

Association of a Rare Variant with Mismatch Negativity in a Region Between *KIAA0319* and *DCDC2* in Dyslexia

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Abstract It has been repeatedly shown that mismatch negativity (MMN), an event related potential measurement, reveals differences between dyslexic children and age-matched controls. MMN reflects the automatic detection of deviance between a stream of incoming sounds presented to the passive listener, and deficits in MMN (i.e. attenuated amplitudes) have been especially reported in dyslexia for detecting differences between speech sounds (e.g./ba/vs./da/). We performed an association analysis in 200 dyslexic children. This analysis focused on two MMN components, an early MMN (188–300 ms) and a late MMN (300–710 ms), and the dyslexia candidate genes *KIAA0319* and *DCDC2* on chromosome 6. Additionally, we imputed rare variants located in this region based on the 1000 genomes project. We identified four rare variants that were significantly associated with the late MMN component. For three of these variants, which were in high LD to each other, genotyping confirmed

the association signal. Our results point to an association between late MMN and rare variants in a candidate gene region for dyslexia.

Keywords Mismatch negativity · Dyslexia · Speech perception · Rare variants · Imputing

Introduction

Dyslexia is one of the most prevalent learning disorders, affecting about 5–7% of school-aged children. Dyslexia can have a large impact on the psychosocial development of the affected children. For example, as a consequence of repeatedly experiencing failure in school (Shaywitz et al. 2008), children with dyslexia often leave the school system without completing their high school requirements or develop psychiatric disorders, such as depression and anxiety disorder (Goldston et al. 2007; Mugnaini et al. 2009). Understanding the etiology of dyslexia is a critical step in order to improve the efficiency of therapeutic strategies and remedial interventions for dyslexic children, adolescents and adults.

Molecular genetic work has had a strong impact on the understanding of dyslexia (Paracchini et al. 2008; Scerri and Schulte-Körne 2010). To date, linkage studies in families with a history of dyslexia have identified nine chromosomal regions—DYX1 through DYX9—that are listed by the Human Gene Nomenclature Committee (HGNC) (Bruford et al. 2008). These regions are located on chromosomes 15q21 (DYX1), 6p21-p22 (DYX2), 2p15-p16 (DYX3), 6q11-q12 (DYX4), 3p12-q13 (DYX5), 18p11 (DYX6), 11p15 (DYX7), 1p34-p36 (DYX8), and Xq27 (DYX9) (Scerri and Schulte-Körne 2010). Many of these genes play a functional role in neuronal migration

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(Galaburda et al. 2006). The most convincing findings have been reported for DYX2 at 6p21-p22, where independent studies have identified the region by linkage analysis and association between dyslexia and genetic variants in the genes *Doublecortin-Domain-Containing-Protein-2* (*DCDC2*, MIM 605755) and *KIAA0319* (MIM 609269) have been reported (Cope et al. 2005; Deffenbacher et al. 2004; Francks et al. 2004; Meng et al. 2005; Schumacher et al. 2006). These genes are located only 150 kb from one another on 6p22.2. Both genes, *KIAA0319* and *DCDC2*, are expressed in brain regions (i.e. temporal and occipital, inferior temporo-occipital region of the left hemisphere) that show functional relevance for cognitive processes such as grapheme phoneme association (van Atteveldt et al. 2004) and speech perception related to reading and spelling development (Blau et al. 2009; Schulte-Körne et al. 2001b). It has been suggested that a down-regulation of *DCDC2* and *KIAA0319* in dyslexia might result in a disruption of neuronal migration within the cerebral cortex during the prenatal period leading to a variety of neocortical malformations (Meng et al. 2005; Paracchini et al. 2008; Burbridge et al. 2008). In line with this, earlier findings of molecular layer ectopias and periventricular heterotopias in dyslexic brains led to the postulation that dyslexia might arise from a disruption in neuronal migration (Galaburda and Kemper 1979; Galaburda et al. 1985; Humphreys et al. 1990).

Speech perception in dyslexia

The investigation of brain functions using event related potentials (ERPs) reveals evidence for altered cortical networks and neuro-cognitive functions in dyslexic individuals (Maurer et al. 2007; McCandliss and Noble 2003). Altered perception of speech sounds in superior temporal cortices in children and adults with dyslexia (Alonso-Bua et al. 2006; Corbera et al. 2006; Hommet et al. 2009; Maurer et al. 2009; Schulte-Körne et al. 2001b; Schulte-Körne et al. 1998a; Sharma et al. 2006) and in the brain-stem (Hornickel et al. 2009) have been reported (for review see Schulte-Körne and Bruder 2010). Even in preschool children at risk for dyslexia, speech discrimination ability measured with ERPs correlated with reading and spelling ability in the first and second grades (Leppänen et al. 1999; Guttorm et al. 2009; Lovio et al. 2010).

