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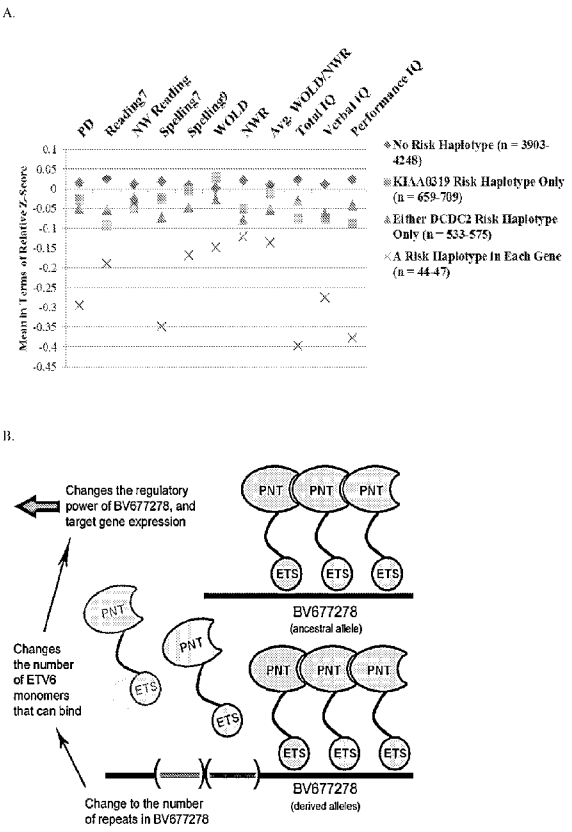
(54) **Title**: ASSESSING RISK OF READING AND LANGUAGE IMPAIRMENT

(57) **Abstract**: Described herein are the association BV677278 (READ1) with reading disability and language impairment, as well as the synergistic interaction of DCDC2 risk haplotypes or alleles with KIAA0319 risk allele.

FIG. 3



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ASSESSING RISK OF READING AND LANGUAGE IMPAIRMENT

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/723,774, filed November 7, 2012. The teachings of the referenced application are incorporated by reference herein in their entirety.

FEDERAL FUNDING

This invention was made with government support under R01NS043530 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Specific learning disabilities (LDs) are disorders characterized by unexpected difficulty with a specific mode of learning, despite adequate IQ and educational opportunity. LDs can involve reading, math, writing, and speech skills, among others, but the most common involve language. The National Institute of Child Health and Development (NICHD) estimates 15-20% of Americans have a language-based LD, of which reading disability (RD) afflicts the majority (1). RD, also known as dyslexia, is a specific impairment in processing written language (2). Another LD, language impairment (LI), is characterized by difficulty processing and expressing spoken language (3). These LDs are frequently comorbid; children with LI have increased risk of developing RD (3). Because reading and language skills are fundamental to academic success, affected individuals are at risk for adverse psychological outcomes, as well as limited educational and occupational prospects (2). Additionally, the prevalence of these LDs makes the cost of remediation burdensome to the educational system (4). Intervention is more effective the earlier it is administered (2), making early detection of high-risk individuals an attractive prospect.

SUMMARY

As described herein, two haplotypes, both in the same six-marker haplotype block in the reading disability (RD) risk gene *DCDC2*, are associated, respectively, with reading disability and language impairment (LI). Each of the haplotypes is in very strong linkage disequilibrium with an allele of BV677278 (also known as READ1), which is a polymorphic compound STR associated with reading disability and capable of modulating expression from the *DCDC2* promoter. BV677278 (READ1) has been shown to specifically bind ETV6, a potent transcriptional regulator and proto-oncogene, in vivo. BV677278 binds the brain-expressed

nuclear protein with very high specificity and is capable of modulating reporter gene expression from the *DCDC2* promoter in an allele-specific manner. Activation patterns in reading-related areas of the brain, as measured by functional magnetic resonance imaging, are influenced by BV677278 alleles. Work described herein shows that BV677278 is associated with both reading and language, and that at least two BV677278 alleles have a deleterious effect on reading and language. Allele 5 is important for dyslexia (RD) and allele 6 is important for language impairment. BV677278 has been renamed and is also referred to herein as READ1, which stands for "regulatory element associated with dyslexia 1." The two terms are used interchangeably.

As also described herein, the DCDC2 risk haplotypes or alleles interact with a KIAA0319 risk haplotype in a synergistic manner. The synergy between the BV677278 (READ1) alleles or the DCDC2 risk haplotypes and the KIAA0319 haplotypes in decreasing performance in phoneme deletion (very important to reading), spelling, and total IQ and performance IQ, has not previously been described. Together, the effect is 3-fold to 8-fold greater than if the deleterious version of either risk allele or haplotype is present.

In particular, described herein is a six-marker haplotype block within DCDC2, of which two haplotypes (CGCGAG and GACGAG) associated with very poor performance on a phoneme deletion task and composite language measure, respectively. The two haplotypes show strong association with their respective phenotypes: CGCGAG with RD and GACGAG with LI. Carriers of the CGCGAG haplotype, on average, showed significantly poorer performance on eight reading-related measures, compared to non-carriers, and carriers of the GACGAG haplotype showed significantly lower average performance on the WOLD/WR composite language measure.

Further described herein are methods of identifying or aiding in identifying an individual who is at risk of developing at least one (a, one or more) learning disability (LD), in which a sample obtained from the individual is assayed for the presence of at least one (a, one or more) haplotype in the DYX2 locus (chromosome 6p; 6p22) that is associated with susceptibility for developing at least one (a, one or more) LD in humans. The presence of at least one haplotype that is associated with susceptibility for developing at least one (a, one or more) LD in humans in the DYX2 locus indicates that the individual is at risk for developing at least one (a, one or more) LD. In some embodiments, the at least one LD is reading disability (RD) or language

impairment (LI). The at least one haplotype can be located in the DCDC2 gene within the DYX2 locus or in the KIAA0319 gene within the DYX2 locus. Alternatively, a sample is assayed (analyzed) for the presence of a haplotype located in the DCDC2 gene within the DYX2 locus and the presence of a haplotype located in the KIAA0319 gene within the DYX2 locus. The at least one haplotype can comprise (a) CGCGAG, GACGAG or both in a DCDC2 gene within the DYX2 locus; (b) one or more single nucleotide polymorphisms (SNPs) associated with a variant KIAA0319, such as rs4504469, rs2038137, or rs2143340 or any combination of two or three of rs4504469, rs2038137 and rs2143340; or (c) any combination of the haplotypes in (a) and (b). Also described herein are methods of treating an individual suspected or identified as having a LD. Treatment can include, for example, inhibiting ETV6 in the individual, and/or providing services designed to address or remedy certain aspects associated with a LD, such as RD or LI, such as providing intervention, including services and materials, including but not limited to using special teaching techniques; making classroom modifications, such as providing extra time to complete tasks and taped tests to permit the individual to hear, rather than read the tests; using books on tape; using word-processing programs with spell-check features; helping the individual learn through multisensory experiences; teaching coping tools; and providing services to strengthen the individual's ability to recognize and pronounce words.

Described herein is a method of determining if a sample obtained from an individual comprises nucleic acid which comprises a haplotype associated with susceptibility for developing a learning disability (LD) in humans, comprising assaying a sample that comprises nucleic acid from the individual for the presence in the DYX2 locus of at least one of the following markers: (a) CGCGAG in a DCDC2 gene; (b) CACGAG in a DCDC2 gene; (c) both CGCGAG and CACGAG in a DCDC2 gene; (d) rs4504469 in a KIAA0319 gene; (e) rs2038137 in a KIAA0319 gene; (f) rs2143340 in a KIAA0319 gene; (g) any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene; and (h) any combination of CGCGAG in a DCDC2 gene; CACGAG in a DCDC2 gene; both CGCGAG and CACGAG in a DCDC2 gene; rs4504469 in a KIAA0319 gene; rs2038137 in a KIAA03109 gene; rs2143340 in a KIAA0319 gene; and any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene, wherein if the sample comprises at least one of (a) – (h), the sample comprises a haplotype associated with susceptibility for developing a learning disability in humans. In one embodiment, the sample is assayed for at least one marker of (a), (b) and (c) and at least one marker of (d), (e), (f) and (g). In various embodiments, the sample is assayed (analyzed) for two or more markers of (a), (b) and (c) and two or more markers of (d), (e), (f)

and (g); only markers of (a), (b) and (c); only markers of (d), (e), (f) and (g); or any combination of the markers of (a)-(h). The LD can be RD or LI or both RD and LI. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods, and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of a haplotype associated with susceptibility for developing a learning disability (LD) in humans determined. In one embodiment, the method further comprises assaying the sample for allele 5 of DCDC2 gene, as presented herein (e.g., SEQ ID NO:35); allele 6 of DCDC2 gene, as presented herein (e.g., SEQ ID NO:36) or both.

Another embodiment is a method of assaying a sample for a marker of a haplotype associated with susceptibility for developing a learning disability (LD) in humans, comprising: (a) obtaining a sample comprising nucleic acid from an individual; and (b) determining if the sample comprises at least one of the following: (i) CGCGAG in a DCDC2 gene in the DYX2 locus; (ii) CACGAG in a DCDC2 gene in the DYX2 locus; (iii) both CGCGAG and CACGAG in a DCDC2 gene in the DYX2 locus; (iv) rs4504469 in a KIAA0319 gene in the DYX2 locus; (v) rs2038137 in a KIAA0319 gene in the DYX2 locus; (vi) rs2143340 in a KIAA0319 gene in the DYX2 locus; (vii) any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene in the DYX2 locus; and (viii) any combination of CGCGAG in a DCDC2 gene; CACGAG in a DCDC2 gene in the DYX2 locus; both CGCGAG and CACGAG in a DCDC2 gene in the DYX2 locus; rs4504469 in a KIAA0319 gene in the DYX2 locus; rs2038137 in a KIAA03109 gene in the DYX2 locus; rs2143340 in a KIAA0319 gene in the DYX2 locus; and any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene in the DYX2 locus, wherein if the sample comprises at least one marker of (i) – (vii), the sample comprises a haplotype associated with susceptibility for developing a learning disability in humans. In one embodiment, the sample is assayed for at least one marker of (i), (ii) and (iii) and at least one marker of (iv), (v), (vi) and (vii). The LD is reading disability (RD) or language impairment (LI) or both RD and LI. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the

presence or absence of a haplotype associated with susceptibility for developing a learning disability (LD) in humans determined.

A further embodiment is a method of determining if a sample obtained from an individual comprises nucleic acid which comprises an allele associated with susceptibility for developing a learning disability (LD) in humans, comprising: assaying a sample that comprises nucleic acid from the individual for the presence of allele 5 of DCDC2 gene in the DYX2 locus (e.g., SEQ ID NO:35), allele 6 of DCDC2 gene in the DYX2 locus (e.g., SEQ ID NO:36), or both allele 5 of DCDC2 gene and allele 6 of DCDC2 gene, wherein if the sample comprises at least one of allele 5 and allele 6, the sample comprises an allele associated with susceptibility for developing a learning disability. The LD is reading disability (RD) or language impairment (LI) or both RD and LI. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of a haplotype associated with susceptibility for developing a learning disability (LD) in humans determined.

A further embodiment is a method of determining if a sample obtained from an individual comprises at least one marker associated with comorbid reading disability (RD) and language impairment (LI) in humans, comprising: (a) obtaining a sample that contains nucleic acid from the individual and (b) assaying the sample for (i) at least one marker in DCDC2 gene in the DYX2 locus for a haplotype associated with susceptibility for developing RD in humans and (ii) at least one marker in KIAA0319 gene in the DXY2 locus for a haplotype associated with susceptibility for developing LI in humans, wherein if the sample comprises a marker of (i) and a marker of (ii), the sample comprises at least one marker associated with comorbid RD and LI. The marker of (b)(i) and the marker of (b)(ii) can be the same marker or two different markers. In one embodiment, the at least one marker of (b)(i) is CGCGAG or GACGAG and the at least one marker of (b)(ii) is rs4504469; rs2038137; or rs2143340. In any of these embodiments, the method can further comprise assaying the sample for allele 5 of DCDC2 gene in the DYX2 locus, allele 6 of DCDC2 gene of DYX2 locus, or both allele 5 of DCDC2 gene in the DYX2 locus and allele 6 of DCDC2 gene in the DYX2 locus. In yet further embodiments, the at least one marker of (b)(i) and the at least one marker of (b)(ii) are selected from: rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555;

rs259521; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs4504469; rs2038137; rs2143340; rs9295626; rs7763790 rs6935076; rs2817201; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs6456624; rs6935076; rs2038137; rs3756821; rs1883593; rs3212236; rs6456621; rs12193738; rs2817198; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; and rs807694. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods, and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of a marker associated with susceptibility for developing a learning disability (LD) in humans determined.

Another embodiment is a method of determining if a sample obtained from an individual comprises a marker associated with language impairment (LI) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: CACGAG in a DCDC2 gene in the DYX2 locus; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs807694; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; and a marker for at least one of the following genes: NEK2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; and PGD. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of a marker associated with susceptibility for developing LI in humans determined.

A further embodiment is a method of determining if a sample obtained from an individual comprises a marker associated with reading disability (RD) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: CGCGAG in a DCDC2 gene in the DYX2 locus; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs4725745; rs12444778; rs1444186; rs2294691; rs10456309; rs1562422; and a marker for at least one of the following

genes: MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF. . Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of a marker associated with susceptibility for developing RD in humans determined.

Another embodiment is a method of determining if nucleic acids (DNA, RNA) in an individual comprise markers of haplotypes that interact in a synergistic manner in resulting in a learning disorder (LD) in humans comprising: (a) obtaining a sample that comprises nucleic acids from an individual and (b) assaying the sample for at least one DCDC2 risk haplotype or DCDC2 risk allele and at least one KIAA0319 risk haplotype, wherein the at least one DCDC2 risk haplotype is CGCGAG or GACGAG, the at least one DCDC2 risk allele is allele 5 of DCDC2 gene in the DYX2 locus (SEQ ID NO:35) or allele 6 of DCDC2 gene in the DYX2 locus (SEQ ID NO:36) and the at least one KIAA0319 risk haplotype is a variant KIAA0319 haplotype comprising a snp which is rs4504469; rs2038137; or rs2143340 and wherein if the sample comprises at least one DCDC2 risk haplotype or at least one DCDC2 risk allele and at least one KIAA0319 risk haplotype, the nucleic acids comprise markers of haplotypes that interact in a synergistic manner in resulting in a LD in humans. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of markers that interact in a synergistic manner in resulting in LD in humans determined.

A further embodiment is a method of identifying or aiding in identifying an individual at risk for developing at least one learning disability (LD), comprising assaying a sample obtained from the individual for the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans, wherein the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans indicates that the individual is at risk for developing a LD. At least one LD is a reading disability or language impairment. The at least one haplotype is located in the DCDC2 gene

within the DYX2 locus or in the KIAA0319 gene within the DYX2 locus. The at least one haplotype can comprise (a) CGCGAG or CACGAG in a DCDC2 gene within the DYX2 locus; or (b) rs4504469, rs2038137, rs2143340, or any combination thereof in a KIAA0319 gene within the DYX2 locus; or (c) any combination of the haplotypes in (a) and (b). Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a haplotype of interest can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans determined. The presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans indicates that the individual is at risk for developing a LD.

Also the subject herein is a method of determining if a sample obtained from an individual comprises a marker for susceptibility for developing a learning disability (LD) that is reading disability (RD) or language impairment (LI), comprising: obtaining a sample that comprises nucleic acid from the individual and determining if the sample comprises at least one marker selected from the group consisting of: rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs12636438; rs1679255; rs9521789; rs476739; rs505277; rs482700; rs7695228; rs867036; rs867035; rs1940309; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs4725745; rs12444778; rs1444186; rs2294691; rs10456309; rs1562422; rs807694; rs3756814; rs3777663; rs9295626; rs7763790; rs6935076; rs9348646; rs2328791; rs2328791; rs2817201, rs9295626; rs4576240; rs17307478, rs9356939, rs7763790, rs6456621; rs6456624, rs6935076, rs2038137, rs3756821, rs1883593, rs3212236; rs3777663, rs3756814, rs6931809, rs6916186, rs6933328, rs17491647; rs2328791; rs33914824a; rs807694a; rs707864a; rs10456301a; rs16889066a; rs9379651a; rs2817201; rs9295626; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs3756821; rs1883593; rs3212236; rs2294691; rs3777663; rs3756814; rs6931809; rs6916186; rs6933328; rs17491647; rs9348646; rs1562422 and a marker for each of

the following genes:R5H2; OR5H6; RRAGA; OR6B3; UMOD; A26C1A; FAM29A; CHRNA1; IFIT5; LOC643905; K2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; PGD; MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF. The LD is reading disability (RD) or language impairment (LI) or both RD and LI. Any sample that contains nucleic acid (e.g., genomic DNA; RNA) that can be analyzed for a marker for susceptibility for developing a learning disability (LD) that is reading disability (RD) or language impairment (LI)can be assayed; methods for analyzing nucleic acids are well known in the art and also described herein. They include, but are not limited to, hybridization-mediated methods and sequencing. The sample can be, for example, blood, cells, or tissue. Alternatively, genomic DNA can be sequenced and the presence or absence of such a marker or markers.

Further described herein is a method of identifying or aiding in identifying an individual at risk for developing at least one learning disability (LD), comprising assaying a sample obtained from the individual for the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans, wherein the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans indicates that the individual is at risk for developing a LD. At least one LD is a reading disability (RD) or language impairment (LI). The at least one haplotype is located in the DCDC2 gene within the DYX2 locus or in the KIAA0319 gene within the DYX2 locus and can comprise (a) CGCGAG, CACGAG, or both CGCGAG and CACGAG in a DCDC2 gene within the DYX2 locus; or (b) rs4504469, rs2038137, rs2143340, or any combination thereof in a KIAA0319 gene within the DYX2 locus; or (c) any combination of the haplotypes in (a) and (b). In the method, the assay comprises a hybridization-mediated method, nucleic acid sequencing, or both a hybridization-mediated method and nucleic acid sequencing. The sample is blood, cells, or tissue.

Another embodiment is a method of identifying an individual as having, or being susceptible to developing, a learning disability (LD), comprising obtaining a sample comprising nucleic acid from an individual; determining whether nucleic acid in the sample comprises a DCDC2 gene haplotype in the DYX2 locus associated with susceptibility for developing reading disability (RD) and a KIAA0319 gene haplotype associated with susceptibility for developing language impairment (LI), wherein the DCDC2 gene haplotype and the KIAA0319 gene haplotype interact synergistically in decreasing performance in phoneme deletion and in resulting in a learning disorder (LD) in humans, wherein if the sample comprises both haplotypes, the

individual is identified as having or being susceptible to developing a LD. In this method, the determining comprises a hybridization-mediated method, nucleic acid sequencing, or both a hybridization-mediated method and nucleic acid sequencing. The sample is blood, cells, or tissue.

