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ORIGINAL ARTICLE

The role of READ1 and *KIAA0319* genetic variations in developmental dyslexia: testing main and interactive effects

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Developmental dyslexia (DD) is a complex heritable condition characterized by impaired reading abilities. Two well-replicated candidate risk factors are as follows: (1) regulatory element associated with dyslexia 1 (READ1), which is located in intron 2 of *DCDC2* and acts as a binding site for protein regulation of *DCDC2* expression; and (2) a three-single-nucleotide polymorphism risk haplotype spanning *KIAA0319*. Phylogenetically similar READ1 variants showed synergistic effects with the *KIAA0319* risk haplotype on reading-related phenotypes in a general population sample. Here we examine the association between different allele classes in READ1, the *KIAA0319* risk haplotype and reading-related traits in a cohort of 368 Italian children with DD and their siblings ($n = 266$) by testing both main and non-additive effects. We replicated the deleterious main effects upon both reading accuracy and speed exerted by the longer READ1 alleles. We further supported the interdependence through non-additive, possibly antagonistic, effects between READ1 and the *KIAA0319* risk haplotype on reading accuracy. By suggesting the presence of common biological processes underlying reading (dis)ability, these findings represent initial support for a generalist effect of the non-additive interdependence between READ1 and the *KIAA0319* risk haplotype. Moreover, our results confirm that using as much information as possible about genetic interdependence among dyslexia-candidate genes can help in clinically assessing the individual risk for DD.
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INTRODUCTION

Developmental dyslexia (DD), the most common learning disability among school-aged children across languages, is a lifelong impairment in reading acquisition, unexplained by deficits in general intelligence, neurological and sensorial conditions, and educational opportunities.[1] It is one of the most common complex neurodevelopmental disorders, which affects 5–12% of the population according to the applied diagnostic criteria, and is often associated with undesirable outcomes[2] as well as with negative social impact and economic burden.[3]

Following earlier descriptions of strong familial aggregation of the disorder,[4] substantial heritability typical of a complex trait has been reported,[5] with estimates ranging from 0.18 to 0.72.[6] Since the early 1980s, at least nine DD risk loci termed DYX1–DYX9 on eight different chromosomes have been mapped (1p36-p34, 2p16-p15, 3p12-q13, 6p22 and 6q13-16.2, 11p15.5, 15q21.3, 18p11.2 and Xq27.3) and the involvement of several genes spanning these regions in the etiology of DD has been reported (*DYX1C1*, *DCDC2*, *KIAA0319*, *C2ORF3*, *MRPL19*, ROBO1, *FAM176A*, *NRSN1*, *KIAA0319L* and *FMR1*).[7] Apart from the DYX loci, additional genes implicated in other disorders, such as *FOXP2*, *CNTNAP2*, *DOCK4* and *GTF2I* on chromosome 7, *GRIN2B* and *SLC2A3* on chromosome 12, *ATP2C2* and *CMIP* on chromosome 16, and *PCNT*, *DIP2A*, *S100B* and *PRMT2* on chromosome 21,[7] have also been associated with reading (dis)ability. Among these genes, nine DD-candidate genes have been replicated in at least one independent sample, that is, *DYX1C1*, *DCDC2*, *KIAA0319*, *C2ORF3*, *MRPL19*, ROBO1, *GRIN2B*, *FOXP2* and *CNTNAP2*.[7]

To date, DYX2 on chromosome 6p21.3 is the most reliable and interesting risk locus. Within this locus, two genes, that is, *KIAA0319* and *DCDC2*, are by far the most replicated.[2,8] Both genes affect neuronal migration, neurite outgrowth, cortical morphogenesis, and ciliary structure and function,[9–19] and they are associated with deficits in specific cognitive functions such as rapid auditory processing, spatial learning and memory in animal studies.[20–22] Interestingly, initial evidence has shown the presence of putative functional genetic variants spanning these genes and influencing gene expression.