Speech perception involves the mapping of basic auditory information onto phonological units. The majority of ERP studies investigating speech processing in dyslexia have focused on the mismatch negativity component (MMN) as an index of successful discrimination between speech sounds, with changes in formant transitions (FT) (spectral changes; e.g./da/vs./ga/) or voice onset timing (VOT) transitions (temporal changes; e.g./ba/vs./pa/). In

most studies, dyslexia was consistently associated with a deviant MMN, including reduced amplitudes and prolonged latencies (for review see Bishop 2007; Kujala et al. 2007; Lyytinen et al. 2004; Schulte-Körne and Bruder 2010) suggesting a disorder in speech perception.

MMN, as its name suggests, is a negative ERP component and is generated by the automatic response of the brain to a mismatch in auditory stimulation. Specifically, MMN is elicited when an infrequent, deviant stimulus interrupts a train of frequently occurring standard stimuli. For example, MMN would occur when a standard stimulus of the speech sound/da/, occurring with a probability of 85%, is interrupted by an infrequently presented deviant speech sound, such as/ba/. It is obtained by subtracting the ERP elicited by the frequently occurring standard stimuli from the ERP associated to the infrequent deviant stimuli. MMN is elicited irrespective of the subject's direction of attention (Näätänen et al. 1978; Näätänen et al. 2007). In fact, in order to direct the subject's attention away from the presented stimulus sequence and to avoid attention related ERP components a behavioural task such as reading or watching a silent movie is normally used (Näätänen and Gaillard 1983). MMN is predominately observed over fronto-central and central scalp electrodes, peaking at 150–250 ms from change onset (Näätänen et al. 2007; Näätänen 1982; Näätänen et al. 1997; Tiitinen et al. 1994). The generators of the MMN were located in the bilateral supra-temporal cortices and in right frontal cortical areas (Rinne et al. 2000). Studying MMN is also useful in determining the short-term memory auditory trace, as it is elicited when at least 2–3 standard stimuli are successively presented (Sams et al. 1985) and when the interstimulus interval (ISI) between standard stimuli and deviant stimuli is below 12 s. Finally, MMN was also recorded for comparisons of native versus non-native language vowel stimuli suggesting the presence of language-specific memory traces for phonemes (Näätänen et al. 1997).

When complex stimuli such as speech sounds, as opposed to simpler sounds such as sinus tones, are involved, a later MMN component with a latency from 400 to 600 ms has been reported (Schulte-Körne et al. 2001b; Schulte-Körne et al. 1998a; Maurer et al. 2003; Maurer et al. 2009; Korpilahti and Lang 1994; Hommet et al. 2009; Froyen et al. 2009; Alonso-Bua et al. 2006). This component was named late MMN (Korpilahti et al. 2001; Froyen et al. 2009) or “Late Discriminative Negativity” (LDN) (Ceponienė et al. 2002; Ceponienė et al. 2004). The latency of this component suggests the involvement of different brain processes than those associated to the earlier, classical MMN component with a latency between 150 and 250 ms. This late MMN component is characterized by a broad negativity over frontal-central areas and is mainly elicited by complex auditory stimuli like syllables and

words. The generator of this component has been measured over right central-parietal areas (Hommet et al. 2009). The functional significance of the late MMN has been less intensively investigated in comparison to the classical MMN component, however its significance is believed to be related to attention related processes (Shestakova et al. 2003), to long term memory (Zachau et al. 2005) and to letter-speech sound integration (Froyen et al. 2009). In dyslexia reduced amplitude of the MMN and the late MMN component to speech stimuli in children (Schulte-Körne et al. 1998a; Lachmann et al. 2005) and adults (Schulte-Körne et al. 2001b) as well as a longer MMN latency in response to speech stimuli (Corbera et al. 2006; Alonso-Bua et al. 2006) were repeatedly found and interpreted as a neurophysiologic correlate of impaired discrimination of speech sounds.