In some embodiments, a method by which an individual is identified as having or being susceptible for developing a learning disability (LD) comprises treating the individual so identified. Treatment comprises providing interventions, including services and materials, including but not limited to: using special teaching techniques; making classroom modifications, such as providing extra time to complete tasks and taped tests to permit the individual to hear, rather than read the tests; using books on tape; using word-processing programs with spell-check features; helping the individual learn through multisensory experiences; teaching coping tools; and providing services to strengthen the individual's ability to recognize and pronounce words. See, for example, nichd.nih.gov/health/topics/learning/conditioninfo. What are the treatments for learning disabilities?

Another embodiment is a method of treating an individual for a learning disability (LD) comprising inhibiting ETV6 in the individual. The individual has RD, LI, or both.

In some embodiments, a method by which an individual is identified as at risk for developing a learning disorder further comprises monitoring the individual identified as at risk for developing a learning disability to assess whether development of a learning disability occurs and, if development occurs, treating the individual, wherein treating comprises providing interventions, including services and materials, including but not limited to: using special teaching techniques; making classroom modifications, such as providing extra time to complete tasks and taped tests to permit the individual to hear, rather than read the tests; using books on tape; using word-processing programs with spell-check features; helping the individual learn through multisensory experiences; teaching coping tools; and providing services to strengthen the individual's ability to recognize and pronounce words.

See, for example,nichd.nih.gov/health/topics/learning/conditioninfo. What are the treatments for learning disabilities?

Also described herein are arrays, such as microarrays (DNA arrays or microarrays). According to one embodiment, an array (e.g., microarray) for identifying or aiding in identifying an individual at risk for developing at least one learning disability (LD) is provided. The array comprises a support having a plurality of discrete regions (e.g., spots), each discrete region having (having affixed thereto) one or more nucleic acid fragment (e.g., probes) spotted or otherwise attached or deposited thereon. Typically, each discrete region bears a reagent, such as nucleic acid (DNA, RNA) that detects a marker (e.g., SNP, haplotype marker, allele, etc) associated with susceptibility for developing a LD (e.g., RD, LI) in humans. The nucleic acid fragments are complementary to nucleic acids (e.g., DNA, such as genomic DNA, or RNA, such as mRNA) that are markers for a variant gene, such as variant DCDC2, KIAA0319 and others named herein, associated with susceptibility for developing at least one LD (e.g., as provided herein). The nucleic acid fragments on a particular discrete region can be of any length and sequence (e.g., that complements the nucleic acid comprising a marker) suitable for the detection of any marker described herein. For example, in some embodiments, a nucleic acid fragment (e.g., probe, SNP probe) is between 10 and 100 nucleotides in length. In some embodiments, a nucleic acid fragment is between about 20 and 80, about 30 and 60, or about 40 and 50 nucleotides (nt) in length. In specific embodiments, the probes are 25 nt, 30 nt, 35 nt. or 40 nt in length. See, for example, LaFramboise, T., Nucl. Acids Res. (2009) 37 (13): 4181-4193. In some embodiments, a particular discrete region comprises a plurality of nucleic acid fragments (e.g., probes, SNP probes), each of which is capable of hybridizing to a particular marker. In some embodiments, the plurality of nucleic acid fragments are of varying lengths (e.g., as described herein) and sequences. In some embodiments, the array detects two or more markers associated with susceptibility for developing a learning disability (LD) in humans, wherein the two or more markers comprise one or more markers in a DCDC2 gene and one or more markers in a KIAA0319 gene. In some embodiments, the one or more markers in a DCDC2 gene are selected from CGCGAG, CACGAG, READ1 allele 5 (SEQ ID NO:35), READ1 allele 6 (SEQ ID NO:36), or any combination of two, three or four of CGCGAG, CACGAG, READ1 allele 5 (SEQ ID NO:35), and READ1 allele 6 (SEQ ID NO:36). In some embodiments, the one or more markers in a KIAA0319 gene are selected from rs4504469, rs2038137, rs2143340, or any combination of two or three of rs4504469, rs2038137 and rs2143340.

In some embodiments, the array (e.g., microarray) detects markers associated with susceptibility for developing language impairment (LI) in humans. In some embodiments, the array comprises discrete regions (e.g., discrete regions comprising one or more nucleic acid fragments) capable

of detecting markers in a DCDC2 gene, such as CACGAG, READ1 allele 6 (SEQ ID NO:36), rs793845, rs2799373, rs793862, rs793834, rs2792682, rs807704, rs707864, rs807694, or any combination thereof. In some embodiments, the array (further) detects one or more markers in a KIAA0319 gene, such as rs12193738, rs2817198, rs10456309, or any combination thereof. In some embodiments, the array further comprises one or more discrete regions comprising nucleic acid fragments spotted on the support that detect one or more markers selected from rs985080, rs1554690, rs2533096, rs6951437, rs344470, rs344468, rs482700, rs7695228, rs1940309, rs505277, rs476739, rs867036, rs867035, rs2071674, rs7694946, rs4823324, and markers for the following genes: NEK2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; and PGD.

In some embodiments, the array (e.g., microarray) detects markers associated with susceptibility for developing a reading disability (RD) in humans. In some embodiments, the array comprises discrete regions (e.g., discrete regions comprising one or more nucleic acid fragments) capable of detecting markers in a DCDC2 gene, CGCGAG and READ1 allele 5 (SEQ ID NO:35), or both. In some embodiments, the array (further) detects one or more markers in a KIAA0319 gene, such as rs10456309. In some embodiments, the array further comprises one or more discrete regions comprising nucleic acid fragments spotted on the support that detect one or more markers selected from rs180950, rs2590673, rs892100, rs1792745, rs12546767, rs12634033, rs892270, rs10887149, rs10041417, rs6792971, rs4725745, rs12444778, rs1444186, rs2294691, rs10456309, rs1562422, and a markers for the following genes: MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

In some embodiments, an array (e.g., microarray) is provided that detects one or more markers associated with susceptibility for developing a LD in humans, wherein the one or more markers are selected from rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs12636438; rs1679255; rs9521789; rs476739; rs505277; rs482700; rs7695228; rs867036; rs867035; rs1940309; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs4725745; rs12444778; rs1444186;

rs2294691; rs10456309; rs1562422; rs807694; rs3756814; rs3777663; rs9295626; rs7763790; rs6935076; rs9348646; rs2328791; rs2328791; rs2817201, rs9295626; rs4576240; rs17307478, rs9356939, rs7763790, rs6456621; rs6456624, rs6935076, rs2038137, rs3756821, rs1883593, rs3212236; rs3777663, rs3756814, rs6931809, rs6916186, rs6933328, rs17491647; rs2328791; rs33914824a; rs807694a; rs707864a; rs10456301a; rs16889066a; rs9379651a; rs2817201; rs9295626; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs3756821; rs1883593; rs3212236; rs2294691; rs3777663; rs3756814; rs6931809; rs6916186; rs6933328; rs17491647; rs9348646; rs1562422 and markers of the following genes:R5H2; OR5H6; RRAGA; OR6B3; UMOD; A26C1A; FAM29A; CHRNA1; IFIT5; LOC643905; K2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; PGD; MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1: (A) Structure of the BV677278 STR. (B) Location of the risk haplotype block within the DCDC2 gene, relative to BV677278. Exons are numbered.

FIG. 2: (A-B) SILAC results for Raji and HeLa cells; two-dimensional interaction plot. (C) ChIP results. α-H3: antibody to a histone H3 variant enriched in actively transcribing genes. β-actin: control amplicon from the β-actin gene. Error bars represent standard deviation among 3 replicates. Double asterisk (**) represents a p-value below 0.01 (one-tailed T-test; see Table S6).

FIG. 3: (A) Effect of risk haplotype carrier status on various reading, language, and cognitive phenotypes (described in Table S1 and Materials and Methods of Examples Section). Data points represent the mean of each group, converted to a z-score relative to the mean of the ALSPAC sample population. Units of the y-axis are fractions of a standard deviation. PD: phoneme deletion task; Reading7: single-word reading at age 7; NW Reading: non-word reading at age 9; Spelling7 and 9: spelling at ages 7 and 9; WOLD: Wechsler Objective Learning Dimensions verbal comprehension task; NWR: non-word repetition task. (B) Model of differential effects of BV677278 alleles. ETV6 monomers must at least homodimerize through their pointed (PNT) domains to bind DNA through their ETS domains, and they may homopolymerize in vivo. Indels of BV677278 repeat units could change the size of the ETV6 polymer, and thus affect target gene expression.

FIG. 4**:** Phylogenetic tree based on multiple alignment of 22 BV677278 alleles. The Clustal W algorithm was used, with default method parameters (IUB matrix, gap penalty = 15, gap extension penalty = 6.66). Clade 1, which contains risk alleles 5 and 6, is the top most branched grouping, in light grey (not including the branch for Allele 22).

FIG. 5: Epistasis of READ1 over KIAA0319 Risk Haplotype. (A) This plot shows the effect of having at least one copy of the denoted READ1 allele alone (e.g., allele 5), at least one copy of KIAHap alone, and at least one copy of each (both), compared to all members of the ALSPAC. (B) This plot shows the protective effects of having a READ1 allele comprising a single copy of Repeat Unit 1 (RU1_1). Data points represent the z-transformed mean of each group, compared to the mean of the entire ALSPAC (Mean_All), on the indicated measures. Units of the y-axis are fractions of a standard deviation. Verbal, Performance, and Total IQ were measured at age 8 by the WISC-III. PD: phoneme deletion task at age 7; Reading 7: single-word reading task at age 7; Reading 9: single-word reading task at age 9; NW Reading: single non-word reading task at age 9, Spelling 7: spelling task at age 7; Spelling 9: spelling task at age 9.

FIG. 6: (A) Schematic of the genes within the DYX2 locus on chromosome 6p21.3. Genes in light grey, *DCDC2* and *KIAA0319*, have replicated associations with written and verbal language phenotypes, namely RD and LI. Regions in dark grey within the genes denote two functional variants, READ1 in *DCDC2* and a risk haplotype with markers in *KIAA0319* and *TDP2*, which have been functionally associated with RD and LI using animal models and molecular techniques. (B) **A**n updated schematic of genes with markers that show replicated associations to RD, LI, and/or IQ. The genes (shown in light grey) have expanded to seven (*DCDC2*, *KIAA0319*, *TDP2*, *ACOT13*, *C6orf62*, *FAM65B*, and *CMAHP*), although linkage disequilibrium may account for multiple associations (particularly for *KIAA0319*, *TDP2*, *ACOT13*, and *C6orf62*).

DETAILED DESCRIPTION

Variant DCDC2 and variant KIAA0319 Polynucleotide Probes and Primers

Provided here are isolated, synthetic and recombinant polynucleotides that detect an alteration in a *DCDC2* gene (referred to as a variant *DCDC2* gene) that is associated with susceptibility to developing a learning disability (LD), such as isolated and recombinant polynucleotides that detect an alteration of *DCDC2* in the DYX2 locus. The variant is, for example, a *DCDC2* risk

haplotype (e.g., CGCGAG, CACGAG), allele 5 of BV677278 (READ1); allele 6 of BV677278 (READ1); or one, two or three or more of the variants associated with susceptibility to developing a learning disability (LD). Also provided are isolated, synthetic and recombinant polynucleotides that detect an alteration in a *KIAA0319* gene (referred to as a variant *KIAA0319* gene) that is associated with susceptibility to developing a learning disability (LD), such as isolated and recombinant polynucleotides that detect an alteration of *KIAA0319* in the DYX2 locus. The variant is, for example, a *KIAA0319* risk haplotype (e.g., rs4504469, rs2038137, rs2143340); or one, two or three or more of the variants associated with susceptibility to developing a learning disability (LD). The LD is a reading disability (RD) or a language impairment (LI). Polynucleotide probes typically have a sequence which is fully or partially complementary to the sequence of the alteration and the flanking region and hybridize to the alteration of interest, and the flanking sequence in a specific manner. A variety of alterations in a *DCDC2* gene or in a *KIAA0319* gene associated with susceptibility for developing LD, such as RD and LI, may be detected by the polynucleotides described herein. For example, a single nucleotide polymorphism (SNP) of a coding region, exon, exon-intron boundary, signal peptide, 5-prime untranslated region, promoter region, enhancer sequence, 3-prime untranslated region or intron that is associated with LD such as RD and LI can be detected. These polymorphisms include, but are not limited to, those that result in changes in the amino acid sequence of the proteins encoded by the *DCDC2* gene and changes in the amino acid sequence of the proteins encoded by the *KIAA0319* gene, produce alternative splice products, create truncated products, introduce a premature stop codon, introduce a cryptic exon, alter the degree or expression to a greater or lesser extent, alter tissue specificity of *DCDC2* and/or *KIAA0319* expression (e.g., at either the mRNA or protein level), introduce changes in the tertiary structure of the proteins encoded by *DCDC2* and/or *KIAA0319*, introduce changes in the binding affinity or specificity of the proteins expressed by *DCDC2* and/or *KIAA0319* or alter the function of the proteins encoded by *DCDC2* and/or *KIAA0319*. The subject polynucleotides include polynucleotides that are variants of the polynucleotides described herein, as long as the variant polynucleotides maintain their ability to specifically detect a variation in the DCDC2 gene that is associated with susceptibility for developing LD, such as RD and/or LI or in the KIAA0319 gene that is associated with susceptibility for developing LD, such as RD and/or LI. Variant polynucleotides may include, for example, sequences that differ by one or more nucleotide substitutions, additions or deletions.

In certain embodiments, the isolated or recombinant polynucleotide is a probe that hybridizes, under stringent conditions, such as highly stringent conditions, to an alteration in the DCDC2 gene that is associated with susceptibility for developing a LD, or to an alteration in the KIAA0319 gene that is associated with susceptibility for developing a LD. A LD can be, for example, a reading disability (RD) or language impairment (LI). As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. The term "probe" refers to a polynucleotide that is capable of hybridizing to another nucleic acid of interest. The polynucleotide may be naturally occurring, as in a purified restriction digest, or it may be produced synthetically, recombinantly or by nucleic acid amplification (e.g., PCR amplification).

It is well known in the art how to perform hybridization experiments with nucleic acid molecules. The skilled artisan is familiar with hybridization conditions and that appropriate stringency conditions which promote DNA hybridization can be varied. Such hybridization conditions are referred to in standard text books such as Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989); and Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992. In one embodiment, the polynucleotides hybridize to a variation in the DCDC2 gene, to a variation in a KIAA0319 gene, or to both a variation in the DCDC2 gene and the KIAA0319 gene (e.g., use of distinct probes that hybridize to each gene, respectivelt). Under highly stringent conditions, essentially no hybridization to unrelated polynucleotides occurs.

Nucleic acid hybridization is affected by such conditions as salt concentration, temperature, organic solvents, base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will readily be appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, or may be in excess of 37°C or 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, or may be less than 500 mM or 200 mM. For example, one could perform the hybridization at 6.0x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0x SSC at 50°C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0x SSC at 50°C to a high stringency of about 0.2x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant

while the other variable is changed. In one embodiment, nucleic acids hybridize under low stringency conditions of 6.0x SSC at room temperature followed by a wash at 2.0x SSC at room temperature. The combination of parameters, however, is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson, *J Mol Biol*. 1968; 31(3):349-70. Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art. One method for obtaining DNA encoding the biosynthetic constructs disclosed herein is by assembly of synthetic oligonucleotides produced in a conventional, automated, oligonucleotide synthesizer.

A polynucleotide probe or primer used in a method described herein may be labeled with a reporter molecule, so that it is detectable in a detection system, including, but not limited to, enzyme (e.g., ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, chemical, and luminescent systems. A polynucleotide probe or primer used in a method described herein may further include a quencher moiety that, when placed very close to a label (e.g., a fluorescent label), causes there to be little or no signal from the label. It is not intended that the present invention be limited to any particular detection system or label.

In another embodiment, the isolated polynucleotide is a primer that hybridizes adjacent, upstream, or downstream to an alteration in the *DCDC2* gene or the *KIAA0319* gene that is associated with susceptibility for developing a LD in humans. For example, a polynucleotide primer can hybridize adjacent, upstream, or downstream to an alteration in the DCDC2 gene or adjacent, upstream, or downstream to an alteration in the KIAA0319 gene that is associated with susceptibility for developing a LD (e.g., RD, LI). As used herein, the term "primer" refers to a polynucleotide that acts as a point of initiation of nucleic acid synthesis when placed under conditions in which synthesis of a primer extension product that is complementary to a nucleic acid strand is induced (e.g., in the presence of nucleotides, an inducing agent such as DNA polymerase, and suitable temperature, pH, and electrolyte concentration). Alternatively, the primer ligates to a proximal nucleic acid when placed under conditions in which ligation of two unlinked nucleic acids is induced (e.g., in the presence of a proximal nucleic acid, an inducing agent such as DNA ligase, and suitable temperature, pH, and electrolyte concentration). A polynucleotide primer may be naturally occurring, as in a purified restriction digest, or may be produced synthetically. The primer is single stranded or double stranded. If double stranded, the primer is treated to separate its strands before being used. The primer can be an

oligodeoxyribonucleotide. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the method used.

One embodiment is a pair of primers that specifically detect an alteration in a *DCDC2* gene or an alteration in a *KIAA0319* gene that is associated with susceptibility for developing a LD. In such a case, the first primer hybridizes upstream from the alteration and a second primer hybridizes downstream from the alteration. One of the primers hybridizes to one strand of a region of DNA that comprises an alteration in the *DCDC2* gene or in the *KIAA0319* gene that is associated with susceptibility for developing LD and the second primer hybridizes to the complementary strand of a region of DNA that comprises an alteration in the *DCDC2* gene or in the *KIAA0319* gene that is associated with susceptibility for developing a LD. As used herein, the term "region of DNA" refers to a sub-chromosomal length of DNA. Other embodiments are pairs of primers that specifically detect alterations in other genes, described herein, that are associated with a susceptibility for developing a learning disability. A further embodiment is a set of three primers useful for distinguishing between two alleles of *DCDC2*, wherein the first allele is a non-deleted *DCDC2* gene (e.g., an allele that does not comprise a deletion of READ1) and the second allele comprises a deletion in the *DCDC2* gene (e.g., comprises allele 39/indicated Del. in Table S4, Example 1) that is associated with susceptibility for LD. The first primer hybridizes to a nucleotide sequence that is common to both alleles, such as a non-allelic nucleotide sequence that is upstream or downstream of the polymorphic sequence in the *DCDC2* gene. The second primer specifically hybridizes to a nucleotide sequence that is unique to a first allele (e.g., a non-deleted *DCDC2* gene). The third primer specifically hybridizes to a nucleotide sequence that is unique to the second allele (e.g., a deletion in the *DCDC2* gene that is associated with susceptibility for RD). Use of the set of three primers results in amplification of a region of DNA that is dependent on which *DCDC2* allele is present in the sample. Alternatively, two primers of the set hybridize to a nucleotide sequence that is common to two alleles of the *DCDC2* gene, such as non-allelic nucleotide sequences that are upstream and downstream of a polymorphic sequence in the *DCDC2* gene, and the third primer specifically hybridizes to one of the two alleles of the *DCDC2* gene.