A haplotype composed by three-single-nucleotide polymorphism spanning *TTRAP*, *THEM2* and *KIAA0319* (hereafter, KIAHap) has been described (identified as 1-1-2, where '1' refers to the common allele and '2' refers to the minor allele of rs4504469, rs2038137 and rs2143340).[23] Compared to the non-risk haplotype, KIAHap is associated with 40% lower levels of expression, splicing or transcript stability of any of the *KIAA0319*, *TTRAP* or *THEM2* genes.[23] Although negative findings have been reported,[24,25] KIAHap has been shown to

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be associated with reading, spelling, orthographic and phonological skills in reading-impaired families,[23,26] as well as in general population samples.[27,28]

A highly polymorphic short-tandem repeat (BV677278) harbored in a 168-base pair (bp) purine-rich region in intron 2 of the *DCDC2* gene has been described.[9] This non-coding region might serve as a regulatory node, as it contains 131 putative transcription factor-binding sites, is rather conserved across species and enhances activity, as it changes reporter gene expression.[29] Recently, Powers *et al.*[30] identified the BV677278-binding protein as transcription factor ETV6, confirmed BV677278 as a regulatory element and proposed 'regulatory element associated with dyslexia 1' (READ1) as new name. By its own nature, READ1 could substantially act as a modifier of *DCDC2* gene expression.[31] A naturally occurring deletion encompassing READ1 has been reported and associated with DD and DD-related phenotypes.[9,32–35] Interestingly, READ1 includes both deleterious and protective alleles for reading and language deficits.[31] In particular, alleles 5 and 6 were significantly associated with, respectively, severe reading and language impairments,[31] and have been shown to be in linkage disequilibrium with two six-marker risk haplotypes within the same *DCDC2* block.[30] When alleles 5 and 6 were combined, the resulting composite allele was associated with both reading and language deficits.[31] The same is true of 'clade 1', which represents a composite including alleles 5 and 6, phylogenetically related rare alleles and 'long alleles', that is, alleles >105 bp in length, regardless of their structure.[31] On the other hand, another group of READ1 alleles ('allele 3', 'RU1-1', that is, variants with only one iteration of the 13-bp repeat unit 1; and 'short alleles', that is, alleles with <90 bp regardless of their structure) suggested a protective effect for severe reading deficits.[31]

Barring a few exceptions,[30,31,33,36,37] *DCDC2* and *KIAA0319* have only been investigated independently until now. Nevertheless, significant interactions among single-nucleotide polymorphisms spanning these two genes on DD-related phenotypes have been reported in three independent samples.[33,36,37] Recently, Powers *et al.*[30,31] focused their attention on KIAHap and READ1 and questioned whether they might interact genetically. In a large general population sample, subjects having both a *DCDC2* risk haplotype and KIAHap showed deficits on several reading, language and cognitive measures greater than the sum of the deficits associated with the risk haplotype in one gene or the other, indicating a synergistic interaction between these two variants.[30] Similarly, nominally significant data showed that having both KIAHap and different READ1 allelic classes led to both worse and better performance in reading and language tasks than would be expected by an additive effect, confirming a synergistic regulatory effect between these two functional genetic risk variants.[31] Interestingly, this genetic interaction is unequal for different READ1 allelic classes. Detrimental READ1 allelic classes (that is, 'allele 5', 'allele 6', 'alleles 5 and 6', and 'clade 1') synergize with KIAHap to reduce performance on neuropsychological traits. By contrast, protective READ1 alleles (that is, 'allele 3' and 'RU1-1') epistatically suppress KIAHap's deleterious effect.[31] Moreover, family data suggest that these variants genetically interact *in trans*.[31] These findings provided initial support for the regulation, in magnitude and/or in direction, of the effects of any single-gene variation by other genetic variants, potentially at the biological level.[30,31,38,39] Moreover, these data advocate that studying the etiology of DD can be helped by the investigation of the influences of familial transmission of non-additive interactions between candidate genes,[31,37] explaining part of its missing heritability.[40] It is indeed plausible to hypothesize that an interactive model can explain the underlying mechanism responsible for complex traits, such as DD.[41–43] Finally, these results support the importance of focusing on selective, putative functional genetic variants spanning DD-candidate genes, leading to a better comprehension of the biological mechanisms underlying reading (dis)ability.[7,31]

Understanding the extent to which genes affecting normal variation in learning abilities also affect learning disabilities is crucial to figure out how much genetic influences overlap across the entire phenotypic liability distribution.[6] Previous studies showed that READ1 and KIAHap may represent good candidates for these shared genetic influences, as—as stated above—they showed significant association both in clinical[9,23,32–36,44] and epidemiological[27,28] samples.

In the context of these studies, we examined the associations between different allele classes in READ1 and KIAHap, which have previously been associated with language and reading phenotypes in a large general population sample,[30,31] and reading-related traits in a nuclear family-based sample of children with DD, by testing both main and non-additive effects.

