (correlating with rs12533005-G) was nominally associated with decreased *FOXP2* expression ($p=0.018$), accounting for 4.1 % of gene expression variability (Supplemental figure II). Within the additive model representing the effect of rs10249531-C on *FOXP2* gene expression levels, we found a regression coefficient beta of -0.024. Within our sample, there was no evidence for age-dependent expression differences.

fMRI results

Among analysed SNPs of *FOXP2*, rs12533005 showed the strongest signs of association with dyslexia. Therefore, the functional relevance of this SNP in language and speech processing was further analysed using fMRI.

Intragroup results

Individuals of the fMRI-sample were grouped according to disorder state: (1) dyslexics (n=19), (2) controls (n=14); and according to carriage of rs12533005-G: (3) subjects

with genetic risk (n=25), (4) subjects without genetic risk (i.e. individuals homozygous for rs12533005-C, n=8). Intragroup results for the contrast (A) rhyming vs. fixation for groups (1) and (2) showed in each group activations in the expected (phonological) reading network comprising occipital and inferior temporal brain areas, the angular gyrus, the insula and inferior frontal brain areas (Figure 1). The same areas were found to be activated when groups were defined according to (3) and (4). For contrast (B), rhyming vs. slashes, activity in the same network could be observed.

Main effects and interactions

In contrast (A) there was only a single cluster of right middle frontal brain areas associated with the main effect “disorder”. For main effect “genetic risk” there was an association in a superior temporal cluster bordering to the angular gyrus. For the interaction effect “disorder x genetic risk” a left sided cluster in the Rolandic operculum could be observed.

In contrast (B) there were three clusters associated with the main effect “disorder”: one left sided cluster in the white matter close to the insula, one cluster in the gray matter close to the anterior commissure and the third in the right cerebellum. For the main effect “genetic risk” various small clusters within the right precuneus, the left and right nucleus caudatus, right-sided superior frontal, the left and right temporal pole, left-sided postcentral, and, finally, in the left-sided fusiform gyrus could be observed. For the interaction effect “disorder” x “genetic risk” clusters in the left and right precuneus as well as in the right superior medial gyrus could be observed. Main effects and interactions for contrasts (A) and (B) are depicted in Figure 2.

Post-hoc t-tests

Post-hoc t-tests were applied to investigate the direction of main effects and interactions. The post-hoc t-tests for the contrast (A) for controls vs. dyslexics revealed no significant effects, whereas the t-test for dyslexics vs. controls showed a small overactivation in the right-sided fusiform gyrus. The t-test of no genetic risk vs. genetic risk (i.e. non-carriage vs. carriage of rs12533005-G) showed a prominent overactivation in the supramarginal and angular gyri as well as three small clusters of overactivation in (I) an area bordering on the inferior frontal gyrus, (II) the superior occipital gyrus and (III) the lingual gyrus for subjects with no genetic risk. The t-test of genetic risk vs. no genetic risk did not show any significant effects.

Post-hoc t-tests for contrast (B) for controls vs. dyslexics showed two clusters in the white matter left and right middle frontal, the t-test for dyslexics vs. controls showed two small clusters left-sided in the lingual, fusiform, and middle occipital gyrus. The t-tests for non-genetic risk vs. genetic risk and vice versa showed no significant clusters.

Discussion

Imaging genetics links genetic variants and their functional relevance in brain activation. To our knowledge we report for the first time results of imaging genetics in dyslexia. We initially performed a genetic association analysis of *FOXP2* variants to prioritise markers for use in fMRI analysis. We selected *FOXP2* tagging SNPs and the coding variant R553H, a mutation initially found to be associated with severe speech and language disorder in a Pakistani family by Lai et al.⁸. No other coding variants of *FOXP2* were investigated as no non-synonymously coding SNPs were reported in dbSNP (Build 130) and no non-synonymously coding SNPs in *FOXP2* were found in dyslexics in a previous study⁷. The coding mutation R553H was not found in any individual in our study, strengthening the hypothesis that R553H is a rare mutation

present only in some sparse families and not of relevance for diseases in the general population³⁸. Of studied variants, only SNP rs12533005 showed nominal significant association with dyslexia ($p=0.016$). As *FOXP2* haplotypes did not show stronger association than single marker rs12533005, this variant was chosen for imaging genetics analysis.

Since rs12533005 is an intronic SNP, it does not change the protein sequence of *FOXP2*. It may be a marker for a yet unknown functional variant or it may modify regulation of gene expression. Notably, according to the PupaSuite database³⁹, rs12533005 is classified to be situated in a conserved region. Three TF-binding sites (ATBF1, LEF1, and MEL1) are predicted to be present in carriers of risk variant rs12533005-G, but not for rs12533005-C. MEL1 and ATBF1 play a role in regulatory transcription processes in the mammalian central nervous system⁴⁰. Positive MEL1 regulation indicates a multistep regulatory network aimed at expression of specific neuronal repressors⁴¹. ATBF1 acts as repressor of gene expression by down-regulating AT-rich enhancer elements^{42,43} and LEF1 seems to be involved especially in downregulation of E-cadherin, which is important for cellular polarity and adhesion processes during embryonic development in general⁴⁴ as well as embryonic brain morphogenesis⁴⁵.

To verify the results of our *in silico* analysis we examined differential allelic expression of *FOXP2* rs12533005 in 138 human resected hippocampi. In this dataset, the most relevant proxy for rs12533005 – which was not contained on the Illumina SNP array used by us - was rs10249531. We found significant reduction of *FOXP2* gene expression for rs10249531-C, which is associated with rs12533005-G (Supplemental figure II). While there is no perfect linkage disequilibrium between the two SNPs, our data suggest differential allelic expression of *FOXP2* depending on rs12533005 alleles.

This supports our *in silico* prediction for a repressor binding site generated by rs12533005. We note that hippocampal tissues were derived from epileptic patients. However, we believe that the identified differential allelic expression of FOXP2 is not connected to a specific epilepsy-related process as neither the FOXP2 locus nor FOXP2 expression levels are reported to be associated with epilepsy. Based on these promising data we examined the role of rs12533005 in reading related brain activation.