Variant DCDC2 Polynucleotide Probes and Primers

The polynucleotides may be used in any assay that permits detection of a variation in the *DCDC2* gene that is associated with susceptibility for developing a LD (e.g., RD, LI). Such methods may encompass, for example, hybridization-mediated, ligation-mediated, or primer

extension-mediated methods of detection. Furthermore, any combination of these methods may be utilized in the invention.

In one embodiment, the polynucleotides detect an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD by amplifying a region of DNA that comprises the alteration in the gene. Any method of amplification may be used. In a second embodiment, the polynucleotides detect an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD by amplifying a region of DNA that comprises the alteration in the gene. In one specific embodiment, a region of DNA comprising an alteration is amplified by using polymerase chain reaction (PCR). (Ann. Rev. Biochem., 61:131-156 (1992)); Gilliland et al, Proc. Natl. Acad. Sci., 87: 2725-2729 (1990); Bevan et al, PCR Methods and Applications, 1: 222-228 (1992); Green et al, PCR Methods and Applications, 1: 77-90 (1991); Blackwell et al, Science, 250: 1104-1110 (1990). PCR refers, for example, to the method of Mullis (See e.g., U.S. Pat. Nos. 4,683,195 4,683,202, and 4,965,188, herein incorporated by reference), which describes a method for increasing the concentration of a region of DNA, in a mixture of genomic DNA, without cloning or purification. For example, the polynucleotide primers described herein of the invention are combined with a DNA mixture (or any polynucleotide sequence that can be amplified with the polynucleotide primers), wherein the DNA comprises the *DCDC2* gene and/or the *KIAA0319* gene. The mixture also includes the amplification reagents (e.g., deoxyribonucleotide triphosphates, buffer, etc.) necessary for the thermal cycling reaction. According to standard PCR methods, the mixture undergoes a series of denaturation, primer annealing, and polymerase extension steps to amplify the region of DNA that comprises a variation in the *DCDC2* gene and/or a variation in the *KIAA0319* gene. The length of the amplified region of DNA is determined by the relative positions of the primers with respect to each other and, therefore, this length is a controllable parameter. For example, hybridization of the primers may occur such that the ends of the primers proximal to the mutation are separated by 1 to 10,000 base pairs (e.g., 10 base pairs (bp) 50 bp, 200 bp, 500 bp, 1,000 bp, 2,500 bp, 5,000 bp, or 10,000 bp).

Standard instrumentation is used for amplification of DNA and detection of amplified DNA. For example, a wide variety of instrumentation has been developed for carrying out nucleic acid amplifications, particularly PCR, e.g. Johnson et al, U.S. Pat. No. 5,038,852 (computer-controlled thermal cycler); Wittwer et al, Nucleic Acids Research, 17: 4353-4357 (1989) (capillary tube PCR); Hallsby, U.S. Pat. No. 5,187,084 (air-based temperature control); Garner

et al, Biotechniques, 14: 112-115 (1993) (high-throughput PCR in 864-well plates); Wilding et al, International application No. PCT/US93/04039 (PCR in micro-machined structures); Schnipelsky et al, European patent application No. 90301061.9 (publ. No. 0381501 A2) (disposable, single use PCR device). In certain embodiments, real-time PCR or other methods known in the art, such as the Taqman assay, is used.

Amplified DNA may be analyzed by several different methods. Such methods for analyzing the amplified DNA include, but are not limited to, (Sanger) sequencing of the DNA, determining the size of the fragment by electrophoresis or chromatography, hybridization with a labeled probe, hybridization to a DNA array or microarray, incorporation of biotinylated primers followed by avidin-enzyme conjugate detection, or incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment. In one embodiment, the amplified DNA is analyzed by gel electrophoresis. Methods of gel electrophoresis are well known in the art. See for example, Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992. Amplified DNA can be visualized, for example, by fluorescent or radioactive means. The DNA may also be transferred to a solid support such as a nitrocellulose membrane and subjected to Southern Blotting following gel electrophoresis. In one aspect, the DNA is analyzed by electrophoresis and exposed to ethidium bromide and visualized under ultra-violet light.

In one aspect, the alteration in the *DCDC2* gene that is associated with susceptibility for developing RD is a deletion. The deletion may be detected using polynucleotide primers described herein. For example, a set of three primers may be used to distinguish between an allele of the *DCDC2* gene that comprises a deletion and a wildtype *DCDC2* gene. Use of the set of three primers results in amplification of a region of DNA that is dependent on which *DCDC2* allele is present in the sample. In some instances, a deletion is protective, such as allele 39/Del in Table S4 of Example 1. In some embodiments, alterations or variants are protective.

In another embodiment, amplified DNA is analyzed by DNA sequencing. DNA sequence determination may be performed by standard methods such as dideoxy chain termination technology (Sanger sequencing) and gel-electrophoresis, or by other methods such as by pyrosequencing (Biotage AB, Uppsala, Sweden). The nucleic acid sequence of the amplified DNA can be compared to the nucleic acid sequence of wild type DNA to identify whether a

variation in the *DCDC2* and/or *KIAA0319* gene that is associated with susceptibility for developing LD is present.

In another embodiment, the polynucleotides of the disclosure detect an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD by hybridization-mediated methods. In a further embodiment, the polynucleotides detect an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD by hybridization-mediated methods. In one embodiment, a polynucleotide probe hybridizes to an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD (and to flanking nucleotides), but not to a wild type *DCDC2* gene. In another embodiment, a polynucleotide probe hybridizes to an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD (and flanking nucleotides), but not to a wild type *KIAA0319* gene. The polynucleotide probe may comprise nucleotides that are fluorescently, radioactively, or chemically labeled to facilitate detection of hybridization. Hybridization may be performed and detected by standard methods known in the art, such as by Northern blotting, Southern blotting, fluorescent in situ hybridization (FISH), or hybridization to polynucleotides on a solid support (e.g., DNA arrays, microarrays, cDNA arrays, or Affymetrix chips). In one embodiment, the polynucleotide probe is used to hybridize genomic DNA by FISH. FISH can be used, for example, in metaphase cells, to detect a deletion in genomic DNA. Using FISH, genomic DNA is denatured to separate the complementary strands within the DNA double helix structure. The polynucleotide probe is combined with the denatured genomic DNA. If an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD or an alteration in the KIAA0319 gene that is associated with susceptibility for developing a LD is present, the probe will hybridize to the genomic DNA. The probe signal (e.g., fluorescence) can be detected through a fluorescent microscope for the presence or absence of signal. The absence of signal indicates the absence of an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD (e.g., RD, LI) or the absence of an alteration in the KIAA0319 gene that is associated with susceptibility for developing a LD (e.g., RD, LI). Alternatively, presence of signal can be used to determine the absence of an alteration in the DCDC2 or KIAA0319 gene.

In another embodiment, the polynucleotides detect an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD (e.g., RD, LI) or an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD (e.g., RD, LI) by primer extension with DNA polymerase. In one embodiment, a polynucleotide primer hybridizes

immediately adjacent to the alteration. A single base sequencing reaction using labeled dideoxynucleotide terminators may be used to detect the alteration. If an alteration is present, the labeled terminator will be incorporated into extension product; if an alteration is not present, the labeled terminator will not be incorporated. In another aspect, a polynucleotide primer hybridizes to an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD or an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD. The primer, or a portion thereof, will not hybridize to a wild type *DCDC2* or wild type *KIAA0319* gene. If an alteration is present, primer extension occurs; if an alteration is not present, primer extension does not occur. The primers and/or nucleotides may further include fluorescent, radioactive, or chemical probes. A primer labeled by primer extension may be detected by measuring the intensity of the extension product, such as by gel electrophoresis, mass spectrometry, or any other method for detecting fluorescent, radioactive, or chemical labels.

In another embodiment, the polynucleotides detect an alteration in the *DCDC2* gene that is associated with susceptibility for developing a LD or an alteration in the *KIAA0319* gene that is associated with susceptibility for developing a LD by ligation. In one aspect, a polynucleotide primer hybridizes to a variation in the *DCDC2* gene that is associated with susceptibility for developing a LD or to a variation in the *KIAA0319* gene that is associated with susceptibility for developing a LD. The primer will not hybridize to the wild type gene (e.g., wild type *DCDC2* gene). A second polynucleotide that hybridizes to a region of the *DCDC2* gene immediately adjacent to the first primer or to a region of the *KIAA0319* gene immediately adjacent to the first primer is also provided. One, or both, of the polynucleotide primers may be fluorescently, radioactively, or chemically labeled. Ligation of the two polynucleotide primers occurs in the presence of DNA ligase if an alteration in the gene (e.g., an alteration *DCDC2* or *KIAA0319* gene) that is associated with susceptibility for developing a LD is present. Ligation may be detected by gel electrophoresis, mass spectrometry, or by measuring the intensity of fluorescent, radioactive, or chemical labels.

EXAMPLES

The following examples are for illustrative purposes and are not intended to be limiting in any way.

Example 1

Materials and Methods

Subject Recruitment, Data and DNA Collection, and Data Management.

Subject recruitment and collection of phenotype data and DNA for the ALSPAC cohort was done by the ALSPAC team, under the supervision of S.M. Ring and these data were managed for this study by L.L. Miller.

The Avon Longitudinal Study of Parents and Children (ALSPAC)

The ALSPAC is a prospective birth cohort based in the Avon region of the United Kingdom. It consists of children mostly of northern European descent, born in 1991 and 1992. Children were recruited before birth; recruitment of their pregnant mothers resulted in a total of 15,458 fetuses, of whom 14,701 were alive at 1 year of age. Details regarding the participants, recruitment, and study methodologies are described in detail elsewhere (bristol.ac.uk/alspac) The children of the ALSPAC have been extensively phenotyped from before birth to early adulthood. An update on the status of the cohort was published in 2012. (S11). The reading, language, and cognitive measures used for this study were collected at ages 7, 8, and 9 years. DNA samples from 10,676 of these children were available for genotyping. Of this subset, the number of children who completed the language and cognitive measures varies by measure, but is generally 5200-5600 subjects.

ALSPAC Reading Measures

Reading measures in the ALSPAC include a phoneme deletion task at age 7, single-word reading at ages 7 and 9, spelling at ages 7 and 9, single non-word reading at age 9, and passage comprehension, speed and accuracy at age 9. The phoneme deletion task measures phoneme awareness (S12), which is widely considered to be a core deficit in RD (S13). The child listens to a word spoken aloud, and is then asked to remove a specific phoneme from that word to make a new word (e.g. what word is created when the /b/ sound is removed from the word 'block'? 'Lock.'). This task is also known as the Auditory Analysis Test, and was developed by Rosner and Simon (S14). Single-word reading was assessed at age 7 using the reading subtest of the Wechsler Objective Reading Dimensions (WORD) (S15). At age 7 and 9, spelling was assessed; the child was asked to spell a set of 15 age-adjusted words (S15). At age 9, single-word reading was again assessed by asking the child to read ten real words and ten non-words aloud. The words and non-words used are a subset of a larger list of words and non-words taken from research conducted by Terezinha Nunes and others at Oxford (S16). Reading speed, accuracy,

and comprehension scores were ascertained at age 9, using the Neale Analysis of Reading Ability (NARA-II) (S17). All three measures are standardized. The child read passages from a booklet aloud and immediately afterward was asked questions about what he/she read to assess reading comprehension. Accuracy was measured by counting the number of mistakes (mispronunciations, substitutions, etc.) the child made and converting to a standardized score. Reading speed was number of words per minute.

ALSPAC Language Measures

The language measures focused on for this study were ascertained at 8 years of age. The first of these is a non-word repetition (NWR) task, wherein the child is asked to repeat recorded non-words. This task measures short-term phonological memory and processing (S18). The second is a subtest of the Wechsler Objective Language Dimensions that measures language comprehension (WOLD Comp) (S19). For this task, the child is asked a series of questions about a paragraph describing a picture, which was read aloud by an examiner. Children with LI consistently perform poorly on these measures (S20, S21).

ALSPAC IQ Assessment

Verbal, performance, and total IQ were assessed at age 8, using the Wechsler Intelligence Scale for Children (WISC-III).

Ethical Approval

Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee, the Local UK Research Ethics Committee, and the Yale Human Investigation Committee.

DYX2 TagSNP Panel Design and Genotyping.

TagSNPs designed to capture the common variation in the DYX2 locus were selected using the association study design server of Han et. al. (design.cs.ucla.edu) (33). SNPs were genotyped on the Sequenom platform, in collaboration with the Yale Center for Genome Analysis (West Haven, CT), as per standard protocols. Call rate and descriptive statistics for the SNPs described herein are listed in Table S4. rs4504469, rs2038137, and rs2143340 were genotyped by Scerri et. al., as described (24).). Minor allele frequency for all tagSNPs was greater than or equal to 0.05. Average power to capture known common variants (MAF>0.05) within DYX2 using this panel was estimated at 83.44% a priori. A number of other SNPs were included in addition to

the tagSNP panel, including several that had been previously associated with RD and coding SNPs in DCDC2.

Haplotype-Based Association Analysis.

Linkage disequilibrium was assessed and haplotypes defined using the Haploview software package, version 4.2 (34). Markers that deviated substantially from Hardy-Weinberg equilibrium, or that had a call rate <85%, were not used for haplotype analysis. The four-gamete rule option was used to demarcate haplotype blocks, which resulted in 44 haplotype blocks covering the DYX2 locus. Association analysis was performed with individual haplotypes that had frequencies of 0.01 or greater (208 total), using the Plink software package, version 1.07 (S4). The association analyses were performed using chi squared and logistic regression test statistics (—hap-assoc and –hap-logistic options). Individuals who were not identified as non-Hispanic white, who had a total IQ below 75, or whose DNA sample returned an average call rate below 85% for SNPs that passed quality control, were excluded from association analysis. To correct for multiple testing, a Bonferroni correction with the alpha level set at 0.05 was applied, treating each of the 208 haplotypes as an individual test; the threshold level is therefore 0.05/208 = 2.4038x10-4. As each phenotype constituted an independent hypothesis (phonological awareness and language), this threshold was not doubled to account for there being two phenotypes.

BV677278 Genotyping.

Carriers of the DCDC2 haplotypes of interest that could be phased unequivocally (using Plink's –hap-phase function) were genotyped for the BV677278 STR. BV677278 is genotyped by PCR amplification and subsequent Sanger sequencing. Alleles are called by an in-house Perl script developed by Y. Kong. The Perl script is available upon request.

Amplification Primers

STR_F: 5'-TGTAAAACGACGGCCAGTTGTTGAATCCCAGACCACAA-3' (SEQ ID NO:1)

STR_R: 5'-ATCCCGATGAAATGAAAAGG-3' (SEQ ID NO:2)

M13F Sequencing Primer

5'-TGTAAAACGACGGCCAGT (SEQ ID NO:3)

Amplification Reaction Mixture (per 10µl reaction)

10X PCR Buffer (Qiagen): 1µl

$MgCl_2$ (25mM) (Qiagen): 0.4µl

dNTPs (10mM): 0.25µl

Primer STR_F (10µM): 0.25µl

Primer STR_R (10µM): 0.25µl

HotStarTaq™ (Qiagen, diluted to 0.5units/µl in Taq dilution buffer): 0.20µl

Template DNA: 1µl (~10ng/µl)

Nuclease-free H_20: 6.65µl

Amplification Reaction

1. 15 minutes, 95°C
2. 30 seconds, 95°C
3. 30 seconds, 65°C
   -Decrease 1°C/cycle
4. 60 seconds, 72°C
5. GoTo step 2, 9 times
6. 30 seconds, 95°C
7. 30 seconds, 56°C
8. 60 seconds, 72°C
9. GoTo step 6, 34 times
10. 5 minutes, 72°C
11. ∞, 4°C

PCR Purification and Sequencing

PCR products were purified using ExoSAP-IT® enzyme mix, according to the manufacturer's protocol. Purified amplicons were then mixed with M13F sequencing primer, and sequenced. Sanger sequencing was performed at the Yale W.M. Keck DNA Sequencing Facility, as per their standard sequencing protocol.

Genotype Calling

Alleles were called from the electropherograms, using an in-house Perl script developed by Y. Kong for the purpose.

Microdeletion Genotyping.

Carriers of the *DCDC2* haplotypes of interest were also genotyped for the 2,445bp *DCDC2* microdeletion. This deletion encompasses the entire BV677278 STR within its breakpoints, so it must be genotyped in addition to BV677278 to get an accurate genotype for apparent BV677278 homozygotes. The microdeletion is genotyped by allele-specific PCR and agarose electrophoresis. The three-primer reaction generates a ~600bp amplicon from intact chromosomes (no deletion), and a ~200bp amplicon from chromosomes with the deletion, allowing heterozygotes and both homozygotes to be readily distinguishable from one another.

Amplification Primers

Primer Del_F: 5'-AGCCTGCCTACCACAGAGAA-3' (SEQ ID NO:4)

Primer Del_RC: 5'-GGAACAACCTCACAGAAATGG-3' (SEQ ID NO:5)

Primer Del_RD: 5'-TGAAACCCCGTCTCTACTGAA-3' (SEQ ID NO:6)

Amplification Reaction Mixture (per 10µl reaction)

10X PCR Buffer (Qiagen): 1µl

$MgCl_2$ (25mM) (Qiagen): 0.4µl

dNTPs (10mM): 0.25µl

Primer Del_F (10µM): 0.30µl

Primer Del_RC (10µM): 0.20µl

Primer Del_RD: 0.20µl

HotStarTaq™ (Qiagen, diluted to 0.5units/µl in Taq dilution buffer): 0.20µl

Template DNA: 1µl (~10ng/µl)

Nuclease-free H_20: 6.45µl

Amplification Reaction

The amplification reaction for the microdeletion is the same as for the BV677278 STR (see above).

Agarose Electrophoresis

PCR products were electrophoresed on 1% agarose gels, using standard 1X TBE buffer with ethidium bromide (0.2µg/mL), via standard methods, at 100-150V depending on gel size. Gels were imaged on a UV transilluminator, and documented with a Bio-Rad Gel Doc™ XR imaging system. Genotypes were called from the gels manually.

Protein Identification by SILAC-Based Mass Spectrometry.