MATERIALS AND METHODS

The Scientific Review Board and the Ethical Committee of the Scientific Institute, IRCCS Eugenio Medea approved the protocol.

Sample

This study is based on an ongoing project about the genetic etiology of DD.[35,37,45–50] Details about the ascertainment scheme have been described elsewhere.[45] Parental written informed consent was obtained for all recruited subjects. Nuclear families were recruited if probands met the criteria for DD according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition,[51] confirmed by an extensive medical assessment and a battery of tests evaluating cognitive abilities (that is, the Wechsler Intelligence Scale for Children, revised[52] or the Wechsler Intelligence Scale for Children, third edition[53]), text, single-word and single-nonword reading,[54–56] writing under dictation of words, nonwords and sentences containing homophones,[56] forward/backward letter/digit spans,[57] phonemic elision and blending,[58] language,[49,59] and mathematical abilities.[60,61] Standardized scores on the Italian population are available for all tests (Supplementary Information 1). The probands' affection status was defined as follows: (1) an absolute score at least 2 s.d.'s below the expected mean grade level on accuracy or speed in a timed text-reading, unrelated word- or nonword-reading tests; (2) total intelligence quotient (IQ) $\geqslant 85$; and (3) no neurological and sensorial disorders. As vocabulary and block design show a high correlation (r) with, respectively, verbal IQ ($r=0.82$) and performance IQ ($r=0.73$),[52,53] only these subtests were administered to fully biological siblings. Siblings were included if they were over 6 and under 18 years old, if the mean score of their vocabulary and block design subtests was above 7 (that is, -1 s.d.), irrespective of their reading scores, and if they had no neurological and sensorial disorders. DNA was extracted from either mouthwash or blood samples in both probands and their biological siblings.

Phenotype description

For the current investigation, we considered parameters that tap both the number of errors in reading accuracy (acc) and reading speed (Supplementary Information 1): (a) text reading (TR), TRacc and TRspeed;[54,55] (b) single-word reading (SWR), SWRacc and SWRspeed;[56] and (c) single-nonword reading (SNWR), SNWRacc and SNWRspeed.[56] Descriptive statistics are reported in Supplementary Table 1.

Genotyping

Genotyping data for markers spanning *DCDC2* and *KIAA0319* were available from previous studies and are described elsewhere.[35,50] Briefly, we performed the *DCDC2* intron 2 deletion's genotyping as described by Meng *et al.*[9] In particular, READ1 and the common 2445-bp deletion were genotyped by allelic-specific amplification with a combination of three primers in one reaction. We examined allele classes spanning READ1, which have previously

Table 1 Significant associations of READ1 and KIAHap from the one-way MANCOVA[a] upon the selected reading-related phenotypes in the total sample ($n = 634$)

	Mean	*s.d.*	*Mean*	*s.d.*	*Roy's largest root*				
	No risk (n = *491*)		*Allele 6* (n = *62*)		*F*	P-*value*	*F*	P-*value*	*Effect size*[b]
TRacc	−0.853	1.296	−1.414	1.592	3.181	0.004	14.127	<0.001	0.025
SWRacc	−1.782	2.102	−2.312	2.248			5.428	0.020	0.010
SWRspeed	−2.066	2.405	−2.544	2.531			4.119	0.043	0.007
SNWRacc	−1.145	1.633	−1.728	2.059			10.595	0.001	0.019
	No risk (n = *451*)		*Alleles 5 and 6* (n = *102*)						
TRacc	−0.842	1.294	−1.244	1.503	2.27	0.036	8.674	0.003	0.016
SWRspeed	−2.014	2.378	−2.585	2.566			5.514	0.019	0.010
SNWRspeed	−1.668	1.947	−2.136	2.101			5.255	0.022	0.009

Abbreviations: acc, accuracy (number of errors); MANCOVA, multivariate analysis of covariance; READ1, regulatory element associated with dyslexia 1; TR, text reading; SWR, single-word reading; SNWR, single-nonword reading.
[a]Relatedness was entered as covariate.
[b]Partial η^2.

been shown to affect reading-related measures.[31] Specifically, we considered: (i) allele 3; (ii) allele 5; (iii) allele 6; (iv) combined allele 5 and allele 6 (hereafter, 'alleles 5 and 6'); (v) 'clade 1'; and (vi) 'RU1-1'.