Using fMRI we found for the contrast of the “rhyming” vs. “fixation” task a significant main effect of activation difference for the factor “risk” (i.e. comparison of subjects being carriers and non-carriers of the risk variant rs12533005-G). Subsequent one-tailed post-hoc t-tests indicate that the main effect for the factor “risk” can be explained by an overactivation of the non-carriers of the risk-variant in two temporo-parietal brain areas: the angular and the supramarginal gyrus. This finding is in line with former fMRI research¹⁸: The angular as well as the supramarginal gyrus are significantly involved in phonological language processing and showed a higher activation in normal readers compared to dyslexics¹⁸. Thus our observation is consistent with the interpretation that these temporo-parietal brain areas show a functional deficit in carriers of the putative risk variant compared with non-carriers. Therefore our results point to a possible role of SNP rs12533005 in reading related brain activation in the dorsal temporal cortex.

The interaction of factors “disorder” x “genetic risk” showed significant activation differences in the Rolandic operculum, a brain region strongly involved in motoric speech production. Post-hoc t-tests revealed for the comparison of non risk carriers vs. risk carriers a small cluster of overactivations in an area next to the inferior frontal gyrus. The interaction effect “disorder” x “genetic risk” can probably be explained by stronger activation of non-risk carriers in these inferior frontal areas. This result is in line with earlier findings of an effect of a *FOXP2* mutation on the function of inferior

frontal brain areas¹⁰. Yet, due to the incomplete overlap of the cluster localisation between interaction effect and the post-hoc t-test result, our findings from interaction analysis may require further investigation.

Based on our results, it could be speculated that in presence of the risk variant rs12533005-G repressor binding sites are created, leading to decreased *FOXP2* expression. As very tightly regulated *FOXP2* expression in the developing brain seems to be required for development of speech and linguistic functions⁴⁶, repression might result in neuronal dysfunction giving a possible explanation for our fMRI findings.

We aimed to increase power to detect true positives by integrating genetic and functional data. Yet, given the only moderate sample sizes of the studied populations and no multiple testing correction, our findings need replication.

Our results hint on a possible role of *FOXP2* variants in German dyslexics. The functional link may be found in *FOXP2* influencing function of a left hemispheric brain area involved in spoken (phonological memory) as well as written language (grapheme-phoneme correspondence and the mental lexicon).

While replication of our results in an independent, larger cohort is definitely needed, our results further corroborate the role of *FOXP2* in speech and language development, hint on the relevance of genetic variants of *FOXP2* for reading and spelling, and demonstrate the application of imaging genetics in the investigation of dyslexia.

Conflict of Interest

The authors declare no conflict of interest.

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Reference List

- 1 Mattay VS, Goldberg TE: Imaging genetic influences in human brain function. *Curr Opin Neurobiol* 2004; **14**: 239-247.
- 2 Hariri AR, Mattay VS, Tessitore A *et al.*: Serotonin transporter genetic variation and the response of the human amygdala. *Science* 2002; **297**: 400-403.
- 3 Fan J, Fossella J, Sommer T, Wu Y, Posner MI: Mapping the genetic variation of executive attention onto brain activity. *Proc Natl Acad Sci U S A* 2003; **100**: 7406-7411.
- 4 Egan MF, Goldberg TE, Kolachana BS *et al.*: Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 2001; **98**: 6917-6922.
- 5 Schulte-Körne G, Remschmidt H: Lese-Rechtschreibstörung (Legasthenie) - Symptomatik, Diagnostik, Ursachen, Verlauf und Behandlung. *Deutsches Ärzteblatt* 2003; **100**: 396-408.
- 6 Harlaar N, Spinath FM, Dale PS, Plomin R: Genetic influences on early word recognition abilities and disabilities: a study of 7-year-old twins. *J Child Psychol Psychiatry* 2005; **46**: 373-384.
- 7 Kaminen N, Hannula-Jouppi K, Kestila M *et al.*: A genome scan for developmental dyslexia confirms linkage to chromosome 2p11 and suggests a new locus on 7q32. *J Med Genet* 2003; **40**: 340-345.

- 8 Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP: A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 2001; **413**: 519-523.
- 9 Belton E, Salmond CH, Watkins KE, Vargha-Khadem F, Gadian DG: Bilateral brain abnormalities associated with dominantly inherited verbal and orofacial dyspraxia. *Hum Brain Mapp* 2003; **18**: 194-200.
- 10 Liegeois F, Baldeweg T, Connelly A *et al.*: Language fMRI abnormalities associated with FOXP2 gene mutation. *Nat Neurosci* 2003; **6**: 1230-1237.
- 11 Fee MS, Scharff C: The songbird as a model for the generation and learning of complex sequential behaviors. *ILAR J* 2010; **51**: 362-377.
- 12 Fisher SE, Scharff C: FOXP2 as a molecular window into speech and language. *Trends Genet* 2009; **25**: 166-177.
- 13 Frayer DW, Fiore I, Lalueza-Fox C, Radovic J, Bondioli L: Right handed Neandertals: Vindija and beyond. *J Anthropol Sci* 2010; **88**: 113-127.
- 14 Enard W, Gehre S, Hammerschmidt K *et al.*: A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell* 2009; **137**: 961-971.
- 15 Vernes SC, Newbury DF, Abrahams BS *et al.*: A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008; **359**: 2337-2345.
- 16 Vargha-Khadem F, Watkins KE, Price CJ *et al.*: Neural basis of an inherited speech and language disorder. *Proc Natl Acad Sci U S A* 1998; **95**: 12695-12700.
- 17 Shaywitz SE, Shaywitz BA, Pugh KR *et al.*: Functional disruption in the organization of the brain for reading in dyslexia. *Proc Natl Acad Sci U S A* 1998; **95**: 2636-2641.
- 18 Ligges C, Blanz B: [Survey of fMRI results regarding a phonological deficit in children and adults with dyslexia: fundamental deficit or indication of compensation?]. *Z Kinder Jugendpsychiatr Psychother* 2007; **35**: 107-115.
- 19 Maisog JM, Einbinder ER, Flowers DL, Turkeltaub PE, Eden GF: A meta-analysis of functional neuroimaging studies of dyslexia. *Ann N Y Acad Sci* 2008; **1145**: 237-259.
- 20 Paulesu E, Demonet JF, Fazio F *et al.*: Dyslexia: cultural diversity and biological unity. *Science* 2001; **291**: 2165-2167.
- 21 Brickenkamp R, Zillmer E: The d2 test of attention. Göttingen, Hogrefe, 2002.
- 22 Marx H: Knuspels Leseaufgaben (KNUSPEL-L). Goettingen, Hogrefe, 1998.
- 23 Weiss RH: Grundintelligenztest Skala 2. Goettingen, Hogrefe, 1998.