Raji and HeLa cells were SILAC-labeled with with Lys-8 and Arg-10 (Eurisotop) or their naturally-occurring counterparts Lys-0, Arg-10 (Sigma), as described (20). Heavy nuclear lysate prepared from these cells was incubated with a biotinylated oligonucleotide probe identical to a segment of BV677278 that had been previously shown to bind a nuclear protein with high specificity (15). Light nuclear lysate was incubated with a biotinylated scrambled probe previously shown not to bind the nuclear protein of interest (15). The resulting oligonucleotide-protein complexes were pulled down with streptavidin-conjugated beads and subjected to quantitative mass spectrometry, as described previously (36). The reverse experiment was also done (binding probe with light lysate, scrambled probe with heavy lysate), resulting in the two-dimensional interaction plots in Fig. 3A-B. The above experiment is described in more detail as follows:

SILAC labeling of HeLa and Raji cells

Raji cells were labeled for at least 8 generations in DMEM (-Arg, -Lys) medium containing 10% dialyzed fetal bovine serum (Gibco) supplemented with 58 mg/L 13C615N4 L-arginine and 34 mg/L 13C615N2 L-lysine (Eurisotop) or the corresponding non-labeled amino acids. For Raji, cell extracts were prepared as described in Wu et. al.(S5). HeLa S3 cells were SILAC-labeled in RPMI 1640 (-Arg, -Lys) medium containing 10% dialyzed fetal bovine serum (Gibco) supplemented with 84 mg/L 13C615N4 L-arginine and 40 mg/L 13C615N2 L-lysine (Eurisotop) or the corresponding non-labeled amino acids, respectively. For HeLa S3, three consecutive batches of cells were independently harvested and cell extracts prepared as described by Dignam et. al.(S6).

SILAC, DNA pulldown of proteins, and quantitative mass spectrometry were performed as previously described (S7), using the cell lines Raji and HeLa. The binding pulldown probe is a concatamer of two copies of the EMSA3 probe used in the EMSA experiments we reported in 2011, while the scrambled probe is a concatamer of two copies of the EMSA3-Scram1 probe from the same experiments (S8).

Oligonucleotides:

EMSA3_for:

5'-TTGAGAGGAAGGAAAGGAAGGATCCCTGAGAGGAAGGAAAGGAAGGA-3'

(SEQ ID NO:7)

EMSA3_rev:

5'-AATCCTTCCTTTCCTTCCTCTCAGGGATCCTTCCTTTCCTTCCTCTC-3'

(SEQ ID NO:8)

EMSA3/Scram1_for:

5'-TTGAGAGAGAGAGAGAGAGAGATCCCTGAGAGAGAGAGAGAGAGAGA-3'

(SEQ ID NO:9)

EMSA3/Scram1_rev:

5'-AATCTCTCTCTCTCTCTCTCTCAGGGATCTCTCTCTCTCTCTCTCTC-3'

(SEQ ID NO:10)

DNA-Pulldown

25 μg of annealed, concatenated and desthiobiotinylated DNA probes was bound to 75 μl of Dynabeads MyOne C1 (Life Technologies). Excess oligonucleotides were removed and beads were incubated with 400 μg of SILAC-labelled nuclear extracts in protein binding buffer (150 mM NaCl, 50 mM Tris–HCl (pH 8), 0.5% NP-40, 10 mM $MgCl_2$, protease inhibitor cocktail; Roche). After 1 h of on a rotation wheel at 4°C, the beads were washed three times, combined and DNA-protein complexes eluted in protein binding buffer containing 16 mM biotin. The supernatant was precipitated with 4 volumes (v/v) of ethanol overnight and the proteins pellets by maximum centrifugation on a table top microcentrifuge. The pellet was resolubilized in 8M urea/50 mM Tris pH8.0, reduced with 1 mM DTT, alkylated with 3 mM iodoacetamide and subsequently digested with trypsin (Promega) in 50 mM ammonium bicarbonate pH8 buffer at room temperature overnight. Samples were stored on stage tips and eluted prior to use.

Mass spectrometry

Peptides were separated with a 140 min gradient from 5 to 60 percent acetonitril (EasyHPLC, Thermo Fisher) using a 75 um 15 cm capillary packed with 3.0 um C18 beads (Dr. Maisch) directly mounted to a LTQ-Orbitrap mass spectrometer (Thermo Fisher). The instrument was operated in a data-dependent top10 acquisition modus. The raw data was searched using the MaxQuant software (version 1.2.0.18) suite against the complete IPI human database (v3.68, 87061 entries). Enzyme search specificity was trypsin/p with 2 allowed miscleavages. Carbamidomethylation was set as fixed modification while methionine oxidation and protein N-acetylation was considered as variable modifications. The search was performed with an initial mass tolerance of 7 ppm mass accuracy for the precursor ion and 0.5 Da for the MS/MS spectra.

ChIP-QPCR.

The AbCam ChIP kit (cat. # ab500) was used to perform the ChIP assays described, according to the manufacturer's instructions, but with several modifications described below. For qPCR, the Qiagen QuantiTect® SYBR® Green qPCR kit for ChIP-qPCR was used. Manufacturer's instructions were followed 25pg of template per reaction. All reactions in triplicate. Antibodies, primer sequences, and detailed methods for this experiment are available in the Supporting Information. Quality control data for qPCR is shown in Fig. S3.

Step 1: Cell Fixation and Collection

- For each set of 3 ChIP reactions, 9 million freshly harvested cells (Raji) were used instead of 3 million, with volumes appropriate for 9 million cells, as given in the protocol.

Step 2: Cell Lysis

- After the final PBS wash in the cell fixation step, each aliquot of 9 million cells was carried through the cell lysis step with volumes appropriate for 3 million cells (9 million treated as if it were 3 million—resulting in 3 million cells per ChIP reaction instead of 1 million).
- 100µl Buffer D with protease inhibitors was replaced with ~150µl of the following:

Buffer D2/PI (S9)

10mM Tris

1mM EDTA

0.5mM EGTA

pH = 7

1X Protease inhibitor cocktail

- A Branson 450 probe sonicator was used for sonication. DNA was fragmented with 18 20-second pulses, with the amplitude set to 6 and the DC set to continuous. The samples were kept in ice water during sonication and were allowed to rest 2 minutes on ice between each pulse.

Step 3: Immunoprecipitation

- Followed manufacturer's protocol.
    - α-ETV6 antibody: sc-166835X (Santa Cruz Biotech), 5µg/ChIP
    - Control antibody: ab1791 (AbCam) (α-variant histone H3, enriched in actively transcribing genes, an aliquot of this comes with the kit), 2µg/ChIP

- Instead of the beads provided in the kit, Magna-Bind™ Protein A/G magnetic beads were used and a magnet stand, according to manufacturer's protocol was used, instead of the centrifuge, for immunoprecipitation.

Step 4: DNA Purification

- After reversing the crosslinks and proteinase K treatment, DNA was purified using a Qiagen QIAquick® PCR Purification kit instead of the DNA-purifying slurry provided in the ChIP kit.
- After purification with a QIAquick® column, each final product was eluted with a total of 60µl Buffer EB, in two 30µl elution steps.

  The end product was quantified by fluorescence (Quant-IT™ PicoGreen® dsDNA assay kit, as per manufacturer's protocol).

  qPCR
- The Qiagen QuantiTect® SYBR® Green qPCR kit for ChIP-qPCR was used, following manufacturer's instructions.
- 25pg template per reaction was used.
- 1.25µl of each primer (5µM) was used per reaction.
- A log-5 standard curve was done using input Raji DNA (sonicated, but not subjected to ChIP). See Fig. S3A-B.
- To avoid pipetting error, all samples were diluted to the same concentration as the least-concentrated sample, so the same amount could be added to every reaction. Dilutions were done serially.
- All reactions in triplicate were performed.

qPCR Primers

ChIP-STR (BV677278 amplicon)

Primer ChIPSTR_F: 5'-TCATGCAAAGTTCCAAAACC-3' (SEQ ID NO:11)

Primer ChIPSTR_R: 5'-GATTTCCTCCCTCCCTTCC-3' (SEQ ID NO:12)

These primers capture the entire BV677278 repeat, and generate a ~200bp amplicon.

β-actin (+ control)

Primer βAct_F: 5'-GCCCTAGGCACCAGGGTGTGA-3' (SEQ ID NO:13)

Primer βAct_R: 5'-ACAGGGTGCTCCTCAGGGGC-3' (SEQ ID NO:14)

These primers amplify a ~150bp sequence from the actively transcribing *β-actin* gene.

q*PCR Reaction*

1. 15 minutes, 95°C
2. 30 seconds, 95°C
3. 30 seconds, 65°C
   -Decrease 1°C per cycle
4. 60 seconds, 72°C
5. Plate read
6. GoTo step 2, 9 times
7. 30 seconds, 95°C
8. 30 seconds, 56°C
9. 60 seconds, 72°C
10. Plate read
11. GoTo step 6, 39 times
12. 5 minutes, 72°C
13. Melting Curve
14. ∞, 4°C

*Quality control data for the ChIP experiment reported here are shown in Figure S3.

Enrichment Calculations

Fold enrichment was calculated with respect to the no antibody control (a complete ChIP reaction, but with no antibody—only beads). Briefly, this was done by raising 2 to the negative power of the difference between the C(t) of an experimental condition and its respective no-antibody control: Fold Enrichment = $2^{-[C(t)Exp - C(t)NoAntibody]}$

Results

Two Six-Marker Haplotypes in DCDC2 are Associated with Reduced Performance on Reading and Language Measures

To determine whether DCDC2, KIAA0319, both, or neither gene is responsible for the DYX2 signal, a tagSNP panel was designed to densely cover the DYX2 locus. Haplotype-based association analysis of reading and language in a large, extensively phenotyped birth cohort: the Avon Longitudinal Study of Parents and Children (ALSPAC), was then performed (17). Analysis showed a six-marker haplotype block within *DCDC2*, of which two haplotypes—CGCGAG and GACGAG— associated with very poor performance on a phoneme deletion task

and a composite language measure, respectively (Table 1). For this analysis, RD cases were defined as individuals scoring two or more standard deviations below the mean on the phoneme deletion task, and LI cases as individuals scoring two or more standard deviations below the mean on either of two language measures (WOLD/NWR). The phoneme deletion task measures phonological awareness, which is widely considered to be the core deficit in RD (2). The Wechsler Objective Learning Dimensions (WOLD) verbal comprehension and nonword repetition (NWR) tasks that comprise the WOLD/NWR composite language measure are used to assess deficient language skills; children with LI show consistently poor performance on these measures (18, 19) (see Table S1 and the Materials and Methods for more information on these phenotypic measures). Cases were defined this way to examine association of DYX2 variants with severe RD and LI. The two haplotypes show strong association with their respective phenotypes; the CGCGAG-RD association survived Bonferroni correction for multiple testing and the GACGAG-LI p-value was just below the threshold. However, the associations by themselves are not strong enough to rule out type I error, partly due to the low frequencies of the haplotypes and the small number of cases. Interestingly, however, the effect of these haplotypes is strong enough to reduce mean performance on relevant phenotypic measures in carriers versus non-carriers. Carriers of the CGCGAG haplotype, on average, showed significantly poorer performance on eight reading-related measures compared to non-carriers. Likewise, carriers of the GACGAG haplotype showed significantly lower average performance on the WOLD/NWR composite language measure (Table 2). This quantitative effect indicated that this finding is not a false positive and prompted further analysis. Additionally, this haplotype block resides in close proximity to BV677278, a putatively functional compound short tandem repeat (STR) Applicants reported previously (11) (Fig. 1C). The polymorphism of BV677278 derives from five discrete repeat units that vary in number (Fig. 1A, Table S4). This STR evolves rapidly, as indicated by its high degree of polymorphism among primate species and within *Homo sapiens* (Fig. 1B, Fig. S2, Table S4).

The DCDC2 Risk Haplotypes are in Strong Linkage Disequilibrium with Two Alleles of BV677278

The associated haplotype block is adjacent to BV677278 (Fig. 1C) and whether the two risk haplotypes could be capturing association arising from functional alleles of BV677278 via linkage disequilibrium was assessed. To address this question, all carriers of these haplotypes were subjected to BV677278 genotyping by Sanger sequencing. Of the carriers of the CGCGAG haplotype, 92% also carry BV677278 allele 5. Likewise, 78% of the carriers of GACGAG also

carry BV677278 allele 6 (Table 1). Alleles 5 and 6 are similar in structure to each other, and cluster phylogenetically to the same clade (Table S4, Fig. S1). Indeed, nearly all carriers of these two haplotypes also carry an allele from this clade (Table 1). These results further implicate BV677278 as a RD risk variant and expand it as a possible LI risk variant (11), and together with its apparent regulatory capacity, suggest that these BV677278 alleles are responsible for the risk haplotypes' effects.

BV677278 Specifically Binds the Transcription Factor ETV6

To gain mechanistic insight into the function of BV677278, quantitative mass spectrometry was used to identify the protein(s) that bind to this locus (20). To this end, a biotinylated oligonucleotide probe carrying segments of the BV677278 repeat previously shown to bind a nuclear protein, and a scrambled non-binding control, were incubated with nuclear extracts that had been SILAC-labeled (15). SILAC-labeling involves culturing two parallel populations of cells—one with media containing amino acids labeled with heavy isotopes of carbon and nitrogen, the other with naturally-occurring isotopes. After the label is incorporated, proteins from the two populations ('heavy' and 'light') can be differentiated from each other by quantitative mass spectrometry. The heavy nuclear extract was incubated with the BV677278 probe, and the light nuclear extract with the control probe. The probes were then pulled down with streptavidin-conjugated beads, and the resulting protein mixture was subjected to quantitative mass spectrometry, and proteins were looked for that were significantly enriched by pulldown with the BV677278 probe compared to the control probe (high heavy:light ratio). The experiment was conducted with nuclear extracts derived from either HeLa or Raji cells, and repeated with a label-switch resulting in a two-dimensional interaction plot. These experiments yielded a single candidate, shared by both HeLa and Raji: the transcription factor ETV6 (Fig. 2A-B). To confirm the BV677278-ETV6 interaction, chromatin immunoprecipitation with quantitative PCR was performed (ChIP-qPCR) using α-ETV6 antibody, in Raji cells. Immunoprecipitation with the α-ETV6 antibody showed marked enrichment for the BV677278 amplicon, but not for the control amplicon derived from the gene encoding β-Actin (*ACTB*) (Fig. 2C). These results demonstrated that ETV6 binds the BV677278 region.

The DCDC2 Risk Haplotypes Show a Synergistic Genetic Interaction with a Known RD Risk Haplotype in the gene KIAA0319

Together with Applicant's previous findings, these data implicated BV677278 as a regulatory element. Luciferase assays suggest that BV677278 is capable of modulating expression from the *DCDC2* promoter, but it may regulate other genes (15). A three-marker risk haplotype encompassing the 5' half and upstream sequence of *KIAA0319* has been consistently associated with lowered reading performance (21-24). Additionally, expression of *KIAA0319* in human neural cell lines is reduced with this haplotype, relative to non-risk haplotypes (25). Applicants therefore questioned whether BV677278 might interact genetically with the *KIAA0319* risk haplotype, and examined the effect of carrying both a *DCDC2* (CGCGAG or GACGAG) and the *KIAA0319* risk haplotype on several reading, language, and cognitive measures. Strikingly, subjects carrying risk haplotypes in both genes showed markedly worse mean performance (up to 0.40 standard deviations) on nearly all measures examined (Fig. 3A). This reduction in performance in carriers of both risk haplotypes is, for most of the phenotypes examined, greater than the sum of those of single carriers, indicating a synergistic interaction between these two genes. This result corroborates a previous report, which provided statistical evidence that *DCDC2* and *KIAA0319* interact to influence RD risk (26).

Discussion

Given the remarkable similarity of the human exome to those of other higher primates, it has been hypothesized that rapidly evolving regulatory elements are responsible for the large phenotypic differences we observe. The recently published results of the ENCODE Consortium, which showed most of the non-coding genome to be active and much of the active proportion to be regulatory, lend circumstantial support to this hypothesis (27). Here, Applicants provide evidence of just such a regulatory element affecting reading and language, two exclusively human phenotypes. BV677278 expanded rapidly from gorilla to human, though the sequence flanking it is quite conserved (Fig. 1B), and its presence, length, and sequence vary widely among primate species (Fig. S2).

This element specifically binds ETV6, a transcription factor and proto-oncogene also known as TEL (translocation ETS leukemia). The *ETV6* gene forms oncogenic fusions, often with the *AML1* proto-oncogene, that are frequently seen in leukemia (28). ETV6's effect on transcription is generally repressive via recruitment of a co-repressor complex (29). Monomeric ETV6 has essentially no affinity for its binding sequence; it must at least dimerize to bind DNA (30). There is evidence that ETV6 polymerizes *in vivo*, with the length of the polymer dependent on the number and spacing of binding sites (31). This property suggests that different alleles of

BV677278 bind ETV6 polymers of different lengths, depending on the number of suitably spaced ETV6 binding sites, and that these differences change the regulatory power of the complex (Fig. 3B). Supporting this idea is the structural similarity of alleles 5 and 6: both have the same GGAA insertion in repeat unit 2, relative to the most common allele (Table S4). GGAA is the core binding sequence of ETV6 (30), and this insertion could recruit an additional ETV6 monomer to the complex.

However, whether ETV6 represses transcription in this context, and what genes it targets, are uncertain. Applicants previously reported luciferase assays appear to indicate that some BV677278 alleles activate transcription from the *DCDC2* promoter, and that alleles with very different structures (e.g. 3 and 5, Table S4) activate transcription to a similar extent (15). BV677278's genetic interaction with the *KIAA0319* risk haplotype, and its dramatic effect on phenotype, suggest *KIAA0319* as a target gene *in vivo*. The *KIAA0319* risk haplotype is known to be associated with reduced *KIAA0319* expression, at least in human neural cell lines, suggesting the possibility that it carries a promoter or promoter-proximal variant that increases repression (or decreases activation) by BV677278, resulting in reduced gene expression and possible phenotypic consequences. That reduced IQ was also observed with the *DCDC2-KIAA0319* interaction (Fig. 3A) may reflect pathology at the cellular level (e.g. disrupted neuronal migration), or it may simply reflect the importance of language in measuring IQ. BV677278 genotyping in all members of the ALSPAC and subsequent combinatorial analysis, together with chromatin conformation experiments, will further illuminate BV677278's target genes and mechanism of action.