The genomic region surrounding *KIAA0319*-rs2038137 was sequenced through PCR amplification and direct sequencing (primers available upon request). Genotyping of *KIAA0319*-rs4504469 and *TTRAP*-rs2143340 was performed by single-nucleotide polymorphism genotyping with TaqMan probes (Life Technologies, Waltham, MA, USA) using an allelic discrimination real-time PCR method. The rs4504469, rs2038137 and rs2143340 KIAHap[23] has been modeled in further analyses.

Supplementary Table 2 shows allele frequencies for the allelic classes under consideration.

Statistical analysis

To obtain the individual haplotype phase of *KIAA0319*, the '–hap-phase' command in PLINK v1.07 was used (http://pngu.mgh.harvard.edu/purcell/plink/). We retained the haplotype phase with the most likely phase for each subject (that is, BEST = 1).

Pearson's bivariate correlation analyses were used to assess the degree of relatedness among the selected reading-related phenotypes. On the basis of the observed degrees of inter-correlation among them (mean $r = 0.46$; Supplementary Table 3), traits were jointly analyzed by multivariate analysis of variance to investigate both the main effects of READ1 allelic variants and KIAHap, and the effect of READ1 allelic classes in the presence/absence of KIAHap (hereafter, non-additive effect). As expected in a clinical sample, our data are not normally distributed. Although simulation studies have shown that the false positive rate in multivariate analysis of variance is minimally affected by the violation of the assumption on normal distribution,[62–64] we took care of normalizing the phenotypic distributions by excluding outliers. To reach acceptable limits for asymmetry and kurtosis of ± 2.00,[65,66] we excluded subjects whose phenotypic scores were below the first (TRacc, SWRacc, SWRspeed, SNWRacc and SNWRspeed; $n = 6$) or the fifth (TRspeed; $n = 31$) percentile of the phenotypic distribution (Supplementary Table 4). In both main and non-additive analyses, genetic information was entered as the independent variable upon the selected reading-related phenotypes, which were entered as dependent variables. Partial η^2 values were calculated as a measure of effect size for group mean differences. Finally, to control for non-independence for heritable measures, we considered family relatedness as covariate in further multivariate analysis of covariance by assigning '1' to each proband and '2' to each sibling within each family.[67] All analyses were implemented with SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Phenotypic and genotypic data were available for 368 Italian unrelated children with DD (mean age = 10.52 ± 2.64, male:female ratio = 2.3:1), and 266 siblings (mean age = 12.67 ± 3.48, male:female ratio = 1.4:1), of which 38.0% were affected by DD. All the 634 offspring had already participated in previous studies.[37,49,50]

Main effects: association analysis

Table 1 and Supplementary Table 5 show associations of READ1 allelic classes and KIAHap with the selected reading-related phenotypes. 'Allele 6' is associated with impairment in TRacc, SWRacc, SWRspeed and SNWRacc (Roy's largest root = 3.181, $P = 0.004$; $F_{(1,552)} = 14.127$, partial $\eta^2 = 0.025$, $P < 0.001$; $F_{(1,552)} = 5.428$, partial $\eta^2 = 0.010$, $P = 0.020$; $F_{(1,552)} = 4.119$, partial $\eta^2 = 0.007$, $P = 0.043$; and $F_{(1,552)} = 10.595$, partial $\eta^2 = 0.019$, $P = 0.001$, respectively). 'Alleles 5 and 6' is associated with TRacc, SWRspeed and SNWRspeed (Roy's largest root = 2.270, $P = 0.036$; $F_{(1,552)} = 8.674$, partial $\eta^2 = 0.016$; $P = 0.003$; $F_{(1,552)} = 5.514$, partial $\eta^2 = 0.010$, $P = 0.019$; and $F_{(1,552)} = 5.255$, partial $\eta^2 = 0.009$, $P = 0.022$, respectively). On average, individuals with 'alleles 5 and 6' perform worse in these tasks than subjects without this allelic class. No significant associations have been reported for KIAHap.

To control for the effect of sex, we repeated the same analyses considering 'sex' as covariate. No significant sex effect was found as findings overlapped with the results without this covariate (data not shown).