- 24 Wilcke A, Weissfuss J, Kirsten H *et al.*: The role of gene DCDC2 in German dyslexics. *Ann Dyslexia* 2009; **59**: 1-11.
- 25 Wenzel T, Elssner T, Fahr K *et al.*: Genosnip: SNP genotyping by MALDI-TOF MS using photocleavable oligonucleotides. *Nucleosides Nucleotides Nucleic Acids* 2003; **22**: 1579-1581.
- 26 Kirsten H, Dienst S, Emmrich F, Ahnert P: CalcDalton: a tool for multiplex genotyping primer design for single-base extension reactions using cleavable primers. *Biotechniques* 2006; **40**: 158, 160, 162.
- 27 Kirsten H, Teupser D, Weissfuss J *et al.*: Robustness of single-base extension against mismatches at the site of primer attachment in a clinical assay. *J Mol Med* 2007; **85**: 361-369.
- 28 Gordon D, Haynes C, Blumenfeld J, Finch SJ: PAWE-3D: visualizing power for association with error in case-control genetic studies of complex traits. *Bioinformatics* 2005; **21**: 3935-3937.
- 29 Tasheva ES, Klocke B, Conrad GW: Analysis of transcriptional regulation of the small leucine rich proteoglycans. *Mol Vis* 2004; **10**: 758-772.
- 30 Tewes U, Rossmann P, Schallberger U: Hamburg-Wechsler-Intelligenz-Test für Kinder III (HAWIK III). Bern, Verlag Hans Huber, 2002.
- 31 Zimmermann, P., Gondan, M., & Fimm, B. KiTAP Kinderversion der Testbatterie zur Aufmerksamkeitsprüfung. 2002. Herzogenrath, Vera Fimm - Psychologische Testsysteme.
- 32 Grissemann H , Baumberger W: ZLVT 4-6 Zürcher Leseverständnistest für das 4. bis 6. Schuljahr. Bern, Verlag Hans Huber, 2000.
- 33 Grund M, Haug G, Naumann CL: Diagnostischer Rechtschreibtest für 4. Klassen (DRT 4) (2nd ed.). Göttingen, Beltz, 2004.
- 34 Grund M, Haug G, Naumann CL: Diagnostischer Rechtschreibtest für 5. Klassen (DRT 5). Göttingen, Beltz, 2004.
- 35 Müller R: Diagnostischer Rechtschreibtest für 2. Klassen (DRT 2). Weinheim, Beltz, 1990.
- 36 Rathenow P, Laupenmühle D, Vöge J: Westermann Rechtschreibtest 6+ (2nd ed.). Braunschweig, Westermann, 1981.
- 37 Bickeboller H, Campion D, Brice A *et al.*: Apolipoprotein E and Alzheimer disease: genotype-specific risks by age and sex. *Am J Hum Genet* 1997; **60**: 439-446.
- 38 Sanjuan J, Tolosa A, Gonzalez JC *et al.*: FOXP2 polymorphisms in patients with schizophrenia. *Schizophr Res* 2005; **73**: 253-256.

- 39 Conde L, Vaquerizas JM, Dopazo H *et al.*: PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. *Nucleic Acids Res* 2006; **34**: W621-W625.
- 40 Miura Y, Tam T, Ido A *et al.*: Cloning and characterization of an ATBF1 isoform that expresses in a neuronal differentiation-dependent manner. *J Biol Chem* 1995; **270**: 26840-26848.
- 41 Ohtsuka T, Ishibashi M, Gradwohl G *et al.*: Hes1 and Hes5 as notch effectors in mammalian neuronal differentiation. *EMBO J* 1999; **18**: 2196-2207.
- 42 Sansregret L, Nepveu A: The multiple roles of CUX1: insights from mouse models and cell-based assays. *Gene* 2008; **412**: 84-94.
- 43 Yasuda H, Mizuno A, Tamaoki T, Morinaga T: ATBF1, a multiple-homeodomain zinc finger protein, selectively down-regulates AT-rich elements of the human alpha-fetoprotein gene. *Mol Cell Biol* 1994; **14**: 1395-1401.
- 44 Jamora C, DasGupta R, Kocieniewski P, Fuchs E: Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature* 2003; **422**: 317-322.
- 45 Shimamura K, Takeichi M: Local and transient expression of E-cadherin involved in mouse embryonic brain morphogenesis. *Development* 1992; **116**: 1011-1019.
- 46 Lai CS, Gerrelli D, Monaco AP, Fisher SE, Copp AJ: FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain* 2003; **126**: 2455-2462.

Titles and legends to figures

Figure 1

Intragroup results according to state of disorder and genetic risk

A) Intragroup results for the contrast rhyming vs. fixation, B) Intragroup results for the contrast rhyming vs. slashes. Sample sizes: dyslexics n=19, controls n=14, risk (carriers of rs12533005-G), n=25, no risk (non-carriers of rs12533005-G), n=8.

Figure 2

Main effects and interactions according to state of disorder and genetic risk

A) Results of the F-Test for the contrast rhyming vs. fixation, B) Results of the F-Test for the contrast rhyming vs. letters. Disorder: contrast dyslexics vs. controls. Risk: contrast carriers of rs12533005-G vs. non-carriers. Sample sizes: dyslexics n=19, controls n=14, risk (carriers of rs12533005-G), n=25, no risk (non-carriers of rs12533005-G), n=8.