In recent years an altered early MMN has emerged as a promising endophenotype for studies in psychiatric genetics. Endophenotypes are intermediate phenotypes, also known as biomarkers, that form the causal link between genes and the overt expression of disorders (Cannon and Keller 2006). The MMN is relatively stable and heritable (Winterer et al. 2003). For example, studies of patients diagnosed with schizophrenia and their non-affected siblings found alterations in the MMN in both groups, regardless of the schizophrenia diagnosis, when compared to a control group who had no family history of schizophrenia (Jessen et al. 2001). The heritability of the MMN was found to be high for the peak and mean amplitude, 0.63 and 0.68 respectively. The retest reliability was also high, 0.67 for the peak and 0.66 for the mean amplitude (Hall et al. 2006). The high reliability and heritability of the MMN component indicates that it is a suitable endophenotype. However, heritability data for the late MMN were not available.

In conclusion, both early and late MMN components might be useful endophenotypes for research of susceptibility genes in dyslexia and for understanding gene functions. To elucidate the connection between early MMN, late MMN and known candidate genes for dyslexia, we analyzed whether two of the most prominent candidate genes in dyslexia, *KIAA0319* and *DCDC2*, were not only associated with dyslexia per se, but also with the MMN and late MMN endophenotypes.

Materials and methods

Subjects

A sample of 200 dyslexic German children (150 male and 50 female) was recruited from two outpatient Departments of Child and Adolescent Psychiatry in Marburg and Würzburg. Subjects were aged between 8 and 19 years (mean = 12.53, SD = 2.22, no age restriction was applied

in order to obtain a large sample). Our aim was to investigate the association between neurophysiologic endophenotypes of dyslexia, the MMN and the late MMN components elicited by consonant–vowel–syllables (/da/-/ba/).

Children with potential difficulties in reading or writing and children who had been diagnosed with dyslexia were referred to the investigators by parents, teachers, special educators or practitioners. All parents and probands aged over 14 years gave informed written consent for participation in the study. All children participating in the study were of German descent. The ethics committees at each clinical site approved the study.

Since clinical studies on dyslexia in Germany usually use spelling disorder as an inclusion criterion and our previous findings are also based on this selection (Schulte-Körne et al. 1998b; Schulte-Körne et al. 2007; Schumacher et al. 2006; Ziegler et al. 2005), the probands' spelling ability was used as the criterion for inclusion. Spelling was measured using an age-appropriate spelling-test (writing to dictation), and an observed spelling score was calculated on the basis of a correlation between the proband's IQ and spelling of 0.4 based on correlation analyses in different samples of German school age children (Schulte-Körne 2001; Schulte-Körne et al. 2001a). Children were classified as "affected" when the spelling score (mean = 26.70, SD = 5.01, range = 14–38) and the spelling score expected according to IQ showed a discrepancy of ≥ 2 SD (mean = 2.65, SD = 0.45, range = 2.01–4.27). We also investigated reading fluency by means of a single word and non-word reading test (Salzburger Lese- und Rechtschreibtest, (Moll and Landerl 2010)). This test renders *t*-values for children up to the fourth grade that are distributed with a mean = 50 (SD = 10) in unaffected children (Moll and Landerl 2010). Because there were no standardized German reading tests at or above the fifth grade available, a non-standardized reading test was administered to the older children (Schumacher et al. 2006). Aside from spelling, all of the probands also had a reduced reading fluency.

Families were excluded from the study when either the proband or a sibling presented symptoms of ADHD according to a standardized clinical interview which was conducted with the mother (Unnwehr et al. 1998). Furthermore, families were not included if the proband presented a bilingual education, an IQ <85 , an uncorrected disorder of peripheral hearing or vision, a psychiatric or neurological disorder possibly having an impact on the development of reading and spelling ability or an age greater than 21 years.

ERP measures

EEG was recorded during a passive oddball paradigm using 1700 standard (85%) /da/ and 300 deviant (15%) /ba/ synthetic

syllables (Schulte-Körne et al. 1998a; Schulte-Körne et al. 1999; Schulte-Körne et al. 2001b). The stimuli were synthesized with the Computerized Speech Research Environment (Computerized Speech Research Environment (CSRE) (1995) London:AVAAZ Innovations, Inc) with a sampling rate of 10 kHz. Stimulus length was 240 ms and ISI (offset to onset) was 740 ms. The stimuli were presented in a pseudorandom order with at least five standards between any two deviants with a sound pressure level of 70 dB(A).

Subjects were seated in a comfortable chair in a quiet room. They were specifically instructed not to attend to the presented stimuli, and to aid them in this, a self-selected film was shown silently on a TV monitor.