Non-additive effects

Table 2 and Supplementary Table 6 show non-additive effects obtained by comparing the mean performance of the selected reading-related traits among subjects with different composites of KIAHap and READ1 allelic classes. KIAHap seems to epistatically negate the effect of 'allele 6' on TRacc, SWRacc and SNWRacc (Roy's largest root = 4.100, $P < 0.001$; $F_{(3,552)} = 6.629$, partial $\eta^2 = 0.035$, $P < 0.001$; $F_{(3,552)} = 2.932$, partial $\eta^2 = 0.016$, $P = 0.033$; and $F_{(3,552)} = 4.558$, partial $\eta^2 = 0.024$, $P = 0.004$, respectively). Likewise, when 'allele 5' and 'allele 6' are combined, the resulting composite shows a significant synergistic interaction with KIAHap on TRacc (Roy's largest root = 2.956, $P = 0.008$; $F_{(3,552)} = 4.574$, partial $\eta^2 = 0.024$, $P = 0.004$). Individuals having at least one copy of both

Table 2 Significant synergistic effects between alleles classes of READ1 and KIAHap from the one-way MANCOVA[a] upon the selected reading-related phenotypes in the total sample ($n = 634$)

	Mean	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*	*Roy's largest root*				
	No risk (n = 334)		*KIAHap (n = 157)*		*Allele 6 only (n = 51)*		*KIAHap[a] allele 6 (n = 11)*		*F*	*P-value*	*F*	*P-value*	*Effect size[b]*
TRacc	−0.908	1.281	−0.737	1.323	−1.556	1.609	−0.754	1.390	4.100	<0.001	6.629	<0.001	0.035
SWRacc	−1.829	2.071	−1.682	2.168	−2.519	2.364	−1.354	1.287			2.932	0.033	0.016
SNWRacc	−1.124	1.571	−1.189	1.764	−1.901	2.082	−0.924	1.822			4.558	0.004	0.024
	No risk (n = 302)		*KIAHap (n = 149)*		*Alleles 5 and 6 only (n = 83)*		*KIAHap[a] alleles 5 and 6 (n = 19)*						
TRacc	−0.896	1.268	−0.732	1.341	−1.349	1.550	−0.782	1.201	2.956	0.008	4.574	0.004	0.024
	No risk (n = 306)		*KIAHap (n = 136)*		*Clade 1 only (n = 79)*		*KIAHap[a] clade 1 (n = 32)*						
TRacc	−0.921	1.299	−0.695	1.325	−1.275	1.485	−0.919	1.318	3.094	0.005	4.232	0.006	0.023
SNWRacc	−1.159	1.623	−1.031	1.674	−1.489	1.806	−1.772	2.021			3.997	0.008	0.021

Abbreviations: acc, accuracy (number of errors); MANCOVA, multivariate analysis of covariance; READ1, regulatory element associated with dyslexia 1; TR, text reading; SNWR, single-nonword reading.
Clade 1 represents a composite including alleles 5, 6, 11, 12, 13, 14, 20 and 21 (Powers *et al.*[31]). KIAHap refers to the haplotype phase 1-1-2 encompassing markers rs2038137, rs4504469 and rs2143340 (Francks *et al.*[23]).
[a]Relatedness was entered as covariate.
[b]Partial η^2.

'alleles 5 and 6' and KIAHap, on average, perform like subjects with only the READ1 allelic class on TRacc (Figure 1). 'Clade 1' interacted significantly with KIAHap and the magnitude of the interaction is recapitulated for both TRacc and SNWRacc (Roy's largest root = 3.094, $P = 0.005$; $F_{(3,552)} = 4.232$, partial $\eta^2 = 0.023$, $P = 0.006$; and $F_{(3,595)} = 3.997$, partial $\eta^2 = 0.021$, $P = 0.008$, respectively; Figure 1). The regions of interest on chromosome 6 (that is, READ1 allelic classes and KIAHap) and the modulatory pathways between them are graphically depicted in Figure 2.

The same results were obtained when the same analyses were run with 'sex' as covariate (data not shown).

DISCUSSION

Although evidence of strong genetic effects in DD has been consistently reported, the ultimate goal of the latest research is to understand how non-additive effects among genes affect behavior.[68] Surprisingly, even if the above notions have surfaced in recent years, the implementation of such a framework to explore the non-additive effects of genes is quite new. Therefore, few studies have attempted to take gene-by-gene interaction effects in DD into account.[30,31,33,36,37] With the exception of one work,[37] the above-mentioned studies do not exploit the molecular network involved in neuronal migration and neurite outgrowth underlying the etiology of DD.[69]

Here, in a genetically informed study of a nuclear family-based sample of school-aged children with DD, we explored the hypothesis that different allelic classes within two putative functional genetic variants spanning *DCDC2* and *KIAA0319*, that is, READ1 and KIAHap, could also be associated with deficits in reading across the whole distribution of liability.