Supplemental Figure I

Schematic view of FOXP2 and its haplotype structure

Horizontal lines indicate intronic regions, vertical blue lines exonic regions. Long red lines stand for SNPs studied. **1** *rs12533005* **2** *rs10228350* **3** *rs10268637* **4** *rs4727799*
5 *rs17137124* **6** *rs7782412* **7** *rs12670585* **8** *rs936146* **9** *rs10953766*.

Numbers in squared fields indicate correlation between SNP within the haplotype-structure. The SNPs mentioned above cover the region Chr.7:114.055.805-114.312.968 (coordinates according to hg19).

Supplemental Figure II

Differential allelic expression analysis of FOXP2 based on rs10249531

Differential allelic expression data in hippocampal tissue for rs10249531 (representing the most relevant proxy for rs12533005) and ILMN_1695355 (the corresponding transcript on the Illumina Human Whole Genome chipset at position FOXP2 on chromosome 7 at 114.304.471Mb mapping the last and second last exon at the 3' end), detecting expression of *FOXP2*.

Supplemental Table I

Overview of studied SNPs and their primer sequences

Supplemental Table II

Detailed overview of genetic results for studied SNPs

OR = odds ratio; OR [95%CI] = Odds ratio [95% confidence interval]

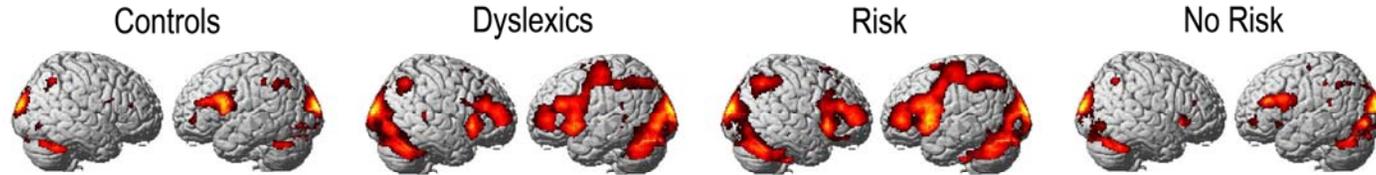
Supplemental Table III

Overview of haplotypes identified by studied SNPs

SNPs in haplotypes are shown in the following order: rs12533005, rs10228350, rs10268637, rs4727799, rs17137124, rs7782412, rs12670585, rs936146, rs10953766.

Intragroup

A) rhyming vs. fixation



B) rhyming vs. slashes

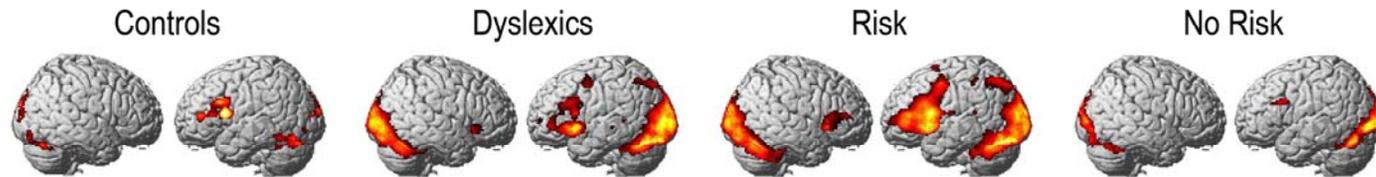


Figure 1 Intragroup results according to disorder state and genetic state

F-Test

A) rhyming vs. fixation

Main Effect Disorder



Main Effect Risk



Interaction Disorder x Risk



B) rhyming vs. slashes

Main Effect Disorder



Main Effect Risk



Interaction Disorder x Risk

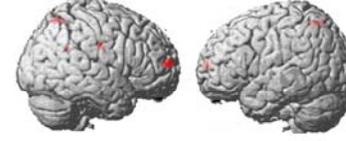
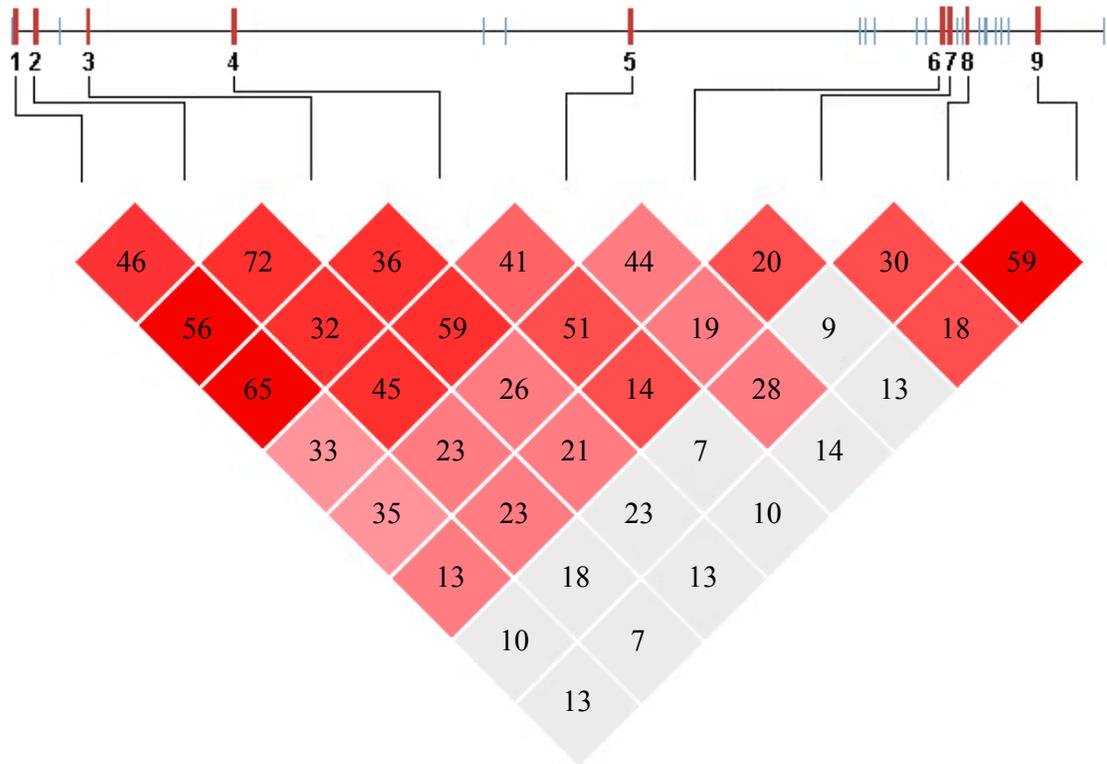
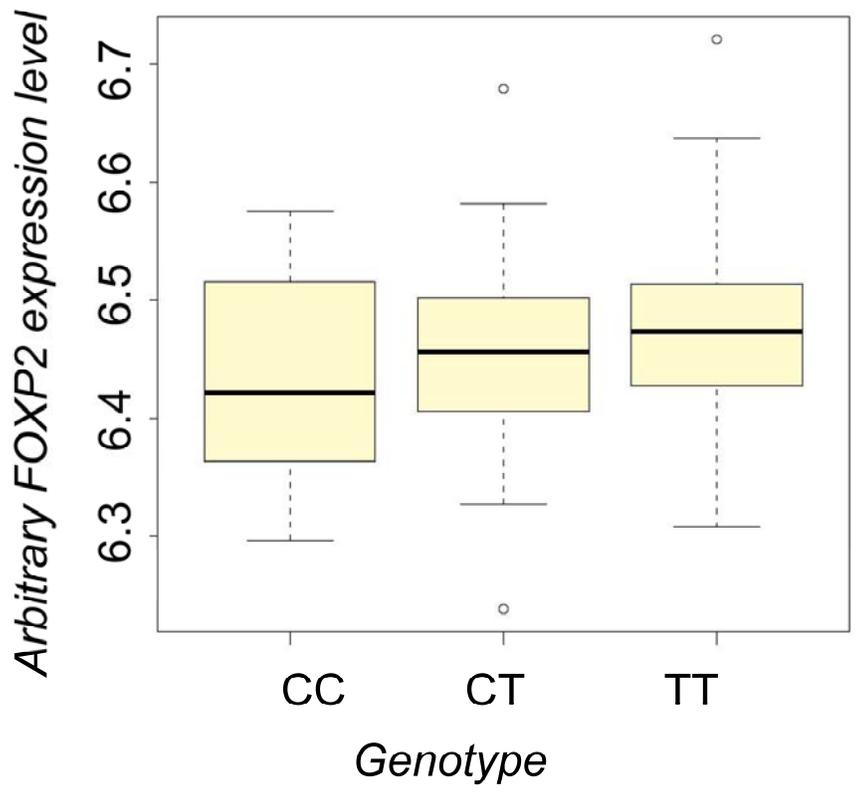