The EEG was recorded with a Neuroscan amplifier at 250 Hz sampling rate at 29 scalp sites of the international 10–20 system: Fp1, Fp2, F7, F3, Fz, F4, F8, FT7, FC3, FCz, FC4, FT8, T3, C3, Cz, C4, T4, TP7, CP3, CP4, TP8, T5, P3, Pz, P4, T6, O1, Oz, O2 plus two additional EOG electrodes below the subjects' right and left eyes and an additional electrode at right mastoid. The reference electrode was placed at the left mastoid. The EEG was offline re-referenced to averaged mastoids, and filtered with a 0.53–40 Hz bandpass using Brainvision Analyzer (www.brainproducts.com). Eye artefacts were corrected by performing an independent component analysis (ICA), with manual identification and exclusion of the eye artefact components. Further artefacts were removed by excluding trials automatically with two gradients (allowed maximum of 50 μ V per sample point; maximum allowed absolute difference 150 μ V in 200 ms) and a max–min (maximum amplitude of $\pm 100 \mu$ V) criteria. Signals were averaged into epochs of 1100 ms, including a pre-stimulus baseline of 100 ms. The MMN was calculated as the difference between the averages of deviant and standard trials. For the average of standard stimuli the first three standard stimuli following each deviant stimulus were omitted. The minimum number of accepted trials was 47. The average number of accepted trials was 271 for deviant stimuli and 372 for standard stimuli. Two components were identified via running *t*-test over the grand average of the whole sample: MMN (measuring peak amplitude, 188–300 ms, mean = -2.27μ V, SD = 1.34) and late MMN (mean amplitude, 300–710 ms, mean = -0.63μ V, SD = 0.91). Analysis of the age versus MMN effects via regression analysis revealed no significant age effect for any of the components. The components are illustrated in Fig. 1. For the two MMN components the mean of nine electrodes over fronto-central sites (F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4) went into further analysis, because in our previous studies this selection was suited to find reliable group differences between dyslexic children and controls and we expected that at these electrodes the cortical activity from

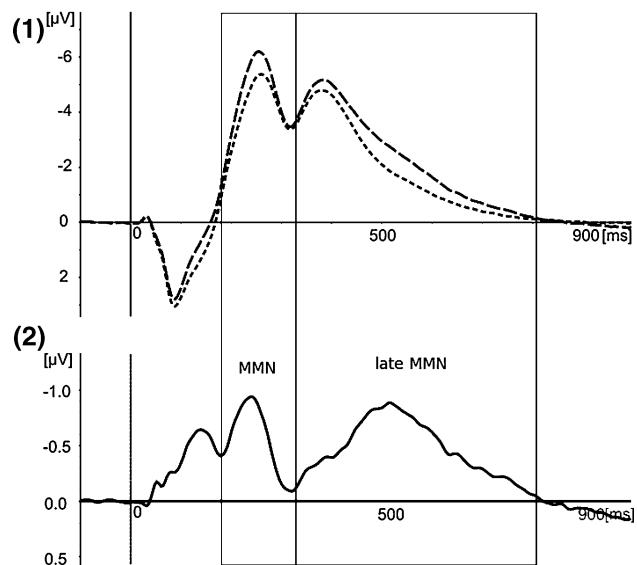


Fig. 1 MMN curves Grand average (mean value of F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4) of **a** the standard/da/ (dotted line) and deviant/ba/ (dashed line) curves and **b** the mismatch negativity demonstrating the MMN (time window 188–300 ms) and the late MMN (time window 300–710 ms)

the bilateral superior temporal cortices were registered and correspond to other studies investigating speech MMN in dyslexic children (Huttunen-Scott et al. 2008).

SNP genotyping

We used Illumina HumanHap300 k Bead Chips. Each chip contained 317503 genomic markers (single nucleotide polymorphism, SNPs) with a mean spacing of 7.9 kb between neighbouring SNPs. Data were analysed using BeadStudio, a software package provided by the manufacturer. SNPs with either a minor allele frequency <1%, a call rate <98% or a deviation from Hardy–Weinberg–Equilibrium ($-\log_{10}(p\text{-value})$) higher than five in the exact test) were removed from further analysis. 297086 SNPs (93.6%) passed these quality criteria. All individuals presented with a genotype call rate of at least 98%.