The results herein replicate the deleterious main effects exerted by the longer READ1 alleles (that is, 'allele 6' and 'alleles 5 and 6') upon accuracy and speed in text, single-word and single-non-word reading.[31] To our knowledge, this study is the first showing the detrimental association between alleles >105 bp in length and reading-related phenotypes in a clinical sample, and it is in line with a previous analysis in general population.[31] These results are also consistent with the hypothesis that changes in READ1 modulates the regulatory effects of the transcription factor ETV6 homopolymer.[30] Moreover, we have not found any significant main effect of the KIAHap upon reading-related traits. Finally, our data provide further evidence that READ1 and KIAHap show interdependence through non-additive effects for reading accuracy. In particular, although our sample size is small and contrary to what was reported in a previous study,[31] KIAHap seems to non-additively overcome the adverse effect of the deleterious longer READ1 'allele 6', suggesting a protective role for KIAHap. In other words, KIAHap appears to epistatically suppress the deleterious effect of 'allele 6'. Likewise, when 'allele 6' is combined with 'allele 5' or with the other rare alleles in the 'clade 1' cluster, the non-additive effect is confirmed. Interestingly, the protective effect of KIAHap seems to be weaker when rare longer READ1 alleles are present. Briefly, in a clinical sample, KIAHap seems to exert a protective role for READ1-linked impairment in reading-related performance. Although these changes in performance are subtle, they show that the effects of KIAHap and READ1 on reading are interdependent, possibly antagonistic, where the effect of each relies upon the other. This supports the "phantom-heritability" hypothesis, which explains the so-called missing heritability of complex traits as a consequence of non-additive interactions between risk variants.[42,43,70] Even if our findings are in accordance with previous works reporting no association between KIAHap and reading-related traits,[24,25,50] and a reading advantage conferred by KIAHap with respect to all other haplotypes in a large sample of twin pairs,[27] other literature data showed that KIAHap is associated with impaired reading performance. These discrepancies may result from differences in entry criteria, sample size and study strategies. Concerning the entry criteria, Francks *et al.*[23] recruited families based on different inclusion criteria within the same study (that is, single-word reading ability below both −2.00 and −1.00 s.d.). The same criteria have been applied by Dennis *et al.*[26] Probands with an IQ ⩾85 and a reading age ⩾2.5 years behind the performance expected for that chronological age have been recruited