The *DCDC2* and *KIAA0319* risk haplotypes have a synergistic effect on reading, language, and cognitive phenotypes. This lends credence to the 'phantom heritability' hypothesis, which explains the so-called missing heritability of continuous traits as resulting from non-additive interactions between risk variants (32). Also supporting this idea is that although carriers of the *DCDC2* risk haplotypes show reduced average performance on phenotypic measures, the standard deviations for these measures were generally similar to those of non-carriers (Table 2). This implies that the magnitude of effect of the risk haplotypes on phenotype lies on a continuum, and is dependent on other, interacting risk variants, as well as environmental and stochastic factors. Additionally, these results may partially explain the missing efficacy of GWAS studies. If rapidly evolving regulatory elements are indeed substantially responsible for continuous phenotypic variation, they would be expected, like BV677278, to show a higher

degree of polymorphism than the average SNP. This would make them difficult to identify by standard single-marker analyses in GWAS, reinforcing the importance of multi-marker, pathway, and gene-gene interaction analyses in the study of complex traits.

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Tables of Example 1

Table 1**:** Association and linkage disequilibrium data for *DCDC2* risk haplotypes. Phenotypes are described in Table S1 and in the Materials and Methods. Cases are defined by a score of less than or equal to 2SD below the mean. P-values that survived Bonferroni correction for multiple testing (α=0.05) are bolded. '% Allele 5,' etc. means 'percentage of haplotype carriers with at least one copy of that allele or group of alleles.' Clade 1, the phylogenetic branch of alleles that includes 5 and 6, is described in Fig. S1.

Association Data						
Haplotype	Phenotype -2SD	n Cases	n Controls	Haplotype Freq.	Odds Ratio	P-value
CGCGAG	Phoneme Del. (RD)	89	5225	0.0236	3.20	**6.068×10^{-5}**
GACGAG	WOLD/NWR (LI)	270	5240	0.0364	1.91	2.84×10^{-4}
LD Data						
Haplotype	n Carriers	% Allele 5	% Allele 6	% Clade 1		
CGCGAG	226	92.0	7.5	94.3		
GACGAG	392	12.0	77.6	91.3		

Table 2: Mean performance on reading and cognitive measures in *DCDC2* risk haplotype carriers vs. non-carriers. The standard deviation is shown in parentheses next to each mean. The number of subjects in each category is shown below that category. P-values are from Student's T-tests comparing the means of carriers and non-carriers of each haplotype; p-values less than 0.05 are marked with an asterisk. NWR/WOLD refers to the average z-score of performance on NWR and WOLD Verbal Comprehension tasks. Phenotypes are described in Table S1 and in the Materials and Methods.

	CGCGAG (RD) Haplotype			GACGAG (LI) Haplotype		
	Carriers	Non-carriers	P-value	Carriers	Non-carriers	P-value
Reading 7	27.34 (9.04)	29.01 (8.77)	0.005*	29.09 (8.62)	28.92 (8.80)	0.728
N	232	4929		358	4803	
Spelling 7	24.38 (13.46)	26.29 (12.33)	0.023*	25.56 (12.77)	26.26 (12.36)	0.305
N	229	4896		355	4770	
Phoneme	19.30 (10.00)	20.80 (9.17)	0.016*	20.61 (9.20)	20.74 (9.21)	0.796
N	230	4909		357	4782	
Reading 9	7.37 (2.71)	7.73 (2.27)	0.020*	7.75 (2.33)	7.72 (2.29)	0.754
N	228	4914		359	4783	
NW Read 9	5.05 (2.58)	5.38 (2.36)	0.043*	5.47 (2.36)	5.36 (2.44)	0.391
N	228	4911		359	4780	
Spelling 9	10.03 (2.58)	10.50 (3.23)	0.031*	10.48 (3.25)	10.48 (3.26)	0.987
N	228	4904		357	4775	
Speed	105.44 (11.76)	106.34 (12.10)	0.299	106.71 (11.77)	106.27 (12.11)	0.524
N	207	4430		326	4311	
Accuracy	102.77 (14.00)	105.22 (13.10)	0.009*	105.18 (13.24)	105.11 (13.15)	0.919
N	208	4438		329	4317	
Read Comp	99.74 (11.67)	101.54 (11.37)	0.026*	101.73 (11.82)	101.44 (11.35)	0.663
N	208	4438		329	4317	
Verbal IQ	107.35 (15.70)	108.97 (15.67)	0.113	108.38 (15.90)	108.94 (15.65)	0.497
N	245	5334		388	5191	
Perf. IQ	101.23 (14.96)	100.28 (16.16)	0.366	101.10 (15.72)	101.19 (16.14)	0.913
N	245	5334		388	5191	
Total IQ	104.58 (14.22)	106.05 (15.26)	0.138	105.62 (14.95)	106.01 (15.23)	0.623
N	245	5334		388	5191	
NWR	7.54 (1.94)	7.58 (1.91)	0.724	7.40 (1.91)	7.55 (1.91)	0.136
N	245	5276		384	5137	
WOLD	7.11 (2.56)	7.33 (2.44)	0.178	7.12 (2.60)	7.33 (2.43)	0.104
N	245	5270		383	5132	
NWR/WOLD	-0.031 (0.82)	0.00 (0.78)	0.532	-0.08 (0.77)	0.01 (0.78)	0.041*
N	245	5281		384	5142	

Table S1: (A) List of phenotypes used. A detailed description of each phenotype is given in the Materials and Methods. **(B)** Case/control definitions used in association analysis.

A.

Phenotype	**Description**
Reading at 7	Wechsler Objective Reading Dimensions (WORD), single-word reading task, age 7
Reading at 9	Single-word reading task, age 9
Phoneme Del	Auditory Analysis task, age 7
Total IQ	Wechsler Intelligence Scale for Children (WISC), Total IQ, age 8
Verbal IQ	WISC Verbal IQ component, age 8
Performance IQ	WISC Performance IQ component, age 8
WOLD	Wechsler Objective Language Dimensions (WOLD), verbal comprehension task, age 8
NWR	Non-word repetition task, age 8
NW Read at 9	Non-word reading task, age 9
Spelling at 7	Single-word spelling task, age 7
Spelling at 9	Single-word spelling task, age 9
Speed	Passage reading, speed, age 9
Accuracy	Passage reading, accuracy of words read, age 9
Reading Comp.	Passage reading, comprehension, age 9

B.

Phenotype	**Description**
Phoneme Del	Cases defined as having a score less than or equal to 2 standard deviations below the mean on the phoneme deletion task
WOLD/NWR	Cases defined as having a score less than or equal to 2 standard deviations below the mean on either the WOLD verbal comprehension task *or* the non-word repetition task

Table S2: Bivariate Pearson correlations among reading and language measures in ALSPAC. Phoneme = Phoneme deletion task at age 7 years; NWR = Nonword Repetition at age 8 years; WOLD = Wechsler Objective Learning Dimensions Verbal Comprehension task at age 8 years; Avg NWR WOLD = average of z-score performance on NWR and WOLD tasks; Reading 7 = Single word reading at age 7 years; Reading 9 = Single word reading at age 9; All IQ measures were collected at age 8 years with the Wechsler Intelligence Scale for Children version III.

	Phoneme	NWR	WOLD Comp.	Avg NWR WOLD	Reading 7	Reading 9	Total IQ	Verbal IQ	Perf. IQ
Phoneme	1								
NWR	0.362	1							
WOLD	0.165	0.214	1						
Avg NWR WOLD	0.338	0.779	0.780	1					
Reading 7	0.688	0.403	0.259	0.425	1				
Reading 9	0.550	0.351	0.202	0.355	0.722	1			
Total IQ	0.406	0.324	0.386	0.455	0.500	0.387	1		
Verbal IQ	0.426	0.346	0.424	0.494	0.536	0.421	0.871	1	
Perf. IQ	0.246	0.192	0.216	0.262	0.292	0.218	0.819	0.435	1

Table S3: Statistics for the SNPs reported here. Statistics were calculated after exclusion of low-call-rate samples (<85% average call rate) and individuals not of European descent. SNPs in normal (not bold) font (SNPs 1-6 in table) comprise the *DCDC2* risk haplotype block; SNPs in bold font (SNPs 7-9 in table) comprise the *KIAA0319* risk haplotype block. SNPs are listed in the order of their respective haplotype (e.g. CGCGAG).

SNP	Call Rate	Major Allele	Major Allele Freq.	Minor Allele	Minor Allele Freq.	HWE p-value
rs33914824	92.6%	C	0.961	G	0.039	0.541
rs807694	94.0%	G	0.952	A	0.047	0.974
rs707864	93.0%	T	0.874	C	0.126	0.012
rs10456301	93.5%	G	0.929	A	0.071	0.814
rs16889066	91.2%	A	0.945	G	0.055	0.134
rs9379651	86.7%	G	0.877	A	0.123	0.720
rs4504469	89.1%	C	0.592	T	0.408	0.054
rs2038137	90.0%	G	0.630	T	0.370	0.611
rs2143340	89.6%	A	0.849	G	0.151	0.583

Table S4: Structures and population frequencies for all BV677278 alleles described to date. Allele frequencies for available alleles were calculated from a previous study (10). Population allele frequencies for alleles 11-22—only frequencies in *DCDC2* risk haplotype carriers are available (see Table S5). 'Del' signifies the 2,445bp microdeletion encompassing BV677278.

Allele	Repeat unit 1 SEQ ID NOs:15 and 16	Repeat unit 2	Repeat unit 3	Repeat unit 4	Const. Region	Repeat unit 5	Const. Region	Allele Freq*	Allele Freq**	Length
1	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.624	0.5536	102
2	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)9 (SEQ ID NO:20)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.003	0.0143	85
3	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)6 (SEQ ID NO:21)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.060	0.0464	85
4	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)6 (SEQ ID NO:21)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.106	0.1429	98
5	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)8 (SEQ ID NO:22)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.028	0.0143	106
6	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)8 (SEQ ID NO:22)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	0.039	0.0571	102
7	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)8 (SEQ ID NO:22)	(GAAA)1	(GGAA)1	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.003	0	102
8	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.003	0	90

9	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.005	0.0179	89
10	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)4 (SEQ ID NO:23)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	0.044	0.0286	90
11	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	0	98
12	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)8 (SEQ ID NO:22)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	0.0036	89
13	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)9 (SEQ ID NO:20)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	0.0071	106
14	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)9 (SEQ ID NO:20)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	110
15	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)5 (SEQ ID NO:24)	(GAAA)2	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	98
16	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)5 (SEQ ID NO:24)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	94*Coriell AfA Plate Only
17	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)4 (SEQ ID NO:23)	(GAAA)2	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	94*Coriell AfA Plate Only
18	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)2	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	106*Coriell AfA Plate Only
19	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)9 (SEQ ID NO:20)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	98
20	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)10 (SEQ ID NO:25)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	114

21	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)6 (SEQ ID NO:21)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	N/A	94
22	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)10 (SEQ ID NO:25)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
23	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)11 (SEQ ID NO:26)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	106
24	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)6 (SEQ ID NO:21)	(GAAA)2	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
25	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)8 (SEQ ID NO:22)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	93
26	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)5 (SEQ ID NO:24)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	N/A	90
27	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)5 (SEQ ID NO:24)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	81
28	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)5 (SEQ ID NO:24)	(GGGA)2	N/A	N/A	106
29	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)5 +(GGGA)1 +(GGAA)1 (SEQ ID NO:27)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
30	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)5 (SEQ ID NO:24)	(GAAA)1	(GGAA)4 (SEQ ID NO:23)	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
31	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)1 +(GGGA)1	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
32	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)8 (SEQ ID NO:22)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	94
33	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)6 (SEQ ID NO:21)	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	N/A	82

34	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)7 (SEQ ID NO:19)	(GAAA)2	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)3 (SEQ ID NO:29)	(GGGA)2	N/A	N/A	102
35	(GAGAGGAAGGAAA)1 +(GAGAGGAAGAAAA)1 (SEQ ID NO:17)	(GGAA)7 (SEQ ID NO:19)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	102
36	(GAGAGGAAGGAAA)1 +(GAGAGGAAGGAA)1 (SEQ ID NO:18)	(GGAA)9 (SEQ ID NO:20)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	109
37	(GAGAGGAAGGAAA)2 (SEQ ID NO:15)	(GGAA)6 (SEQ ID NO:21)	(GAAA)1	(GGAA)2	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)1 +(GAAA)1 +(GGAA)2 (SEQ ID NO:30)	(GGGA)2	N/A	N/A	98
38	(GAGAGGAAGGAAA)1 (SEQ ID NO:16)	(GGAA)10	(GAAA)0	(GGAA)0	GGAAAGAATGAA (SEQ ID NO:28)	(GGAA)4 (SEQ ID NO:23)	(GGGA)2	N/A	N/A	89
39/Del	x	x	x	x	x	x	x	0.085	0.1143	

* Frequency among parents of the Colorado Learning Disability Research Center families.

Sequences of alleles 1-39 from Table S4 of Example 1

Allele 1 (SEQ ID NO: 31)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 2 (SEQ ID NO: 32)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAAGAATGAAGGA
AGGAAGGAAGGAAGGGAGGGA

Allele 3 (SEQ ID NO: 33)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGAATGAAGGA
AGGAAGGAAGGAAGGGAGGGA

Allele 4 (SEQ ID NO: 34)
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AAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 5 (SEQ ID NO: 35)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGG
AAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 6 (SEQ ID NO: 36)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGG
AAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 7 (SEQ ID NO: 37)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 8 (SEQ ID NO: 38)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAAGAATG
AAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 9 (SEQ ID NO: 39)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGAATGA
AGGAAGGAAGGAAGGAAGGGAGGGA

Allele 10 (SEQ ID NO: 40)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGAATG
AAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 11 (SEQ ID NO: 41)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 12 (SEQ ID NO: 42)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGA
ATGAAGGAAGGAAGGAAGGGAGGGA

Allele 13 (SEQ ID NO: 43)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGA
AAGGAAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 14 (SEQ ID NO: 44)
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AAGGAAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 15 (SEQ ID NO: 45)

GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGAAAGAAAGGAAGGAAGG
AAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 16 (SEQ ID NO: 46)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAG
AATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 17 (SEQ ID NO: 47)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGAAAGAAAGGAAGGAAGGAAAG
AATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 18 (SEQ ID NO: 48)
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AAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 19 (SEQ ID NO: 49)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG
AAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 20 (SEQ ID NO: 50)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG
AAGAAAGGAAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 21 (SEQ ID NO: 51)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGG
AAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 22 (SEQ ID NO: 52)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 23 (SEQ ID NO: 53)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGG
AAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 24 (SEQ ID NO: 54)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGAAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 25 (SEQ ID NO: 55)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGA
ATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 26 (SEQ ID NO: 56)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAG
AATGAAGGAAGGAAGGAAGGGAGGGA

Allele 27 (SEQ ID NO: 57)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAAGAATGAAGGAAGGA
AGGAAGGAAGGGAGGGA

Allele 28 (SEQ ID NO: 58)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 29 (SEQ ID NO: 59)
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AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 30 (SEQ ID NO: 60)

GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 31 (SEQ ID NO: 61)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGG
GAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 32 (SEQ ID NO: 62)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAAG
AATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 33 (SEQ ID NO: 63)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAAGAATGAAGG
AAGGAAGGAAGGGAGGGA

Allele 34 (SEQ ID NO: 64)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGAAAGG
AAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGGAGGGA

Allele 35 (SEQ ID NO: 65)
GAGAGGAAGGAAAGAGAGGAAGAAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGG
AAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 36 (SEQ ID NO: 66)
GAGAGGAAGGAAAGAGAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGAA
AGGAAGGAAGGAAAGAATGAAGGAAGGAAGGAAGGAAGGGAGGGA

Allele 37 (SEQ ID NO: 67)
GAGAGGAAGGAAAGAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGAAAGGAAGGAAGG
AAAGAATGAAGGAAGAAAGGAAGGAAGGGAGGGA

Allele 38 (SEQ ID NO: 68)
GAGAGGAAGGAAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAGGAAAGAATGA
AGGAAGGAAGGAAGGAAGGGAGGGA

Table S5: BV677278 allele frequencies in carriers of the CGCGAG and GACGAG haplotypes in the ALSPAC. Alleles belonging to Clade 1 are demarcated with a "1" in parentheses.

Allele	BV677278 Allele Frequency, CGCGAG	BV677278 Allele Frequency, GACGAG
1	0.323	0.367
2	0	0
3	0.024	0.029
4	0.055	0.048
5(1)	0.473	0.061
6(1)	0.038	0.393
7	0	0
8	0	0.001
9	0.002	0.004
10	0.027	0.022
11(1)	0	0.008
12(1)	0	0
13(1)	0	0.009
14(1)	0.007	0.009
15	0.002	0
16	0	0
17	0	0
18	0	0.001
19	0	0
20(1)	0	0.005
21(1)	0	0.003
22	0	0.001
Del	0.049	0.037

Table S6: P-values for ChIP-qPCR experiment (Figure 2B). Values represent one-tailed paired T-tests for fold enrichment between each pair of ChIP conditions specified (three replicates each). STR: BV677278 STR amplicon; β-Actin: control amplicon from the β-actin gene; α-ETV6: ChIP with anti-ETV6 antibody; α-H3: ChIP with anti-variant histone H3 control antibody; NA: no-antibody control ChIP. P-values below 0.05 are shown in bold with an asterisk, p-values below 0.01 have two asterisks.

	α-ETV6, STR	α-ETV6, β-Actin	NA, STR	α-H3, STR	α-H3, β-Actin	NA, β-Actin
α-ETV6, STR	X	**0.00728****	**0.01114***	0.07659	0.06967	**0.01005***
α-ETV6, β-Actin		X	0.19296	0.05375	0.05228	0.11426
NA, STR			X	**0.02079***	0.08653	0.42981
α-H3, STR				X	0.46893	**0.01133***
α-H3, β-Actin					X	0.05509
NA, β-Actin						X

Table S7: One-way ANOVA between groups listed in Figure 3A—carriers of 1) no risk haplotype, 2) the *KIAA0319* risk haplotype, 3) either *DCDC2* haplotype, and 4) a risk haplotype in both genes.

Phenotype (Z-transformed)	ANOVA P-Value
Phoneme Deletion	0.088
Total IQ	0.003
Verbal IQ	0.006
Performance IQ	0.043
Verbal Comprehension (WOLD)	0.562
Nonword Repetition	0.053
Avg WOLD and NWR	0.179

Example 2

[0004] Having established the association between *DCDC2* risk haplotypes and *KIAA0319* risk haplotypes, the relationship of READ1 (a functional RD/LI risk variant within *DCDC2*), and the *KIAA0319* RD risk variant KIAHap (a haplotype covering the 5' half of the RD risk gene *KIAA0319* and some of its upstream sequence and neighboring gene *TDP2*) was investigated. As described herein, READ1 is comprised of five discrete repeat units, each of which varies in number, giving rise to considerable polymorphism. Including the deletion, 39 READ1 alleles have been described thus far, 6 of which are common, and 32 of which are rare (in individuals of European ancestry). These alleles vary in length from 81bp to 114bp.