Genotyping of imputed variants of interest was carried out on the MassArray system on a Sequenom Compact MALDI-TOF device using iPLEX Gold reagents (Sequenom Inc., San Diego, CA) in multiplex reactions. Primer sequences and standard assay conditions are available from the authors upon request. Genotype data were obtained using SpectroTYPER software 4.0 (Sequenom). All SNPs and all individuals presented with a call rate of 100%.

SNP imputation

Imputing of genotypes, i.e. estimation of genotypes based on the LD structure between SNPs in a reference

population, was performed with the imputation software programme MACH 1.0 (<http://www.sph.umich.edu/csg/abecasis/MaCH/tour/imputation.html>). We used public available genotypes from 112 CEU individuals, i.e. Utah residents with Northern and Western European ancestry, which were sequenced within the 1000 Genomes project (<http://www.1000genomes.org/>) as a reference sample and inferred genotypes using a greedy algorithm with 50 iterations in the MCMC procedure.

Statistical analysis

For imputed SNPs, we ran “palinear” as implemented in ProbABEL (<http://mga.bionet.nsc.ru/~yurii/ABEL/>). Genotyped SNPs were analysed for association using linear regression in R (<http://www.r-project.org/>).

Results

We screened *DCDC2* and *KIAA0319* as well as the region in between these genes for association with early MMN and late MMN including 20 kb upstream and downstream (chr6:24,259,963-24,774,362; NCBI Reference Sequence: NM_016356.3 and NM_014809.3). After quality control, 87 genotyped SNPs were present in this region. Additionally 2152 imputed SNPs were analyzed.

We found no association with either early MMN or late MMN based on the genotyped SNPs with a significance level lower than $1.0e^{-3}$. Concerning the imputed SNPs, we detected four variants associated with late MMN and reaching *p*-values $<1.0e^{-3}$. One of these variants was located in the intronic region of *DCDC2* and three were present in the region between *DCDC2* and *KIAA0319*. The corresponding Manhattan plots depicting the association *p*-values between imputed markers and early MMN and late MMN are shown in Fig. 2a, b.

All four variants are rare with minor allele frequencies below 2%. Therefore, the imputation quality, measured as the squared correlation between estimated and true genotypes, is rather low with quality scores ranging from 0.248 to 0.481.

To further elucidate our findings, we genotyped the four variants associated with late MMN and recalculated *p*-values for association. Further information on these variants and a comparison between imputed and genotyped SNPs is given in Table 1.

For all but one variant, findings from the imputation could be confirmed. However, most *p*-values increased as compared to the imputed dataset indicating an over-estimation of effects in the imputed data. One confirmed variant was located in an intron of *DCDC2*, the other two