Figure 2 Main effects and interactions according to disorder state and genetic state



Supplemental figure I Schematic view of FOXP2 and its haplotype structure



Supplemental figure II: Differential allelic expression analysis of FOXP2 based on rs10249531

Gene	SNP rs Number	SNP-Type	PCR-Primer 5'	PCR-Primer 3'	SBE-Primer	expected PCR-Product size
FOXP2	rs12533005	C/G	ACGTTGGATGGTAGTTTTA TATGCATGTTTCATGATTTT	ACGTTGGATGCGAGTATTA AACTATCACACCTATTTGA	bioT TTC CAG G(L)A TAC ACA TTG TGA ATT	172 bp
FOXP2	rs10228350	A/T	ACGTTGGATGTCTTGTA CTGTAGCCAGTATTTATT	ACGTTGGATGAAAGTTTAT TTCACCCTGCTACT	bio TTC TTT AGT TTT TAA GTC CTC (L)TC TTA CAT	199 bp
FOXP2	rs10268637	C/T	ACGTTGGATGTCACTCTCA ACACTTCAATGTTA	ACGTTGGATGTTCTCACTA ATCTCAATTATTCACC	bioG CTA AAT AAT TGT T(L)T TGA ATA GCT TTC	179 bp
FOXP2	rs4727799	C/T	ACGTTGGATGGTGTGGTTA TTTTATTAACATTTGTCT	ACGTTGGATGCATTCCTC ATCCATCCAGTATATA	bioCA ATG AAC CAC (L)AA AAA TAA TCT CTG	156 bp
FOXP2	rs17137124	C/T	ACGTTGGATGAATCTATAC TGGTTAACTGTGAGACA	ACGTTGGATGGGTAAACA AAGAGAATCAGGAG	bioG GAG TTG GCT TT(L) TTT TCA ATC TTA	164 bp
FOXP2	rs7782412	C/T	ACGTTGGATGTTCCCTGCATT TACACCCTAAT	ACGTTGGATGCATGAACTC TTGGGGAACA	bio CCT AAT CTC AGT GTA TAA C(L)G AAG CAT	108 bp
FOXP2	rs12670585	C/T	ACGTTGGATGTTCTGATTGT TGAACCTAGGTATATA	ACGTTGGATGGATCACAGT GAGGCAGAAAA	bioA AAA CAT ATG AAG AG(L) AAT TAA AGT CGT	141 bp
FOXP2	rs936146	C/G	ACGTTGGATGCCTTCTTCA GCACACCACA	ACGTTGGATGGCACAAATC ACTGCCTTG	bioCT TTG AGC AAC CTG AAA (L)GT AGT AAC	148 bp
FOXP2	rs10953766	A/G	ACGTTGGATGCCAACAAC AAGGCAATAATA	ACGTTGGATGGGATCTAAG GACCCTTATAGTTCT	bio CAG CAA CAA GAC TGA C[L]T AAA GGC	152 bp
FOXP2	Mutation R553H	G/A	ACGTTGGATGGGGCTGCCT TATTAGACAATATTA	ACGTTGGATGCCATACTGC TCCTTTAACATTTT	bio TCT TCT GTC TG(L) TTT AGA ATG CAG TAC	132 bp