READ1 does not appear to exist outside of the higher primates, and among higher primate species (and within *Homo sapiens*) its length is highly variable. It appears as though READ1 is a hypermutable, rapidly-evolving element that first appeared in primates and reached its full size in *Homo sapiens* (Powers et al., 2013).

[0005] To determine the effects of individual READ1 alleles *in vivo*, the association of READ1 with reading and language was examined in the Avon Longitudinal Study of Parents and Children (ALSPAC), a large longitudinal birth cohort. Allele 5 of READ1 was strongly associated with Severe RD, while allele 6 of READ1 was strongly associated with Severe LI (Table 1). Furthermore, those individuals who carried at least one copy of allele 5 performed worse on six different reading-related measures, on average, compared to non-carriers of allele 5 ($p<0.05$), while carriers of allele 6 performed worse on a composite language measure, on average, than non-carriers of allele 6 ($p<0.05$). These two relatively common alleles (allele frequencies of 3.6% and 5.0%, respectively) are structurally similar to each other, and cluster to the same clade phylogenetically. Compared to the most common allele (allele 1) both alleles have a GGAA insertion in the same position. By contrast, other common READ1 alleles appeared to have a protective effect on reading and language. Carriers of at least one copy of a shorter READ1 allele (an allele with only one iteration of repeat unit 1 or RU1, as opposed to two iterations in the majority of READ1 alleles) performed better on reading and language tasks relative to non-carriers, although the protective associations with Severe RD and LI were only suggestive (Table 1 of Example 2, below).

Allele	Description	READ1 Alleles	MAF (ALSPAC)	P-Value, Severe RD	OR, Severe Rd	P-Value, Severe LI	OR, Severe LI
3	3 only, protective	3	0.0463	0.179	**0.575***	0.255	0.77
4	4 only	4	0.0924	0.239	1.28	0.141	0.78
5	5 only, deleterious	5	0.0355	**0.000058**	**2.37#**	0.487	0.84
6	6 only, deleterious	6	0.0496	0.0995	**1.53#**	**0.00595**	**1.65**
10	10 only	7	0.0502	0.795	0.919	0.603	0.9
5+6	Major deleterious	5, 6	0.0851	**0.000037**	**1.96#**	**0.000074**	**1.73**

	alleles						
Shorter Alleles	Only one copy of RU1; major protective alleles	2, 3, 9, 12, 25, 27, 38	0.0521	0.0957	**0.506***	0.292	0.8

Table 1: Association results for READ1. This table shows the results of association with READ1 single and compound alleles with Severe RD and Severe LI. Associations were computed under an allelic model. Significant p-values are bolded, and notable odds ratios for increased (#) and reduced (*) risk are shown.

[0006] As described herein, the READ1-binding protein was identified as the potent transcriptional repressor ETV6, whose binding sequence (GGAA) matches the insertion seen in risk alleles 5 and 6. This suggests that alleles 5 and 6 have more ETV6 binding sites available than neutral or protective alleles (particularly the protective shorter alleles). When the properties of ETV6 are considered, the peculiar behavior of READ1 alleles begins to make sense. In its monomeric state, ETV6 is incapable of binding DNA. It must at least homodimerize to displace an autoinhibitory domain blocking its DNA-binding domain in the monomeric state (Green et al., 2010), and is known to be capable of homopolymerization (Tognon et al., 2004). This property suggests an intriguing possibility—that READ1 alleles of different lengths bind different numbers of ETV6 monomers, and this alters the regulatory power of the complex (Figure 3B).

[0007] Likewise, KIAHap is thought to tag a functional promoter or promoter-proximal variant that alters *KIAA0319* gene regulation. There is evidence that this functional variant is the SNP rs9461045. This SNP is in linkage disequilibrium with KIAHap and is associated with reduced reporter gene expression from the *KIAA0319* promoter in both neuronal and non-neuronal cell lines (Dennis et al., 2009; Paracchini et al., 2006). The regulatory nature of KIAHap and the fact that READ1 appears to be an ETV6-binding regulatory element led to the question of whether READ1 risk alleles 5 and 6 interact with KIAHap to affect phenotype. Strikingly, carriers of both a READ1 risk allele and KIAHap showed markedly worse performance in reading, language, and IQ tasks (Figure 5A shows allele 5). This interaction is generally synergistic; that is, the effect of having two risk alleles is greater than the sum of the alleles' individual effects (Figure 5A). Additionally, the protective, shorter alleles of READ1 (e.g., those comprising a single repeat unit 1, "RU1_1") appear to interact epistatically with KIAHap. In individuals with at least one copy of a shorter, RU1_1 allele of READ1, the

deleterious effect of KIAHap is completely negated—mean performance of subjects with both an RU1_1 allele and KIAHap is generally slightly above the population average and resembles that of subjects with an RU1_1 allele alone (Figure 5B). Therefore, it appears that KIAHap synergizes with deleterious READ1 alleles to exacerbate their individual deleterious effects on reading and language, but is epistatically masked by protective READ1 alleles. This suggests that ETV6 and READ1 form a regulatory complex that epistatically regulates *KIAA0319*, and possibly *DCDC2* and other target genes.

Example 3

Characterization of the DYX2 locus on chromosome 6p22 with reading disability, language impairment, and overall cognition

Introduction

Described here is assessment of the relationship of the DYX2 locus with RD, LI, and cognition. A marker panel densely covering the 1.4Mb DYX2 locus was developed and used to assess the association with reading, language, and cognitive measures in subjects from the Avon Longitudinal Study of Parents and Children (ALSPAC). Associations were then replicated in three independent, selected cohorts. Confirming the results of the other Examples described herein, there were associations with known RD risk genes *KIAA0319* and *DCDC2* (Figure 6A). In addition, other markers were identified in or near other DYX2 genes, including *TDP2*, *ACOT13*, *C6orf62*, *FAM65B*, and *CMAHP*. The LD structure of the locus suggests that association hits within *TDP2*, *ACOT13*, and *C6orf62* are capturing a previously reported risk variant in *KIAA0319*. These results further substantiate *KIAA0319* and *DCDC2* as major effector genes in DYX2 and identify *FAM65B* and *CMAHP* as new DYX2 risk genes. Association of DYX2 with multiple neurobehavioral traits suggests risk variants have functional consequences affecting multiple neurological processes.

Methods

Subjects

The discovery cohort in this investigation was the Avon Longitudinal Study of Parents and Children (ALSPAC). The ALSPAC is a population-based birth cohort based in Avon, United Kingdom. Subjects were recruited before birth—a total of 15,458 fetuses were recruited, of

whom 14,701 were alive at 1 year of age. Recruitment, participants, and study methodologies are described in detail elsewhere (bristol.ac.uk/alspac) (Boyd et al. 2012; Golding et al. 2001). DNA samples for genetic analysis were available for 10,259 subjects. Reading, language, and cognitive measures were performed at ages 7, 8, and 9 years. Subjects with IQ $\leq$ 75 on the Wechsler Intelligence Scale for Children (WISC-III) Total IQ were excluded to prevent confounding effects of Intellectual Disability (Eicher et al. 2013a; Eicher et al. 2013b; Powers et al. 2013; Wechsler et al. 2002). To prevent population stratification in genetic analyses, subjects of non-European descent were also excluded. Samples with overall genotype call rates < 0.85 were excluded from analyses, leaving a final sample size of 5579 individuals for LI analyses and 5525 individuals for RD analyses. Ethical approval was obtained from the ALSPAC Ethics and Law Committee, Local UK Research Ethics Committees, and the Yale Human Investigation Committee.

Reading, Language, and Cognitive Measures

Reading measures in the ALSPAC used in this investigation include a phoneme deletion task at age 7 years, single-word reading at ages 7 and 9 years, and single non-word reading at age 9 years (Table 1a). The phoneme deletion task, also known as the Auditory Analysis Test, measures phoneme awareness, a core deficit in RD (Rosner and Simon 1971). For the phoneme deletion task, the child listens to a word spoken aloud and is then asked to remove a specific phoneme from that word to make a new word. Single-word reading was assessed at age 7 years using the reading subtest of the Wechsler Objective Reading Dimensions (WORD) (Rust et al. 1993). At age 9 years, single-word reading was again assessed by asking the child to read ten real words and ten non-words aloud, a subset of a larger list of words and non-words (Nunes et al. 2003). To examine severe cases (Severe RD), cases were defined as having a score 2 or more standard deviations below the mean on the phoneme deletion task (Table 1b). Cases with Moderate RD were also defined as scoring at least 1 standard deviation below the mean on single-word reading at age 7 years, single-word reading at age 9 years, and single non-word reading at age 9 years (Table 1b). A threshold of 1 standard deviation was chosen as measures were included at three time points to isolate individuals with persistently poor decoding skills. Different severity levels were examined because past studies in the DYX2 locus have shown differences in genetic association patterns depending on case severity, particularly with *KIAA0319* associating with more moderate RD case definitions and *DCDC2* with more severe definitions (Paracchini et al. 2008; Powers et al. 2013; Scerri et al. 2011).

[0008] Language measures were collected at age 8 years (Table 1a). An adaptation of the Nonword Repetition Task (NWR), in which subjects repeated recordings of nonwords, assessed short-term phonological memory and processing abilities (Gathercole and Baddeley 1996). Children also completed the Wechsler Objective Language Dimensions (WOLD) verbal comprehension task at age 8 years (Wechsler et al. 1996), where they answered questions about a paragraph read aloud by an examiner describing a presented picture. These measures were looked at because individuals with LI are known to perform consistently poorly on NWR and WOLD tasks (Bishop et al. 1996; Newbury et al. 2009). As with RD, the association of the DYX2 locus with severe and moderate case definitions of LI was also examined. To assess the risk imparted for severe LI, severe LI cases were defined as scoring 2 or more standard deviations below the sample mean on either task (Severe LI) (Table 1b). To assess more moderate deficits, cases were defined as scoring at least 1.5 standard deviations below the sample mean on each task (Moderate NWR and Moderate WOLD) (Table 1b). As fewer measures were used to assess LI related traits, the threshold for case definitions was increased to 1.5 standard deviations to assess more moderate deficits. Verbal, performance, and total IQ were assessed at age 8 years, using the Wechsler Intelligence Scale for Children (WISC-III) (Table 1a). IQ measures were examined as quantitative traits (Table 1b).

Genotyping and Genetic Analyses

A SNP marker panel was developed to capture the common variation in the DYX2 locus. TagSNPs in the locus were selected using the association study design server of Han et al. (Han et al. 2008). The final DYX2 panel contained 195 SNPs with an estimated average power of 83% and 68% to capture known common and rare variants, respectively, in the DYX2 locus spanning approximately 1.4 Mb. Markers were genotyped on the Sequenom platform (San Diego, CA), following manufacturer's guidelines, at the Yale Center for Genome Analysis (West Haven, CT).

Markers that deviated substantially from Hardy-Weinberg equilibrium, or that had an overall call rate <85%, were not used for haplotype-based analysis. In the discovery ALSPAC cohort, single marker SNP analyses of case-control statuses and quantitative traits were completed using SNP & Variation Suite (SVS) v7.6.4 (Bozeman, MT). Linkage disequilibrium was assessed and haplotype blocks were constructed using the four-gamete rule option in HaploView v4.2. Haplotype association tests were performed with haplotypes that had frequencies greater than or equal to 1% using PLINK v1.07 (Barrett et al. 2005; Purcell et al. 2007). Associations with $p<0.001$ are reported for the ALSPAC discovery cohort to present suggestive results. However,

to correct for multiple testing, a Bonferroni threshold of 0.000256 (0.05 divided by 195 markers) was used for discovery association tests in the ALSPAC cohort.

Following discovery analyses in the ALSPAC, associated variants were assessed in three cohorts specifically recruited for either RD or LI: Iowa LI, Italian RD, and Colorado RD (Table 2). The Iowa LI cohort is comprised of 219 LI cases and 209 sex- and age-matched, unrelated controls collected at the University of Iowa. Subjects completed various language measures, including the Woodcock Johnson-III (WJ) and the Gray Oral Reading Test (GORT), which were used to derive a composite language score, which was then dichotomized into case-control status at -1.14 standard deviations (Eicher et al. 2013a; Weismer 2000). The Colorado Learning Disabilities Research Center (CLDRC) cohort consists of 1201 individuals in 293 nuclear families. Families were recruited to the study if at least one child had a history of reading problems. The Italian cohort consists of 878 individuals in 304 nuclear families; these families were recruited via a proband with clinically diagnosed RD. Ethical approval for recruitment and study methodologies were obtained from the Yale Human Investigation Committee and Institutional Review Boards at the University of Iowa, the University of Denver, and Italy. SNPs that had single marker or within-haplotype associations with $p<0.001$ in the ALSPAC were tested for replication in the Iowa LI, Italian RD, and Colorado RD cohorts. Iowa LI was analyzed using SNP & Variation Suite (SVS) v7.6.4 (Bozeman, MT), while the family-based Italian RD and Colorado RD cohorts were examined using PLINK v1.07 (Purcell et al. 2007). Suggestive ALSPAC results were moved forward for replication analyses in order to emphasize replication of associations over statistical corrections for multiple testing. Replications with $p<0.05$ in the Iowa LI, Italian RD, and Colorado RD cohorts are reported.

Results

Association with DYX2 markers was performed in three separate domains: (1) RD, (2) LI, and (3) IQ. For the sake of clarity, associations are presented domain-by-domain, with an emphasis on replication for strength of associations as opposed to correction for multiple testing.

RD

Associations with RD were performed using two different severity definitions: (1) Severe RD and (2) Moderate RD (Table 1b). For Severe RD, associations were found with *DCDC2*, *KIAA0319*, and *TDP2* (Table 3). The association of *DCDC2* and Severe RD is explored fully in Example 1; Powers et al. (2013). *TDP2* marker rs2294691 did not replicate its association in

any of the three replication cohorts (Table 5). *KIAA0319* marker rs10456309 replicated in Iowa LI and Colorado RD cohorts (Table 5). With Moderate RD, there was an association between rs1562422 near the gene *FAM65B* and the pseudogene *CMAHP*, which was replicated in the Colorado RD cohort (Table 3, Table 5).

LI

Association tests were performed on three LI phenotypes: (1) Severe LI, (2) Moderate NWR, and (3) Moderate WOLD (Table 1b). As with Severe RD, there were associations between *DCDC2* and Severe LI. The *DCDC2* haplotype that associated with Severe LI is discussed in Example 1; Powers et al (2013). A marker within this *DCDC2* haplotype, rs807694, showed association with Severe LI and was replicated in the Iowa LI cohort (Table 3, Table 5). With a more moderate case definition, associations were observed with *ACOT13* and *C6orf62* (Table 3), genes neighboring *KIAA0319* and *TDP2*. Both rs3777663 in *ACOT13* and rs3756814 in *C6orf62* showed associations in the Italian RD and Iowa LI cohorts (Table 5).

IQ

Association tests were also performed between DYX2 markers and Verbal IQ, Performance IQ, and Total IQ (Table 1b). Verbal IQ associations included single markers and haplotypes covering the 5' half of *KIAA0319*, rs9348646 in *FAM65B*, and a haplotype spanning *ACOT13* and *C6orf62*, with evidence of replication (Table 4a, Table 4b, Table 5). There was substantial overlap of DYX2 associations with Verbal IQ and associations with RD and LI. These similarities of associations are not unexpected, as the traits are highly correlated and known to capture similar domains (Table 6). The associations of DYX2 with Performance and Total IQ were weaker; there were no associations with Performance IQ and a single, non-replicated association of Total IQ with rs2328791, which is located in a large intergenic region telomeric to *NRSN1* and *DCDC2* (Table 4a, Table 4b, Table 5).

Linkage Disequilibrium within DYX2

In the analyses, replicated associations were observed in the following genes: *DCDC2*, *KIAA0319*, *TDP2*, *ACOT13*, *C6orf62*, *FAM65B*, and the pseudogene *CMAHP*. However, as these SNPs are in close proximity to each other, linkage disequilibrium (LD) was assessed among the marker panel to determine whether the associated SNPs were tagging the same variation in the locus. As described in Examples 1 and 2, *DCDC2* associations tagged READ1 alleles. Within *KIAA0319*, there appear to be two clear LD blocks separating the gene into a 5'

half and a 3' half (data not shown). The 5' half of *KIAA0319* is in strong LD with *TDP2*, *ACOT13*, and *C6orf62*, indicating that associations within these genes may be capturing that same variation (data not shown). Associations in *FAM65B* and *CMAHP* appear to be tagging independent associations (data not shown). Although rs1562422 is located intergenic to *FAM65B* and *CMAHP*, this marker is in strong LD with other markers within the *CMAHP* pseudogene. Integration of the association analyses and LD structure indicate four independent association signals centered on (1) *DCDC2*, (2) the 5' half of *KIAA0319*, (3) *FAM65B*, and (4) *CMAHP*.

In this Example, the relationship of the DYX2 locus with RD, LI, and IQ is characterized (Figure 6B). The results confirm the associations of RD risk genes *KIAA0319* and *DCDC2* to include LI. Additionally, *FAM65B* and *CMAHP* were identified as risk genes for linguistic traits. Markers within the DYX2 locus showed association with numerous communication traits, including RD, LI, and Verbal IQ. There was a marked absence of DYX2 associations with Total and Performance IQ, indicating that the DYX2 locus influences language-related processes to a much greater extent than overall cognitive traits.

The genetic association of DYX2 with RD, LI, and Verbal IQ is the latest example of various neurocognitive and communication processes sharing genetic associations. Applicant and others have shown that these neurobehavioral traits have common genetic contributors, including variants in *FOXP2*, *KIAA0319*, *CMIP*, *ZNF385D*, *CNTNAP2*, and *DCDC2* (Eicher et al 2013b; Newbury et al. 2009; Newbury et al. 2011, Pennington and Bishop 2009, Peter et al. 2011; Pinel et al. 2012; Powers et al. 2013; Scerri et al. 2011; Wilcke et al. 2012). The expansion of DYX2's association from reading to include other language-related processes suggests that the causative variants may affect these traits in a pleiotropic manner, as opposed to influencing written or verbal language exclusively. The findings of this study collude with this 'generalist genes hypothesis,' which is also supported by a recent genome-wide complex trait analysis (GTCA) of cognitive and learning abilities (Trzaskowski et al. 2013). The strong correlations and relatedness among these neurocognitive measures (Table 6) suggest that these DYX2 genes affect central language processes, which in turn manifest themselves phenotypically in various ways, including reading, language, and cognition.