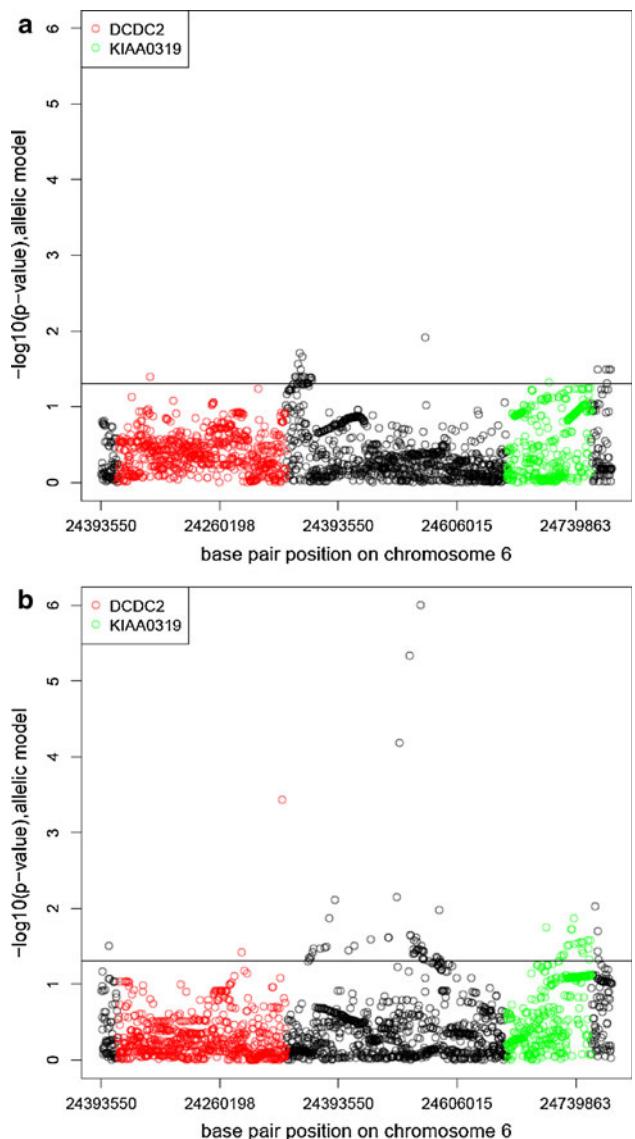


Fig. 2 **a** Manhattan plot for MMN in *KIAA/DCDC2* region. Hits located in *DCDC2* (chr6:24,279,963-24,466,259) are depicted in red, hits located in *KIAA0319* (chr6:24,652,311-24,754,362) are depicted in green. The horizontal line indicates a *p*-value of nominal significance ($p = 0.05$). **b** Manhattan plot for late MMN in *KIAA/DCDC2* region. Hits located in *DCDC2* (chr6:24,279,963-924,466,259) are depicted in red, hits located in *KIAA0319* (chr6:24,652,311-24,754,362) are depicted in green. The horizontal line indicates a *p*-value of nominal significance ($p = 0.05$) (Color figure online)

were in the intergenic region between *DCDC2* and *KIAA0319*. As depicted in Fig. 3a–c, in all cases, the minor allele was associated with an attenuated late MMN amplitude.

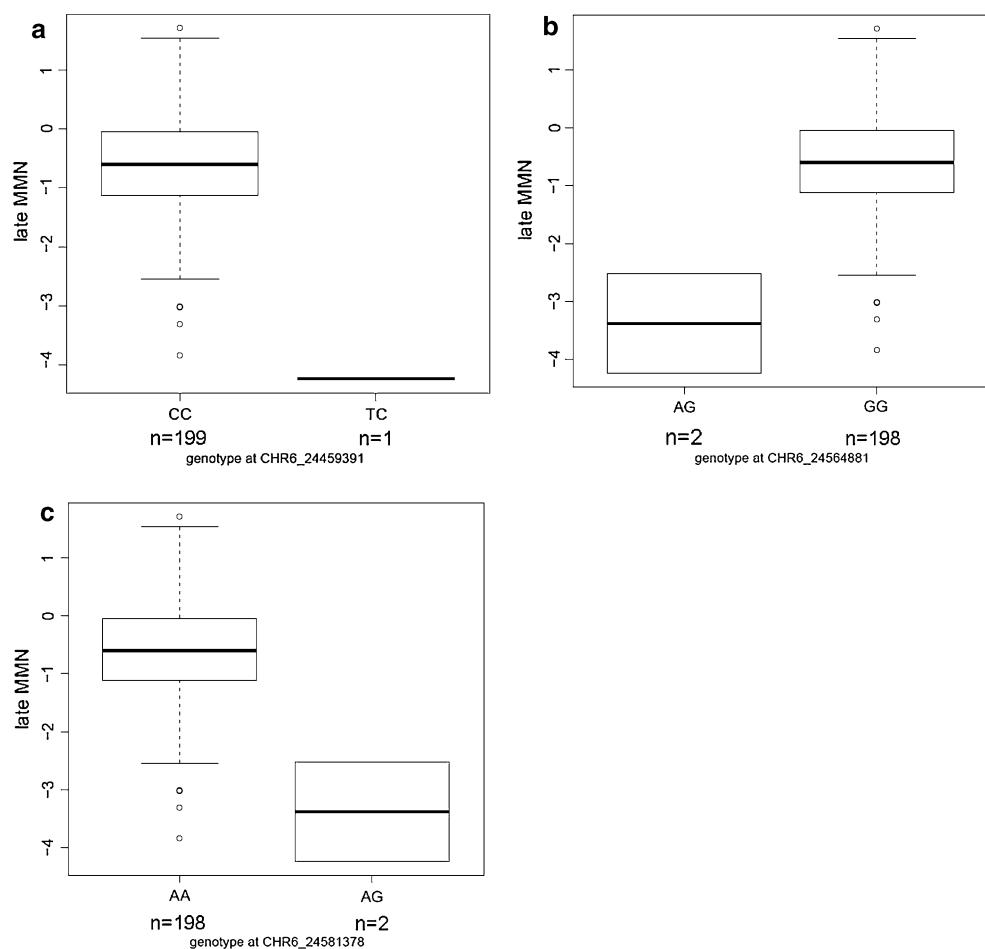
Concerning the LD structure between the variants (see Table 2), all markers were in high LD to each other. Chr6:24564881 and chr6:24581378 were in complete LD to each other, which means that they depict exactly the same signal, reaching a *p*-value of $1.04e^{-05}$.