Supplemental table I Overview of studied SNPs and their primer sequences

	rs12533005	rs10228350	rs10268637	rs4727799	rs17137124	rs7782412	rs12670585	rs936146	rs10953766
Minor allele	C	T	T	G	A	C	A	C	A
Major allele	G	A	C	A	G	T	G	G	G
Cases homozygous minor genotype	10 (16.4%)	8 (14.5%)	14 (23%)	8 (13.3%)	14 (24.6%)	10 (16.4%)	5 (8.2%)	8 (13.3%)	8 (13.1%)
Cases heterozygote genotype	25 (41%)	27 (49.1%)	30 (49.2%)	22 (36.7%)	25 (43.9%)	32 (52.5%)	24 (39.3%)	37 (61.7%)	33 (54.1%)
Cases homozygous major genotype	26 (42.6%)	20 (36.4%)	17 (27.9%)	30 (50%)	18 (31.6%)	19 (31.1%)	32 (52.5%)	15 (25%)	20 (32.8%)
Controls homozygous minor genotype	37 (20.1%)	26 (15.1%)	29 (15.9%)	29 (15.8%)	41 (23.2%)	40 (22.3%)	16 (8.7%)	42 (23.2%)	43 (23.4%)
Controls heterozygote genotype	99 (53.8%)	87 (50.6%)	95 (52.2%)	85 (46.2%)	87 (49.2%)	77 (43%)	71 (38.8%)	81 (44.8%)	75 (40.8%)
Controls homozygous major genotype	48 (26.1%)	59 (34.3%)	58 (31.9%)	70 (38%)	49 (27.7%)	62 (34.6%)	96 (52.5%)	58 (32%)	66 (35.9%)
Cases major allele frequency	63%	61%	52%	68%	54%	57%	72%	56%	60%
Controls major allele frequency	53%	60%	58%	61%	52%	56%	72%	54%	56%
OR [95%CI]	1.52 [1-2.3]	1.06 [0.7-1.6]	0.8 [0.5-1.2]	1.37 [0.9-2.1]	1.05 [0.7-1.6]	1.05 [0.7-1.6]	1.01 [0.6-1.6]	1.06 [0.7-1.6]	1.16 [0.8-1.8]
p-value allelic OR	0.052	0.811	0.289	0.158	0.827	0.821	0.984	0.792	0.490
OR major allele dominant model	1.28 [0.60-2.77]	1.05 [0.44-2.47]	0.64 [0.31-1.30]	1.22 [0.52-2.83]	0.93 [0.46-1.86]	1.47 [0.68-3.15]	1.07 [0.38-3.06]	1.96 [0.86-4.46]	2.02 [0.9-4.58]
p-value dominant OR	0.528	0.952	0.215	0.657	0.844	0.327	0.898	0.103	0.091
OR major allele recessive model	2.10 [1.15-3.85]	1.09 [0.58-2.06]	0.83 [0.44-1.57]	1.63 [0.91-2.93]	1.21 [0.63-2.31]	0.85 [0.46-1.59]	1.00 [0.56-1.79]	0.71 [0.36-1.37]	0.87 [0.5-1.6]
p-value recessive OR	0.016	0.786	0.565	0.103	0.577	0.624	1.000	0.303	0.669
Cochran-Armitage test p-value	0.049	0.804	0.279	0.172	0.833	0.832	0.984	0.793	0.512

Supplemental table II Detailed overview of the genetic results for the studied SNPs

OR = Odds ratio, OR [95%CI] = Odds ratio [95% confidence interval]

Haplotype	Case-Control-frequencies	Chi-Square	p-value (uncorrected)
CACGACGGA	0.198, 0.237	0.8	0.371
GTTAGTACG	0.198, 0.191	0.031	0.8612
GTTAGTGGA	0.032, 0.066	1.92	0.1659
GTTAGCGCG	0.074, 0.047	1.325	0.2498
GACAATGGG	0.038, 0.050	0.271	0.6027
CACGACGCG	0.035, 0.036	0.003	0.9534
GTTAGTGCG	0.034, 0.035	0.004	0.9466
CACAGTGGA	0.017, 0.033	0.866	0.3522
GACAACGGA	0.049, 0.020	2.759	0.0967
GATAGTGCG	0.038, 0.021	1.108	0.2924
CACGGTGCG	0.008, 0.029	1.688	0.1938
CACGACGGG	0.028, 0.021	0.255	0.6135
GACAATGGA	0.013, 0.023	0.4	0.527
CACAGTACG	0.002, 0.026	2.819	0.0932
CACGACACG	0.016, 0.021	0.102	0.7491
GTTAGTAGA	0.037, 0.012	2.963	0.0852
CACAATGGG	0.034, 0.011	2.887	0.0893
GTTAGTGGG	0.025, 0.009	1.742	0.1869