That multiple DYX2 genes showed association with the phenotypes in this study is interesting, and at first glance somewhat unexpected. One possibility is that one or two genes are not solely

responsible for the consistent implication of this locus in reading, language, and cognitive phenotypes, as is largely believed. *KIAA0319* and *DCDC2* are currently known as the two major risk genes in the DYX2 locus. Both have been implicated in both RD and sub-clinical variation in reading performance, using both classical neurobehavioral measures, and more recently, neuroimaging techniques (Eicher and Gruen 2013; Graham and Fisher 2013). Other genes in DYX2 have been associated with RD, but not nearly as often as *DCDC2* and *KIAA0319*. However, with the dense SNP panel described herein, it was possible to observe associations with other DYX2 elements, including *FAM65*B and *CMAHP*.

Another possible explanation for the number of DYX2 genes observed associating with the phenotypes in this study is LD within the DYX2 locus. In fact, LD likely explains the cluster of associations around *KIAA0319*, *TDP2*, *ACOT13*, and *C6orf62*. As described herein, two major LD blocks span *KIAA0319*—one spans the 3' half of the gene, while the other spans the 5' region of *KIAA0319* as well as *ACOT13*, *TDP2*, and part of *C6orf62*. Nearly all of the associations in this study localize to this 5' LD block, which also contains the previously reported *KIAA0319* RD risk haplotype. Because of this LD structure, it is impossible to determine whether the associations in this region are independent, or are capturing the same functional variant. The latter possibility is considered the most likely, and it is believed that the associations in this region are likely tagging the same causative variant captured by the *KIAA0319* RD risk haplotype. Functional study of this region—particularly of the less studied genes *TDP2*, *ACOT13*, and *C6orf62*—will be useful to determine whether these associations are independent or not.

By contrast, the markers within or near *FAM65B* and *CMAHP* appear to be capturing distinct association signals from two different LD blocks (data not shown). The SNP rs9348646, which showed association with Verbal IQ, is located within an intron of *FAM65B* in one LD block, while rs1562422, which showed association with Moderate RD, localized to a separate LD block. While rs1562422 is an intergenic marker located physically between *FAM65B* and *CMAHP*, it shows strong LD with markers in *CMAHP* (data not shown). The LD patterns within the DYX2 locus suggest that associations in *KIAA0319*, *TDP2*, *ACOT13*, and *C6orf62* are tagging the same causative variant, while rs9348646 in *FAM65B* and rs1562422 are independent.

The other DYX2 genes, including *FAM65B* and *CMAHP*, have been studied far less than the risk genes *DCDC2* and *KIAA0319*. Little is known about *FAM65B* in terms of biological function; however, there is evidence that *FAM65B* may influence migration in T lymphocytes (Rougerie et al. 2013). Animal models of *DCDC2* and *KIAA0319* have implicated these genes in migratory processes, albeit in a neural context. *CMAHP*, which encodes a key enzyme in the synthesis of the sialic acids Neu5Ac and Neu5Gc in other mammals, was rendered a pseudogene in humans by an inactivating microdeletion and subsequent fixation of the inactive allele in early human populations (Chou et al. 1998). Although *ACOT13* appears to be tagging variation within *KIAA0319*, the preliminary functional studies of *ACOT13* are intriguing. *ACOT13* was recently associated with lower asymmetric activation of the posterior superior temporal sulcus during reading and phonology tasks (Pinel et al. 2012). The protein product encoded by *ACOT13* has been co-localized with beta-tubulin on microtubules; microtubule binding is postulated to be important to RD, as *DCDC2* contains two doublecortin domains that are thought to bind microtubules (Cheng et al. 2006).

Genes and regulatory elements within the DYX2 locus may contribute interactively to reading and language domains, as seen with the non-additive relationship between putative regulatory variants in *DCDC2* and *KIAA0319* (Example 1; Powers et al. 2013, Ludwig et al. 2008). These risk variants have been shown to influence gene expression, and to interact with each other to substantially influence performance on reading and language tasks. A complex regulatory network, where regulatory elements interact and co-regulate other DYX2 genes and elements, may contribute to reading, language, and cognition. If so, it is likely that the READ1 element in *DCDC2* and the causative variant tagged by the *KIAA0319* risk haplotype have the strongest effects upon gene expression and ultimate neurocognitive phenotype. Supporting this idea is the fact that so many of the association hits described herein—both single-marker and haplotype-based, and with all three phenotypes—localize to the same LD block as the *KIAA0319* risk haplotype. This result, together with the *KIAA0319* risk haplotype's association with reduced *KIAA0319* expression and its synergistic interaction with a regulatory element in an intron of *DCDC2*, indicate the presence of at least one regulatory variant in this region that influences *KIAA0319* expression. The locations of the only other independent hits in the locus (aside from READ1 in *DCDC2*)—an intron of *FAM65B* and downstream of a pseudogene—may suggest additional regulatory regions that influence gene expression. Based on work described herein, *DCDC2* and *KIAA0319* are the major effector genes responsible for DYX2's influence on RD

and LI risk and alteration of gene expression levels or patterns is the mechanism by which this effect is exerted.

Replication of genetic associations in independent cohorts was emphasized in the work described herein, rather than reliance on statistical corrections for multiple testing, for validation of associations in the ALSPAC discovery cohort. The replications of genetic association with the neurocognitive traits of interest, particularly in the varied cohorts in this investigation, provide strong evidence that the results of this study are not due to Type I error. Uncorrected p-values and a statistical threshold correcting for 195 genetic markers (threshold of 0.000256) are also reported in order to present the context of the findings in terms of strength and reliability. Nonetheless, the three replication cohorts were not identical and had inherent differences among themselves and relative to the discovery cohort that may have prevented replication. These differences included: (1) the disorder for which each cohort was selected (RD vs. LI vs. unselected), (2) severity of case definition and recruitment, and 3) country of recruitment (UK vs. US vs. Italy), and language spoken (English vs. Italian). For instance, the use of a regular language such as Italian as opposed to an irregular language such as English may have allowed for easier detection of true reading and language deficiencies. This issue was likely avoided in the discovery ALSPAC cohort due to the large sample size. The observation of multiple replicated associations throughout the DYX2 locus described herein increases confidence in these results.

In summary, the analyses indicate four association signals for RD, LI, and Verbal IQ in the DYX2 locus: *DCDC2*, *KIAA0319*, *FAM65B*, and the pseudogene *CMAHP*. The association results within the *DCDC2* and *KIAA0319* (including *TDP2*, *ACOT13*, and *C6orf62*) areas are in LD with two previously reported risk variants: the READ1 regulatory element in *DCDC2* and the *KIAA0319* risk haplotype in *KIAA0319* and *TDP2*. These results point strongly to variation in *KIAA0319* gene expression as a mediator of DYX2's effect on reading and language phenotypes.

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Figure Legends

Figure 1: Schematic of the genes within the DYX2 locus on chromosome 6p21.3. Genes in blue, *DCDC2* and *KIAA0319*, have replicated associations with written and verbal language phenotypes, namely RD and LI. Regions in red mark two functional variants, READ1 in *DCDC2* and a risk haplotype with markers in *KIAA0319* and *TDP2*, which have been functionally associated with RD and LI using animal models and molecular techniques.

Figure 4: An updated schematic of genes in our study with markers that show replicated associations to RD, LI, and/or IQ. The list of these genes (shown in blue) has expanded to seven (*DCDC2*, *KIAA0319*, *TDP2*, *ACOT13*, *C6orf62*, *FAM65B*, and *CMAHP*), although linkage disequilibrium may account for multiple associations (particularly for *KIAA0319*, *TDP2*, *ACOT13*, and *C6orf62*).

Tables of Example 3

Table 1a: ALSPAC Phenotype Measures

Measure	Domain
Phoneme Deletion (PD) Age 7 Years	Reading (RD)
Single Word Reading (SWR7) Age 7 Years	Reading (RD)
Single Nonword Reading (SNR) Age 7 Years	Reading (RD)
Single Word Reading (SWR9) Age 9 Years	Reading (RD)
Wechsler Objective Language Dimensions (WOLD) Verbal Comprehension Age 8	Language (LI)
Nonword Repetition Task (NWR) Age 8 Years	Language (LI)

Wechsler Intelligence Scale for Children (WISC) Total IQ (TIQ) Age 8 Years	Cognition (IQ)
Wechsler Intelligence Scale for Children (WISC) Verbal IQ (VIQ) Age 8 Years	Cognition (IQ)
Wechsler Intelligence Scale for Children (WISC) Performance IQ (PIQ) Age 8 Years	Cognition (IQ)

Table 1b: Phenotype Definitions for ALSPAC Analyses

Reading (RD)	Phenotype Definition
Severe RD	2 standard deviations below sample mean on the phoneme deletion task
Moderate RD	1 standard deviation below sample mean on SWR7, SNR, and SWR tasks
Language (LI)	Phenotype Definition
Severe LI	2 standard deviations below sample mean on either WOLD and/or NWR tasks
Moderate WOLD	1.5 standard deviations below sample mean on the WOLD task
Moderate NWR	1.5 standard deviations below sample mean on the NWR task
Cognition (IQ)	Phenotype Definition
Total IQ	Quantitative performance on WISC Total IQ task
Verbal IQ	Quantitative performance on WISC Verbal IQ task
Performance IQ	Quantitative performance on WISC Performance IQ task

Table 2: Replication Cohorts

	Iowa LI	Colorado RD	Italy RD
Number of Subjects	428	1201	878
Number of Families	N/A	293	304
Disorder	LI	RD	RD
Cohort-type	Case-control	Family-based	Family-based
Analysis	SVS	TDT (PLINK)	TDT (PLINK)
Association Conditioned on:	Case-control Status	Case-control Status and Discriminant Score	Case-control Status
Case Status Determined on:	Composite score on language measures		Speed or Accuracy on text- or single-word reading task

Table 3: Single marker genetic associations with various RD and LI case-control definitions.

Phenotype	Marker	Gene	BP Location	Model	OR (95% CI)	P-value
Severe RD	rs2294691	*TDP2*	24652843	Allelic	2.0 (1.3-2.9)	0.00050
Severe RD	rs2294691	*TDP2*	24652843	Additive	1.9 (1.3-2.8)	0.00053
Severe RD	rs2294691	*TDP2*	24652843	Dominant	2.3 (1.5-3.7)	0.00018*
Severe RD	rs10456309	*KIAA0319*	24589562	Recessive	10.5 (2.2-49.5)	0.00020*
Moderate RD	rs1562422	*CMAHP*	25044577	Dominant	1.7 (1.2-2.2)	0.00081
Severe LI	rs807694	*DCDC2*	24303383	Additive	1.8 (1.3-2.5)	0.00057
Severe LI	rs807694	*DCDC2*	24303383	Allelic	1.8 (1.3-2.5)	0.00050
Severe LI	rs807694	*DCDC2*	24303383	Dominant	1.9 (1.3-2.7)	0.00062
Moderate WOLD	rs3756814	*C6orf62*	24705835	Additive	0.7 (0.6-0.9)	0.00039
Moderate WOLD	rs3756814	*C6orf62*	24705835	Allelic	0.7 (0.6-0.9)	0.00047
Moderate WOLD	rs3777663	*ACOT13*	24700235	Additive	0.6 (0.5-0.8)	0.00039
Moderate WOLD	rs3777663	*ACOT13*	24700235	Allelic	0.7 (0.5-0.8)	0.00041

*Genetic association survives correction for multiple testing

Table 4: Single marker (Table 4a) and haplotype based (Table 4b) genetic associations with quantitative measure of cognition.

Table 4a: Single marker genetic associations with cognition

Phenotype	Marker	Gene	BP Location	Model	Slope	P-value
Verbal IQ	rs9295626	*KIAA0319*	24587339	Allelic	1.40	0.00041
Verbal IQ	rs9295626	*KIAA0319*	24587339	Additive	1.39	0.00043
Verbal IQ	rs7763790	*KIAA0319*	24615063	Allelic	-1.40	0.00045
Verbal IQ	rs7763790	*KIAA0319*	24615063	Additive	-1.38	0.00048
Verbal IQ	rs6935076	*KIAA0319*	24644322	Allelic	1.16	0.00049
Verbal IQ	rs6935076	*KIAA0319*	24644322	Additive	1.15	0.00052
Verbal IQ	rs9348646	*FAM65B*	24052526	Allelic	-1.14	0.00066
Verbal IQ	rs9348646	*FAM65B*	24052526	Additive	-1.14	0.00066
Total IQ	rs2328791	N/A	23736848	Allelic	-1.21	0.00066
Total IQ	rs2328791	N/A	23736848	Additive	-1.18	0.00075
Total IQ	rs2328791	N/A	23736848	Recessive	-3.36	0.00042

Table 4b: Haplotype based genetic associations with cognition

Markers	Haplotype	Gene	BP Location	Slope	P-value
rs2817201, rs9295626	AT	*KIAA0319*	24585214, 24587339	1.42	0.000378
rs10456309, rs4576240, rs17307478, rs9356939, rs7763790, rs6456621	GGTCAC	*KIAA0319*	24589562, 24596478, 24605024. 24613354, 24615063, 24618511	-1.40	0.000569
rs6456624, rs6935076, rs2038137, rs3756821, rs1883593, rs3212236	AGATA	*KIAA0319*	24639223, 24644322, 24645943, 24646821, 24647191, 24648455	1.81	0.0000145*
rs3777663, rs3756814, rs6931809, rs6916186, rs6933328, rs17491647	TGTGGA	*ACOT13/C6orf62*	24700235, 24705835, 24706770, 24708523, 24710920, 24713723	-1.56	0.000742

*Genetic association survives correction for multiple testing

Table 5: Replication of genetic associations in the Iowa, Italian, and Colorado cohorts.

Marker	Gene	Iowa Case Control		Italy Case Control		Colorado Case Control		Colorado Discriminant Score	
		OR	p	OR	p	OR	p	Slope	p
rs2328791	N/A	1.0	0.813	1.0	1.000	0.9	0.646	0.087	0.447
rs33914824[a]	***DCDC2***	**2.2**	**0.034**	0.9	0.768	1.1	0.847	0.023	0.934
rs807694[a]	***DCDC2***	**1.9**	**0.028**	0.9	0.786	0.9	0.853	-0.025	0.919
rs707864[a]	***DCDC2***	**1.6**	**0.017**	1.0	0.840	1.2	0.446	-0.246	0.101
rs10456301[a]	*DCDC2*	0.9	0.553	1.1	0.811	1.5	0.289	0.221	0.162
rs16889066[a]	*DCDC2*	1.2	0.517	1.0	0.884	1.2	0.622	-0.304	0.150
rs9379651[a]	*DCDC2*	1.1	0.602	1.3	0.225	0.6	0.059	0.205	0.141
rs2817201	*KIAA0319*	1.1	0.733	1.2	0.129	1.0	1.000	0.034	0.787
rs9295626	***KIAA0319***	1.1	0.579	**0.6**	**0.0055**	1.0	0.823	-0.158	0.169
rs10456309	***KIAA0319***	0.5	0.073	0.7	0.189	0.4	0.206	**0.628**	**0.00845**
rs4576240	***KIAA0319***	1.1	0.825	**1.9**	**0.0027**	1.1	0.862	-0.052	0.754
rs17307478	*KIAA0319*	1.0	0.996	1.3	0.292	0.8	0.555	0.039	0.803

rs9356939	***KIAA0319***	**4.0**	**0.018**	0.8	0.069	1.3	0.151	-0.116	0.254
rs7763790	*KIAA0319*	1.0	0.831	1.1	0.627	1.4	0.163	0.014	0.910
rs6456621	***KIAA0319***	**2.2**	**0.019**	1.6	0.405	1.8	0.366	-0.458	0.104
rs3756821	*KIAA0319*	1.2	0.278	1.0	0.842	1.2	0.327	-0.033	0.734
rs1883593	***KIAA0319***	1.3	0.169	**1.6**	**0.0052**	1.3	0.239	-0.108	0.395
rs3212236	*KIAA0319*	1.0	0.883	1.1	0.496	0.9	0.745	-0.124	0.319
rs2294691	*TDP2*	1.1	0.779	1.9	0.0578	1.4	0.491	-0.290	0.247
rs3777663	***ACOT13***	**0.7**	**0.016**	**0.6**	**0.0052**	1.0	0.908	0.101	0.345
rs3756814	***C6orf62***	**0.7**	**0.005**	**0.7**	**0.023**	0.9	0.600	-0.003	0.980
rs6931809	***C6orf62***	**1.4**	**0.023**	**1.4**	**0.017**	1.2	0.491	-0.096	0.382
rs6916186	*C6orf62*	0.9	0.757	1.2	0.413	1.2	0.547	0.112	0.490
rs6933328	***C6orf62***	0.9	0.612	0.9	0.613	1.0	0.827	**0.215**	**0.0437**
rs17491647	*C6orf62*	0.8	0.155	0.7	0.104	1.0	0.901	0.042	0.709
rs9348646	***FAM65B***	0.9	0.358	1.1	0.535	1.4	0.144	**-0.415**	**0.00021**
rs1562422	***CMAHP***	1.0	0.793	1.0	0.796	**0.6**	**0.093**	-0.030	0.840

[a]These markers are part of the six-marker risk haplotype in *DCDC2* fully discussed in Powers et al. 2013.

Table 6: Phenotype correlations in the ALSPAC cohort*

	NWR	WOLD	SWR7	SWR9	SNR	PD	TIQ	VIQ	PIQ
NWR	1								
WOLD	0.214	1							
SWR7	0.403	0.259	1						
SWR9	0.351	0.202	0.722	1					
SNR	0.306	0.149	0.660	0.708	1				
PD	0.362	0.165	0.688	0.550	0.538	1			
TIQ	0.324	0.386	0.500	0.387	0.343	0.406	1		
VIQ	0.346	0.424	0.536	0.421	0.421	0.426	0.871	1	
PIQ	0.192	0.216	0.292	0.218	0.218	0.246	0.819	0.435	1

*All correlations were had $p<0.05$

Example 4

Genome-Wide Association Study of Shared Components of Reading Disability and Language Impairment; *ZNF385D* influences Reading and Language Disorders.

Introduction

Both RD and LI are complex traits that frequently co-occur, leading to the hypothesis that these disorders share genetic etiologies. To test this, a genome wide association study (GWAS) was performed on individuals affected with both RD and LI in the Avon Longitudinal Study of Parents and Children. The strongest associations were seen with markers in *ZNF385D* (OR=1.81, $p=5.45 \times 10^{-7}$) and *COL4A2* (OR=1.71, $p=7.59 \times 10^{-7}$). Markers within *NDST4* showed the strongest associations with LI individually (OR=1.827, $p=1.40 \times 10^{-7}$). Association of *ZNF385D* was replicated using receptive vocabulary measures in the Pediatric Imaging Neurocognitive Genetics study (p=0.00245). Diffusion tensor imaging fiber tract volume data on 16 fiber tracts was then used to examine the implications of replicated markers. *ZNF385D*

was a predictor of overall fiber tract volumes in both hemispheres, as well as global brain volume. In this Example, evidence is presented for *ZNF385D* as a risk gene for RD and LI. The implication of transcription factor *ZNF385D* in RD and LI underscores the importance of transcriptional regulation in the development of higher order neurocognitive traits.