Table 1 Results of association analysis with late MMN

SNP	Position	Gene	Minor allele	MAF ^a imputed data	MAF ^a genotyped data	Rsq ^b	Nominal <i>p</i> -value ^c imputed data	Nominal <i>p</i> -value genotyped data
Chr6:24459391	24459391	DCDC2	T	0.012	0.005	0.248	3.69e ⁻⁰⁴	6.28e ⁻⁰⁵
chr6:24564881	24564881	n.a.	A	0.011	0.010	0.481	6.63e ⁻⁰⁵	1.40e ⁻⁰⁵
chr6:24571041	24571041	n.a.	A	0.008	0.000	0.308	4.55e ⁻⁰⁶	NA ^d
chr6:24581378	24581378	n.a.	G	0.013	0.010	0.439	9.81e ⁻⁰⁷	1.40e ⁻⁰⁵

^a Minor allele frequency^b Quality measure of imputation: squared correlation between imputed and true genotypes^c All association results with a nominal *p*-value < 1.0e⁻⁰³ are depicted^d Genotyped marker is monomorph, therefore no calculation of association *p*-values possible

Fig. 3 **a** Boxplot for late MMN and chr6:24459391. For each genotype group, the median (middle black line) and the quantiles (75% top line, 25% bottom line) are represented by a rectangle. The dashed vertical lines illustrate the distribution (minimum–maximum) of the late MMN amplitude. Outliers, deviating more than 1.5-fold interquartile distance of the median, are represented by a circle. **b** Boxplot for late MMN and chr6:24564881. **c** Boxplot for late MMN and chr6:24581378



The question arises of whether or not this signal is independent on the effect of chr6:24459391 which is also in high LD to chr6:24564881 and chr6:24581378. We used linear regression to investigate this question and included chr6:24581378 and chr6:24459391 as independent variables, and late MMN as the dependent variable. In this combined model only chr6:24581378 was significantly associated with late MMN, chr6:24459391 reached a *p*-value of 0.17. Therefore, we were able to identify one signal in the region between *DCDC2* and *KIAA0319*.

Discussion

Based on imputation, we detected four variants associated with the late component of the speech MMN, three located between *DCDC2* and *KIAA0319*, and one located in the intronic region of *DCDC2*. However, due to the rarity of these variants the imputation quality was rather low. Therefore, in order to confirm our results we genotyped the imputed variants and repeated the statistical analysis. We were able to confirm that three rare variants were in fact

Table 2 LD structure between rare variants

SNP	Chr6:24459391	Chr6:24564881	Chr6:24571041	Chr6:24581378
chr6:24459391	1.0/1.0			
chr6:24564881	1.0/0.5	1.0/1.0		
chr6:24571041 ^a	1.0/1.0	1.0/1.0	1.0/1.0	
chr6:24581378	1.0/0.5	1.0/1.0	1.0/1.0	1.0/1.0

Values are given as D'/r^2

^a Monomorphic marker

associated with the late MMN component. We were further able to demonstrate that these variants were in high LD to each other, which indicates that they depict the same signal. Because these results are based on rare variants, meaning that only one or two individuals carry the risk allele, larger samples will be required to confirm these results. Nonetheless, these results provide an initial clue to a possible association between the late MMN and rare variants located in the *DCDC2/KIAA0319* region.

In previous association studies for *KIAA0319*, the most significant associations were found to be clustered around the first intron and the predicted promoter region of this gene. Cope et al. (2005) identified three SNPs located in *KIAA0319* to be associated with dyslexia in two case–control samples from the UK. None of these SNPs were associated with the early MMN or late MMN in our sample. Francks et al. (Francks et al. 2004) reported a three SNP haplotype in the *KIAA0319* region which was associated with reading disability, but these SNPs were also not replicated in our sample. In the present study however, we focused on imputation based association results and therefore haplotype association analysis was not performed.