Supplemental Table III Overview of haplotypes identified by studied SNPs

Intragroup

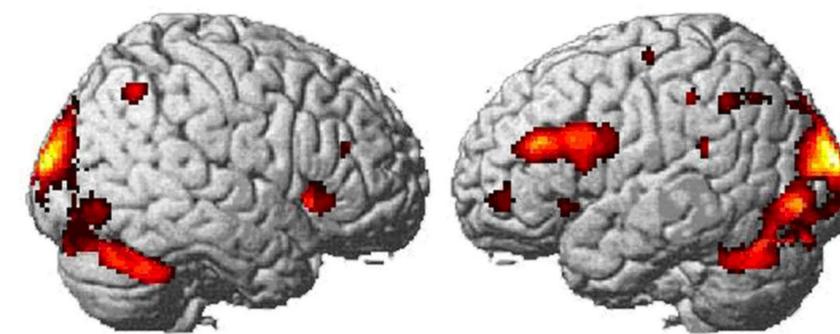
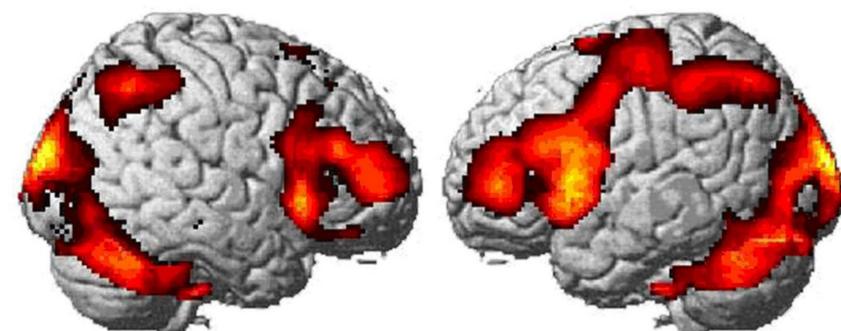
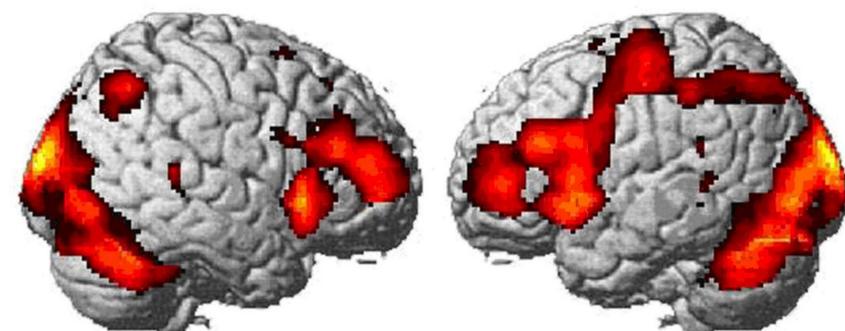
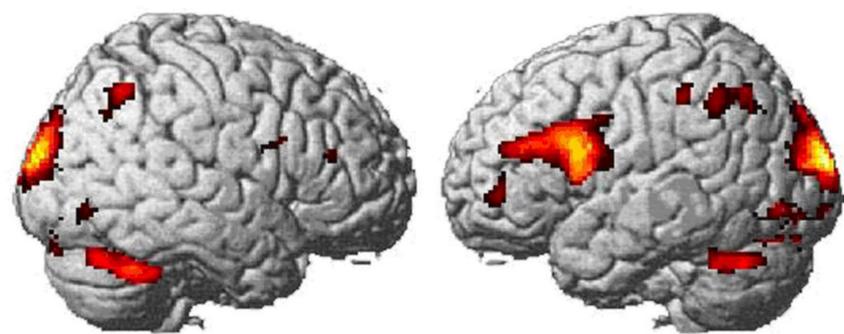
A) rhyming vs. fixation

Controls

Dyslexics

Risk

No Risk



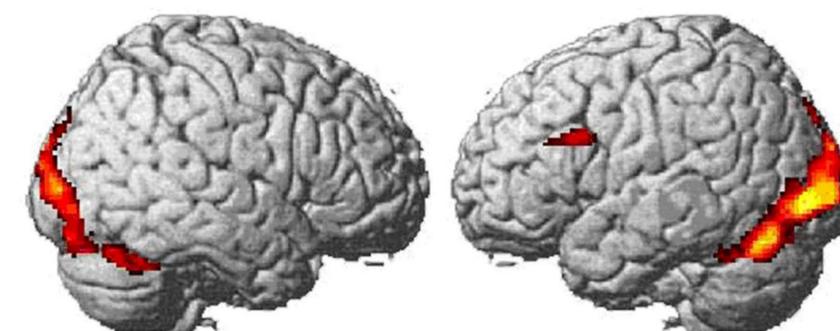
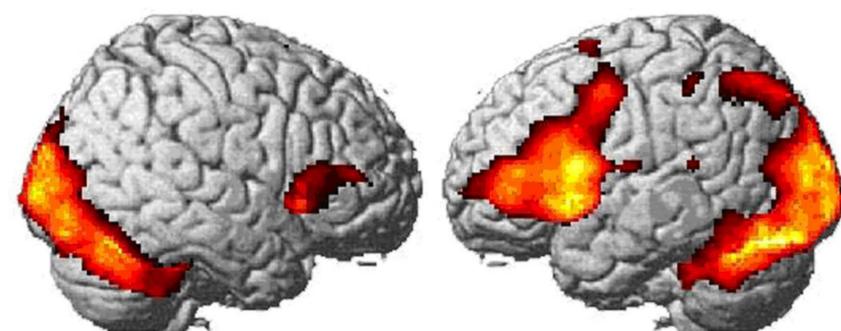
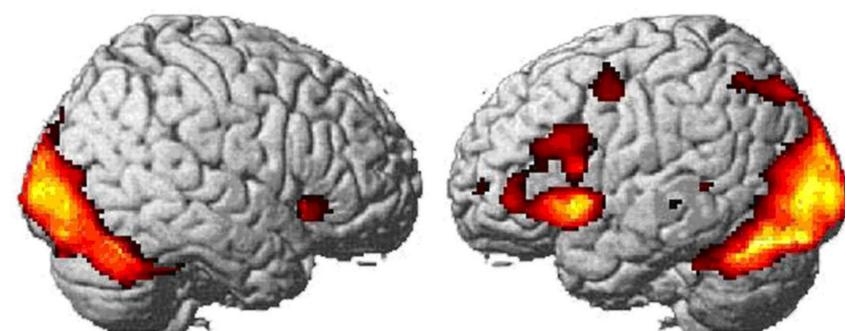
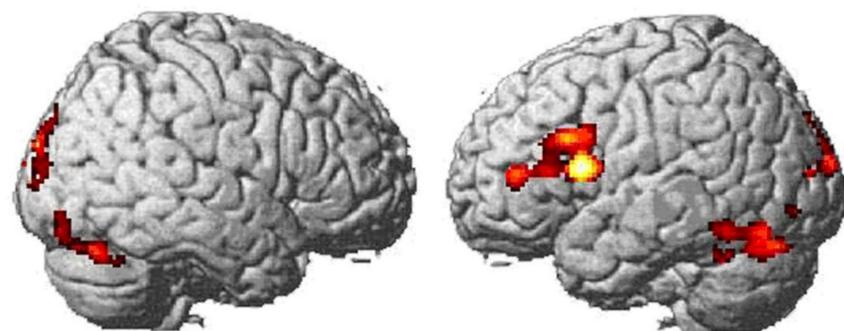
B) rhyming vs. slashes