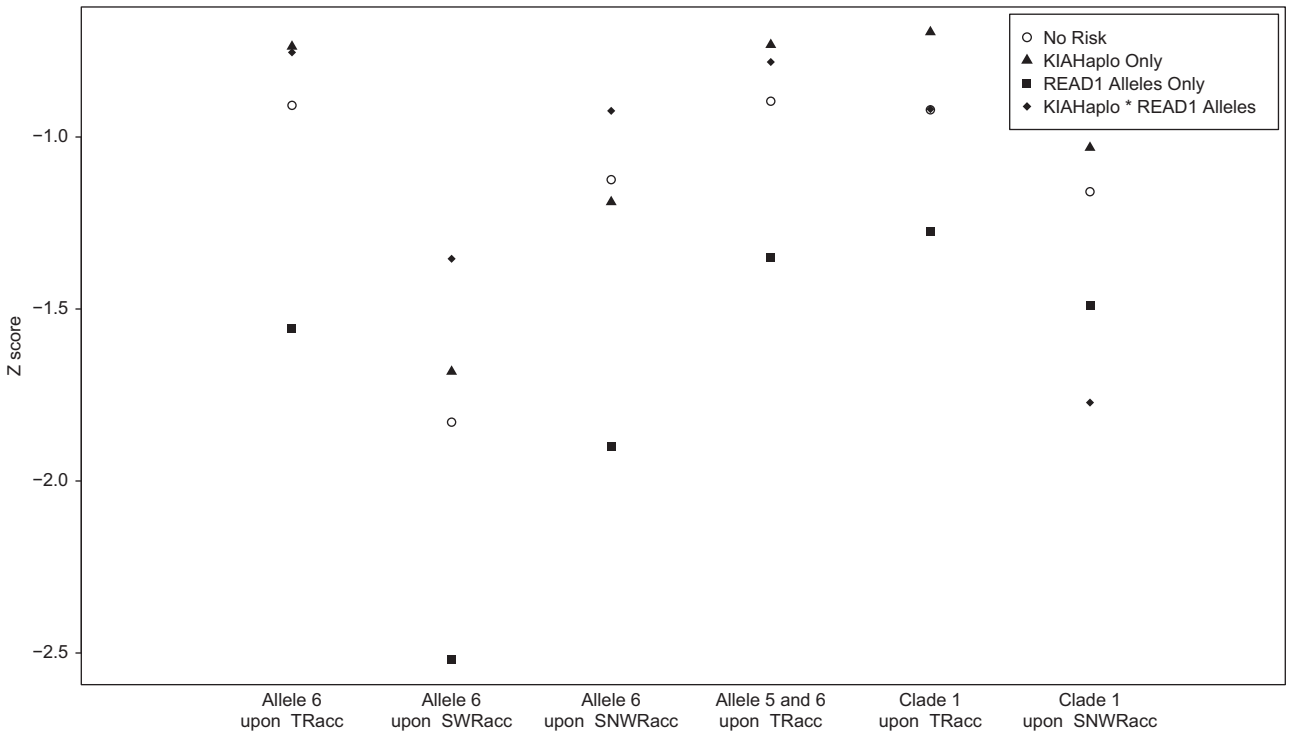

Figure 1 READ1–KIAHap interactions for the selected allele classes in the total sample ($n=634$). These charts show the effect of the denoted READ1 single or composite allele on phenotype in the presence and absence of KIAHap. Each point shows the *z*-score of the denoted allele class on the denoted measure, relative to the entire developmental dyslexia sample; units of the *y* axis are fractions of an s.d. READ1, regulatory element associated with dyslexia 1. Allele classes: KIAHap alone, individuals positive for KIAHap but negative for the indicated READ1 allele; READ1 allele alone, individuals positive for the indicated READ1 allele but negative for KIAHap; no risk, individuals negative for both; KIAHaplo*READ1, non-additive effect, individuals positive for both. Phenotypes: acc, accuracy (number of errors); TR, text reading; SNWR, single-nonword reading.

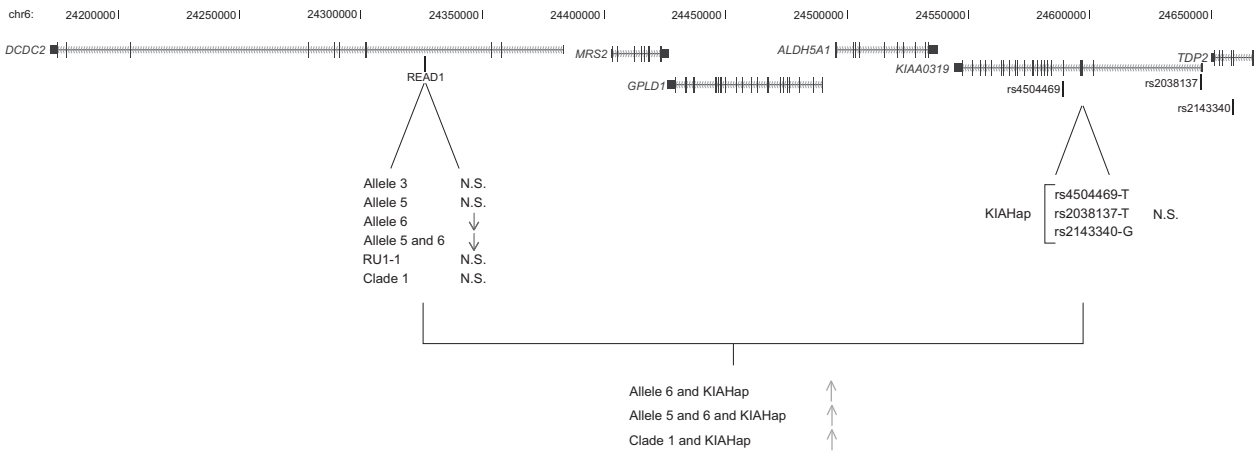

Figure 2 Genomic location and role of READ1–KIAHap. Schematic representation of genomic region surrounding *DCDC2* and *KIAA0319* in the human reference genome GRCh37. Location of READ1 allelic classes and KIAHap is also reported. READ1 allelic classes analyzed in this study, KIAHap and their non-additive effects are shown in boxes. The protective or deleterious effect on reading-related phenotypes is indicated with green and red arrows, respectively. NS, not significant; READ1, regulatory element associated with dyslexia 1. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