To our knowledge, association between both MMN components and SNPs located in *KIAA0319* or *DCDC2* has not yet been studied. Our finding of an association of rare variants with late MMN in a speech processing paradigm might lead to the conclusion that these variants are of specific significance for the late component. Recently we found an association with a SNP on chromosome 4 and the late MMN component in dyslexia. This SNP had a trans-regulation effect on *SLC2A3*, a gene on chromosome 12, and the predominant facilitative glucose transporter in neurons (Roeske et al. 2009). We identified a risk haplotype, which leads to a reduced expression of the *SLC2A3* and which might impact speech processing in children with dyslexia. The present study and the study by Roeske et al. provide evidence for a genetic influence on the late MMN component elicited by speech and do not show any effects related to the classical MMN in the early latency window. Therefore, we hypothesize that those neurophysiologic correlates of speech perception in dyslexia which are under genetic influence are mainly related to later cognitive processes.

The overall significance of the late MMN component is still unclear. Unfortunately, some studies investigating speech MMN in children with dyslexia did not analyse this component although this component was visible in the grand average (Huttunen-Scott et al. 2008). In studies where the late MMN to speech sounds was investigated there was clear evidence for lower amplitudes in participants with dyslexia (Hommet et al. 2009; Schulte-Körne et al. 1998a). Therefore, we conclude that, at least in auditory speech perception in dyslexia, the late MMN component is a relevant neurophysiologic correlate.

In comparison to the classical MMN, which has been registered in 6 month old children and is relatively stable throughout the lifetime, the late MMN emerges in school-aged children and decreases in amplitude in adulthood (Hommet et al. 2009; Cheour et al. 2001). Furthermore, the topography of the late MMN changes with age. In children, the generator was found to be mainly located in the centro-parietal regions of the right hemisphere, whereas in adults a more central distribution was described (Cheour et al. 2001; Hommet et al. 2009). In both the present study and our previous study (Roeske et al. 2009) we did not confirm lateralisation of the late MMN component. Because of the later latency of this MMN component in comparison to the earlier MMN, which is associated with the perception of the sensory difference of two speech sounds, different speech related processes are most likely associated with its activity. This could be a semantic process (Korpilahti et al. 2001), an attention related discrimination process (Shestakova et al. 2003) or a memory related automatic processing of speech information (Hommet et al. 2009).

To date, a British study is the only investigation which has sufficiently covered both genes at the same time (Harold et al. 2006). While no evidence for association between dyslexia and *DCDC2* was obtained, association for *KIAA0319* was observed. Also, a weak interaction between both genes was detected. Our group has also found nominal significant interaction between both loci in a trio sample from Germany when considering the phenotype word reading (Ludwig et al. 2008). The fact that interactions between markers located in *DCDC2* and markers located in *KIAA0319* (with regard to dyslexia or dyslexia related phenotypes) were observed points to an association

and interplay between these two genes rather than just an additive association effect of both genes.

Currently, there is no study where association for *KIAA0319* and *DCDC2* has been found in the same sample. It is interesting that the signal in the present study is located between *KIAA0319* and *DCDC2*. All identified variants are rare and have not been described in other studies. Replication and functional analysis will be necessary but our results could hint at a possible link between *KIAA0319* and *DCDC2*. The location of the finding between these two genes may point to a possible reconciliatory interpretation of the findings brought forward by the various groups working in the region, sometimes showing more evidence for *KIAA0319*, sometimes more for *DCDC2*. Rare variants located in this gene region, as well as genome-wide, have not yet been explored in detail. Looking more closely into these areas in other samples of dyslexia might reveal further insight into dyslexia and its endophenotypes.

Our results, which await replication, suggest an involvement of rare variants in the speech perception of dyslexic children. In future studies and given that larger samples can be studied, it would be interesting to include rare variants on a genome-wide level in association studies regarding dyslexia and dyslexia related endophenotypes.

However, it should be noted that in the present study we have only investigated dyslexic children and did not explore these effects in typically reading children. Thus, the identified effect might also be observed in a control group. If so, such a finding would indicate that our results are not specific for dyslexia per se, but would mean that the rare variants involved are rather indicative of the general processing of speech sounds. To enlighten this point, it is therefore necessary to confirm or reject this finding in a control population.

Furthermore, the investigation of functional evidence for these rare variants in animal models might be a very promising step for the understanding of one causal mechanism relevant for dyslexia. Most candidate genes (i.e. *DCDC2*, *KIAA0319*, *DYX1C1*) play a functional role in neuronal migration. As this functional impairment is found in most brain tissues, it is not specific enough to explain the circumscribed deficits in dyslexia like speech processing deficits.

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