Controls

Dyslexics

Risk

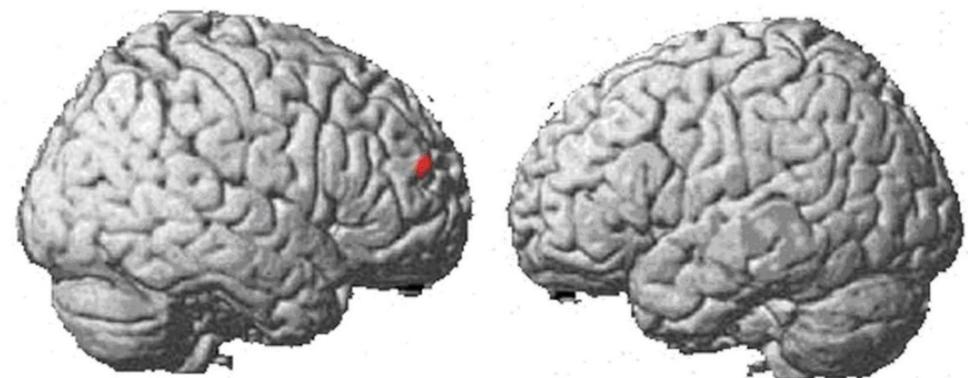
No Risk



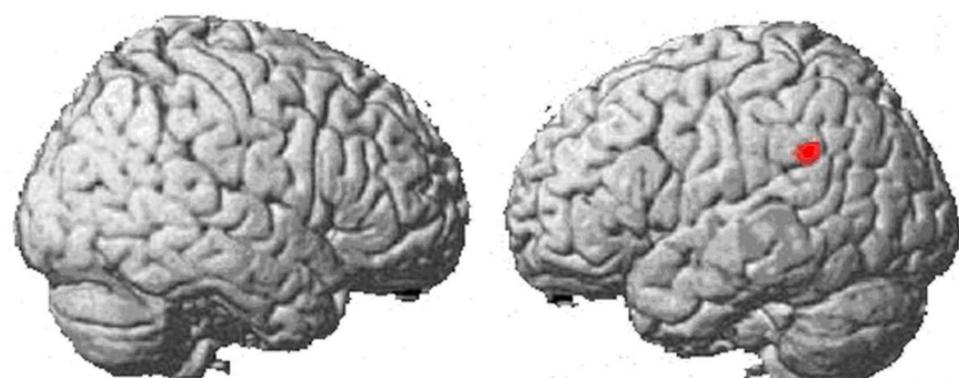
F-Test

A) rhyming vs. fixation

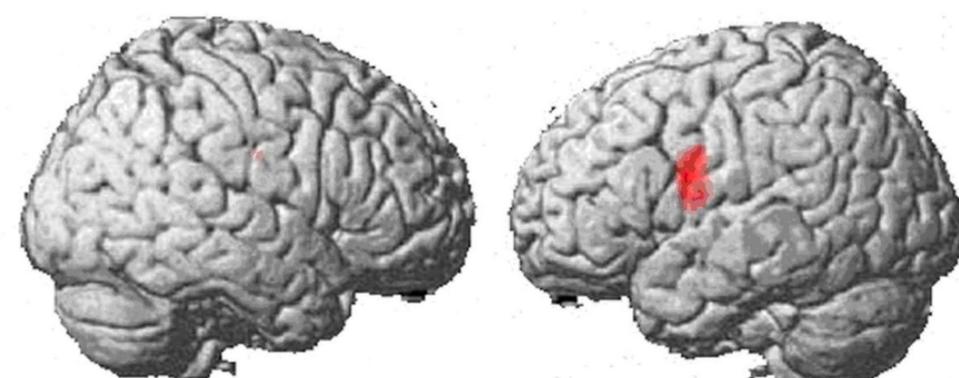
Main Effect Disorder



Main Effect Risk

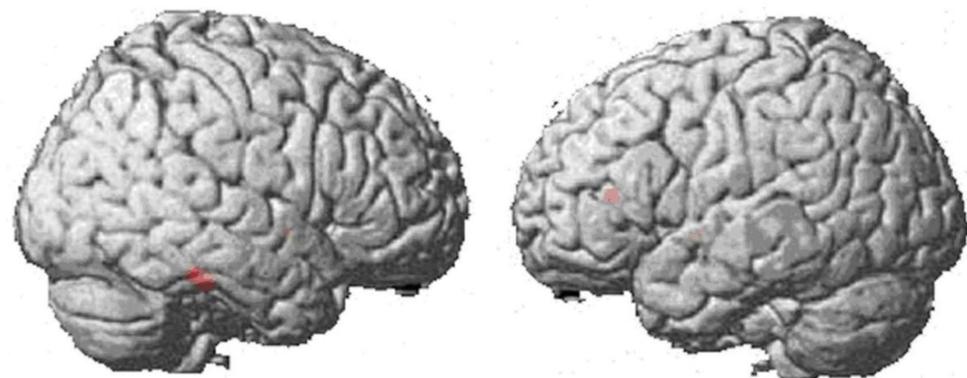


Interaction Disorder x Risk

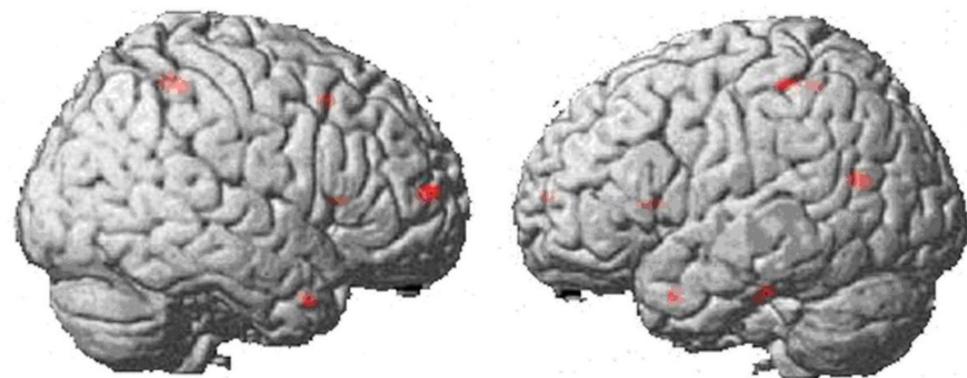


B) rhyming vs. slashes

Main Effect Disorder



Main Effect Risk



Interaction Disorder x Risk