by Cope *et al.*[24] Lim *et al.*[25] classified children as having DD if their literacy composite score and at least one cognitive composite score were at least 1 s.d. below the means of their respective ages. Study strategies and sample size may be other reasons for the variation in association results. Trios[23] and nuclear families with DD,[25] general population samples composed by both unrelated individuals[28,30,31] and twin pairs,[27] as well as combined strategies,[24] have been used to investigate the effects of KIAHap upon reading-related traits. Although the sample size used in this study is equivalent to those of previous works,[23–27] it is small compared to the large general population cohort used by Powers *et al.*[30,31]

Several independent findings point to a regulatory interaction between KIAHap and READ1 at different levels of analysis. At the chromosomal level, a recent model suggests that the two variants

interact in *cis* physically (that is, the copy of *KIAA0319* is directly regulated by each READ1 allele on the same chromosome), but in *trans* genetically (that is, when both a detrimental READ1 allele and KIAHap are present, *KIAA0319* expression drops below the minimal threshold in many cells and substantially increases the risk of impairment in language and reading).[31] At the macroscopic, molecular level, *DCDC2* and *KIAA0319* have been shown to fit into a neurodevelopmental and theoretical network encompassing several etiological cascades involved in neuronal migration and neurite outgrowth.[69] According to the hypothesis supporting DD as a neuronal migration disorder,[71] the corresponding proteins could be directly or indirectly related to the regulation and modulation of cytoskeletal microtubules and actin filaments underlying neuronal migration and/or neurites/axons outgrowth.[69] In particular, at the neuronal cell membrane the *KIAA0319* proteins may function as receptors for netrin-like molecules, regulating the rate and direction of outgrowth of neurite/axon and the neurons, and glial fibers' direct adhesion during neuronal migration.[69] This process may lead to signaling through CDC42 (a protein regulating several cellular functions such as migration, cell morphology, cell cycle progression and endocytosis) and ERK1 and ERK2 (cytoplasmic extracellular-signal-regulated kinase proteins), changing neurite outgrowth and the cytoskeletal organization of microtubules and actin filaments. Moreover, the activation of ERK1/2 proteins also regulates the activity of the *DCDC2* protein by phosphorylating its DCX domain, which in turn directly binds and modulates actin filaments and neuronal microtubules involved in neuronal migration and outgrowth of neurite/axon.[69]

In summary, these findings likely provide evidence for the existence of common biological processes underlying reading (dis)ability and lend preliminary support for a generalist non-additive effects between READ1 and KIAHap in a sizable nuclear family-based sample of children with DD. This emphasizes the importance of the role of common allelic variants in modulating the range of human neurocognitive functions and affecting the overlap between typical variation and the low end of the normal distribution of ability.[6] If confirmed, this implies that the same genes affect learning (dis)abilities and that there are no etiologically distinct disabilities. Moreover, our findings confirm that investigation of the genetic interdependence among DD-candidate genes with pleiotropic effects may be used in the clinic to assess individual risk in complex disorders, such as DD. Indeed, it is important to account for genetic complexity as a promising strategy for individualized interventions based on genetic information.[72] Finally, these results highlight the limitations of traditional single-marker analyses in genome-wide association studies, supporting the importance of multi-marker and gene–gene interaction analyses, and the use of intermediate phenotypes in the study of complex traits.[7,30]

Nevertheless, our study has some inherent limitations. First, even if the overall sample size is appreciable and comparable to those of previous works,[23–27] non-additive effects' groups are smaller compared to those used by Powers *et al*.[31] Although findings are recapitulated when single alleles are combined into composite clusters yielding larger subsample sizes, our results may reflect spurious associations due to low power. Larger independent clinical samples are therefore needed to confirm our findings. Second, further studies should take into account the influences of familial transmission of non-additive interactions between KIAHap and READ1. Third, replications in samples with languages with non-transparent orthography are needed, as stratification of the genetic risk across language groups may be observed due to not only the underlying genetic effect but also the linguistic environment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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