Methods

ALSPAC.

Subject recruitment and collection of phenotype and genetic data for the ALSPAC cohort was completed by the ALSPAC team. The ALSPAC is a prospective population-based, birth cohort based in the Avon region of the United Kingdom. It consists mainly of children of northern European descent, born in 1991 and 1992. Children were recruited before birth; recruitment of their pregnant mothers resulted in a total of 15,458 fetuses, of whom 14,701 were alive at 1 year of age. Details regarding the participants, recruitment, and study methodologies are described in detail elsewhere (http://www.bristol.ac.uk/alspac) (Boyd et al., 2012; Golding et al., 2001). The children of the ALSPAC have been extensively phenotyped from before birth to early adulthood. Ethical approval was obtained from the ALSPAC Ethics and Law Committee, Local UK Research Ethics Committees, and the Yale Human Investigation Committee.

Reading and Language Measures.

The reading, language, and cognitive measures used for this study were collected at ages 7, 8, and 9 years. Subjects with IQ $\leq$ 75 on the Wechsler Intelligence Scale for Children (WISC-III) Total IQ, completed at age 8 years, were excluded from the presented analyses (Weschler et al. 1992). Reading measures in the ALSPAC include a phoneme deletion task at age 7, single-word reading at ages 7 and 9 years, single non-word reading at age 9 years, and reading passage comprehension at age 9 years. The phoneme deletion task measures phoneme awareness, widely considered to be a core deficit in both RD and LI (Pennington 2006; Pennington & Bishop, 2009). For the phoneme deletion task, also known as the Auditory Analysis Test, the child listens to a word spoken aloud, and is then asked to remove a specific phoneme from that word to make a new word (Rosner & Simon, 1971). Single-word reading was assessed at age 7 using the reading subtest of the Wechsler Objective Reading Dimensions (WORD). At age 9, single-word and nonword reading were assessed by asking the child to read ten real words and ten non-words aloud from a subset of a larger list of words and non-words taken from research conducted by Terezinha Nunes and colleagues (Rust et al., 1993). Reading comprehension scores were ascertained at age 9, using the Neale Analysis of Reading Ability (NARA-II) (Neale 1997). Two additional language measures, nonword repetition and verbal comprehension tasks,

were collected during clinical interviews at age 8 years. An adaptation of the Nonword Repetition Task (NWR), in which subjects repeated recordings of nonwords, was used to assess short-term phonological memory and processing (Gathercole & Baddeley, 1996). Children also completed the Wechsler Objective Language Dimensions (WOLD) verbal comprehension task, where they answered questions about a paragraph read aloud by an examiner describing a presented picture (Weschler 1996). Z-scores were calculated for each subject on each individual measure.

Case Definitions.

Applicant aimed to capture persistently poor performers in various reading and verbal language domains as RD and LI cases in the case definitions (Table 1). Therefore, RD cases were defined as having a z-score less than or equal to -1 on at least 3 out of the 5 following tasks: single word reading at age 7 years, phoneme deletion at age 7 years, single word reading at age 9 years, nonword reading at age 9 years, and reading comprehension at age 9 years. There were 527 subjects defined as RD cases. LI cases were defined as having a z-score less than or equal to -1 on at least 2 out of the 3 following tasks: phoneme deletion at age 7 years, verbal comprehension at age 8 years, and nonword repetition at age 8 years. There were 337 subjects defined as LI cases. As phoneme awareness is important in both RD and LI, it was inclouded as part of the case definition for both RD and LI to reflect clinical presentation. There were 174 individuals affected with both RD and LI, with a male to female ratio of 1.7:1. In the further characterization of observed associations, subsets of cases were created with no comorbidity. There were 163 LI cases excluding those with comorbid RD, and 353 RD cases excluding those with comorbid LI. For all analyses, controls were defined as ALSPAC subjects of European ancestry who completed all the necessary neurobehavioral assessments but did not meet the criteria for case status.

Genotyping and Analysis.

Subjects were genotyped on Illumina HumanHap 550 bead arrays (San Diego, CA). Subjects were excluded if the percentage of missing genotypes was greater than 2% (n=6). To prevent possible population stratification, only subjects of European ancestry were included. In the primary analysis of RD and LI individuals, there were 174 cases and 4117 controls. There were a total of 500,527 SNPs genotyped before quality assessment and quality control. Markers were removed if Hardy-Weinberg equilibrium $p \leq 0.0001$ (n=93) or if missingness was greater than 10% (n=19). All markers had a minor allele frequency greater than 0.01. All genetic analyses

were performed using logistic regression in PLINK v1.07 (Purcell et al., 2007). To correct for multiple testing, a Bonferroni corrected threshold was set of $\alpha = 1.00 \times 10^{-7} = 0.05 / 500{,}000$ markers tested.

Following the initial analyses examining cases with both RD and LI, RD and LI case definitions were further examined individually (e.g., LI excluding those with comorbid RD, and RD excluding those with comorbid LI). These analyses were completed to determine whether a single disorder (RD or LI) was driving association signals in the comorbid RD and LI analysis. The associations of markers within several previously identified RD and/or LI risk genes were also examined, including those recently reported in Luciano et al., in order to present their results with these phenotypic definitions. These genes included: *ABCC13*, *ATP2C2*, *BC0307918*, *CMIP*, *CNTNAP2*, *DAZAP1*, *DCDC2*, *DYX1C1*, *FOXP2*, *KIAA0319*, *KIAA0319L*, *PRKCH*, *ROBO1*, and *TDP2*.

Gene-based analyses were performed on each phenotype (comorbid RD and LI, as well as RD and LI individually) using the VEGAS program, similar to the Luciano et al. study (Liu et al., 2010; Luciano et al., 2013). To correct for multiple testing, a Bonferroni corrected threshold was set of $\alpha = 2.84 \times 10^{-6} = 0.05 / 17{,}610$ genes tested.

PING Replication Analyses.

Replication analyses were completed in the PING study. Details on the recruitment, ascertainment, neurobehavioral, genetic, and neuroimaging methods and data acquisition in the PING study are described in detail elsewhere, but are summarized briefly below (Akshoomoff et al., 2013, Brown et al., 2012; Fjell et al., 2012; Walhovd et al., 2012). The PING study is a cross-sectional cohort of typically developing children between the ages of 3 and 20 years. Subjects were screened for history of major developmental, psychiatric, and/or neurological disorders, brain injury, or medical conditions that affect development. However, subjects were not excluded due to learning disabilities such as RD and LI. The human research protections programs and institutional review boards at the 10 institutions (Weil Cornell Medical College, University of California at Davis, University of Hawaii, Kennedy Krieger Institute, Massachusetts General Hospital, University of California at Los Angeles, University of California at San Diego, University of Massachusetts Medical School, University of Southern California, and Yale University) participating in the PING study approved all experimental and consenting procedures. For individuals under 18 years of age, parental informed consent and

child assent (for those 7 to 17 years of age) were obtained. All participants age 18 years and older gave their written informed consent.

Subjects completed the validated study version of the NIH Toolbox Cognition Battery, in which two language- and reading- related tasks were completed: the Oral Reading Recognition Test and Picture Vocabulary Test (Akshoomoff et al., 2013; Weintraub et al., 2013). In the Oral Reading Recognition Test, a word or letter is presented on the computer screen and the participant is asked to read it aloud. Responses are recorded as correct or incorrect by the examiner, who views accepted pronunciations on a separate computer screen. The Picture Vocabulary Test is a measure of receptive vocabulary and administered in a computerized adaptive format. The participant is presented with an auditory recording of a word and four images on the computer screen; the task is to touch the image that most closely represents the meaning of the word.

Subjects were genotyped on the Illumina Human660W-Quad BeadChip (San Diego, CA), with markers used for replication analyses passing quality control filters (sample call rate > 98%, SNP call rate > 95%, minor allele frequency > 5%). A reference panel was constructed as described elsewhere (Brown et al., 2012; Fjell et al., 2012; Walhovd et al., 2012). To assess ancestry and admixture proportions in the PING participants, a supervised clustering approach implemented in the ADMIXTURE software (Alexander et al., 2009) was used and clustered participant data into six clusters corresponding to six major continental populations: African, Central Asian, East Asian, European, Native American, and Oceanic. Implementation of ancestry and admixture proportions in the PING subjects is described in detail elsewhere (Brown et al., 2012; Fjell et al., 2012; Walhovd et al., 2012). To prevent possible population stratification, only subjects with a European genetic ancestry factor (GAF) of 1 were included in genetic analysis of behavior. These 440 individuals of European ancestry (mean age of 11.5 [standard deviation = 4.8] years, 53.0% male) were analyzed using quantitative performance on the Oral Reading Recognition and Picture Vocabulary scores with PLINK v1.07, with age included as a covariate (Purcell et al., 2007). To correct for multiple testing (20 total tests = 10 SNPs x 2 language measures), we set statistical thresholds using the false discovery rate with α=0.05 (Benjamini & Hochberg, 1995).

PING Imaging Analysis.

PING imaging techniques, data acquisition, and analyses are discussed in depth elsewhere and briefly below (Brown et al., 2012; Fjell et al., 2012; Walhovd et al., 2012). Across the ten sites and 12 scanners, a standardized multiple modality high-resolution structural MRI protocol was implemented, involving 3D T1- and T2-weighted volumes and a set of diffusion-weighted scans. At the University of California at San Diego, data were obtained on a GE 3T SignaHD× scanner and a 3T Discovery 750× scanner (GE Healthcare) using eight-channel phased array head coils. The protocol included a conventional three-plane localizer, a sagittal 3D inversion recovery spoiled gradient echo T1-weighted volume optimized for maximum gray/white matter contrast (echo time = 3.5 ms, repetition time = 8.1 ms, inversion time = 640 ms, flip angle = 8°, receiver bandwidth = ±31.25 kHz, FOV = 24 cm, frequency = 256, phase = 192, slice thickness = 1.2 mm), and two axial 2D diffusion tensor imaging (DTI) pepolar scans (30-directions bvalue = 1,000, TE = 83 ms, TR = 13,600 ms, frequency = 96, phase = 96, slice thickness = 2.5 mm). Acquisition protocols with pulse sequence parameters identical or near identical to those protocols used at the University of California at San Diego were installed on scanners at the other nine sites. Data were acquired on all scanners to estimate relaxation rates and measure and correct for scanner-specific gradient coil nonlinear warping. Image files in DICOM format were processed with an automated processing stream written in MATLAB (Natick, MA) and C++ by the UCSD Multimodal Imaging Laboratory. T1-weighted structural images were corrected for distortions caused by gradient nonlinearities, coregistered, averaged, and rigidly resampled into alignment with an atlas brain. Image postprocessing and analysis were performed using a fully automated set of tools available in the FreeSurfer software suite (http://surfer.nmr.mgh.harvard.edu/) as well as an atlas-based method for delineating and labeling WM fiber tracts (Fischl, 2012).

Diffusion tensor imaging.

Diffusion-weighted images were corrected for eddy current distortion using a least squares inverse and iterative conjugate gradient descent method to solve for the 12 scaling and translation parameters describing eddy current distortions across the entire diffusion MRI scan, explicitly taking into account the orientations and amplitudes of the diffusion gradient (Zhuang et al., 2006). Head motion was corrected by registering each diffusion-weighted image to a corresponding image synthesized from a tensor fit to the data (Hagler et al., 2009). Diffusion MRI data were corrected for spatial and intensity distortions caused by B0 magnetic field in-homogeneities using the reversing gradient method (Holland et al., 2010). Distortions caused by gradient nonlinearities were corrected by applying a predefined, scanner-specific, nonlinear

transformation (Jovicich et al., 2006). Diffusion-weighted images were automatically registered to T1-weighted structural images using mutual information (Wells et al., 1996) and rigidly resampled into a standard orientation relative to the T1-weighted images with isotropic 2-mm voxels. Cubic interpolation was used for all resampling steps. Conventional DTI methods were used to calculate diffusion measures (Basser et al., 1994; Pierpaoli et al., 1996). Scanning duration for the DTI sequence was 4:24 min. White matter fiber tracts were labeled using a probabilistic-atlas based segmentation method (Hagler et al., 2009). Voxels containing primarily gray matter or cerebral spinal fluid, identified using FreeSurfer's automated brain segmentation, were excluded from analysis (Fischl et al., 2002). Fiber tract volumes were calculated as the number of voxels with probability greater than 0.08, the value that provided optimal correspondence in volume between atlas-derived regions of interest and manually traced fiber tracts.

Statistical Analyses.

Imaging-genetics analyses were performed in individuals of European genetic ancestry. Scanner, age, handedness, socioeconomic status, and sex were included as covariates in all analyses (Akshoomoff et al., 2013; Brown et al., 2012; Fjell et al., 2012; Walhovd et al., 2012). 332 subjects of European genetic ancestry had completed imaging measures that passed PING quality control. Fiber tract volumes in 16 tracts of interest were tested by multiple regression analyses in R using the PING data portal (https://mmil-dataportal.ucsd.edu).

Results

SNP and Gene-Based Associations

The ten strongest GWAS associations with comorbid RD and LI in ALSPAC are presented in Table 2. The strongest associations were observed with *ZNF385D* (OR=1.81, p=5.45 x 10^{-7}) and *COL4A2* (OR=1.71, p=7.59 x 10^{-7}) (Table 2). Next, RD and LI were examined individually—with no comorbid cases included—determining whether one disorder was driving these associations. The ten strongest associations for RD cases and LI cases individually are presented in Table 3 and Table 4, respectively. The strongest associations with LI were with markers in *NDST4* (OR=1.83, p=1.40 x 10^{-7}) (Table 3). Markers on chromosome 10 (OR=1.43, p=5.16x10^{-6}), chromosome 8 (OR=1.70, p=5.85x10^{-6}), and the *OPA3* gene (OR=1.53, p=6.92x10^{-6}) had the strongest associations with RD (Table 4). Markers with p<0.01 within genes previously implicated in RD and/or LI are presented in Supplemental Table 1 for each phenotype. The strongest associations with these markers were seen for *KIAA0319* with

comorbid RD and LI (rs16889556, p=0.0005177), *FOXP2* with comorbid RD and LI (rs1530680, 0.0001702), *CNTNAP2* with LI (rs6951437, p=0.0000462) and *DCDC2* with LI (rs793834, 0.0002679) (Supplemental Table 1a-1c). Gene-based analyses were completed on each phenotype (comorbid RD and LI, RD individually, and LI individually), and the ten strongest gene-based associations are presented in Supplemental Table 2. None of the gene-based associations survived correction for multiple-testing; however, the strongest associations were seen with: (1) *OR5H2*, *OR5H6*, and *RRAGA* with comorbid RD and LI, (2) *NEK2*, *DLEC1*, and *NARS* with LI, and (3) *MAP4*, *OR2L8*, and *CRYBA4* with RD. Markers with the strongest p-values in discovery analyses in *ZNF385D*, *COL4A2*, and *NDST4* were carried forward for replication analysis in PING. We observed replication of two markers within *ZNF385D* and performance on the Picture Vocabulary Test (p=0.00245 and 0.004173) (Table 5). However, markers did not replicate with the Oral Reading Recognition Test (p>0.05).

Imaging-Genetics of *ZNF385D*

To follow-up on the replicated associations of ZNF385D, the effects of these variants on fiber tract volumes previously implicated in written and verbal language were examined. Before doing so, fiber tract volume was first determined as a predictor of performance on Oral Reading Recognition and Picture Vocabulary Tests (data not shown). Within subjects of only European genetic ancestry, ZNF385D genotypes were predictors of overall fiber tract volume and as well as fiber tract volumes in the right and left hemispheres (Table 6). ZNF385D SNPs were also predictors bilaterally within the inferior longitudinal fasiculus (ILF), inferior fronto-occipto fasiculus (IFO), and temporal superior longitudinal fasiculus (tSLF) in this subset (Table 6). To discern whether these associations between ZNF385D and fiber tract volumes reflect global brain volume differences among genotype, the relationship of ZNF385D with both total brain segmentation and total cortical volumes was examined. Associations for both measures were found with rs1679255 (p=0.00072 and 0.00027, respectively) and rs12636438 (p=0.000259 and 0.000069, respectively). The effects appeared to be additive in nature, with heterozygous individuals having intermediate phenotypes relative to those homozygous for the major allele and to those homozygous for the minor allele. In fact, when these total brain volume measures were inserted into the model as a covariate, ZNF385D associations with DTI fiber tract volumes were no longer present.

As described herein, genes were identified that contribute to the common co-occurrence of RD and LI. In the discovery analyses, associations of *ZNF385D* and *COL4A2* were found in

comorbid cases, and of *NDST4* with LI. Next, associations of *ZNF385D* with performance were observed on a vocabulary measure, but not on an oral reading measure, in PING. Association with performance on a vocabulary measure, although not exactly recapitulating the comorbidity phenotype, does provide further evidence for the contribution of *ZNF385D* to language. To gain functional understanding, the effects of replicated *ZNF385D* markers on the volumes of language-related fiber tracts were interrogated. *ZNF385D* markers associated bilaterally with overall fiber tract volumes, as well as with overall brain volume.

Studies have shown that RD and LI share genetic contributors (Trzaskowski et al. 2013). However, specific genes that contribute to both RD and LI have only recently begun to be examined. These studies have only used a candidate gene approach to examine this shared genetic etiology. Such an approach has been successful in showing the shared contribution of *DCDC2*, *KIAA0319*, *FOXP2*, *CNTNAP2*, among others, to both RD and LI (Eicher & Gruen, 2013; Graham & Fisher, 2013; Newbury et al., 2009; Newbury et al., 2010; Pinel et al., 2012; Rice et al., 2009; Scerri et al., 2011). In fact, markers within *KIAA0319*, *FOXP2*, and *CNTNAP2* (along with *BC0307918*) showed nominal association with comorbid RD and LI in the analyses ($p<0.01$) described herein. RD/LI risk genes also showed a tendency to associate with LI individually (*DCDC2*, *KIAA0319*, and *CNTNAP2*) and with RD individually (*CNTNAP2* and *CMIP*) ($p<0.01$). The lack of replication for other RD/LI risk genes and differences specifically between this study and those of Scerri et al. (2011) and Luciano et al. (2013) are likely a results of different case definitions and numbers, as the instant case classifications were designed to capture as wide a range as possible of reading- and language-impaired subjects as opposed to using highly specific neurocognitive measures.

A glaring omission in the genetic investigations of RD and LI is the lack of hypothesis-free methods. These methods allow for discovery of new genes because they do not rely on pre-selected candidates. Here, the GWAS analyses indicate that *ZNF385D* contributes to comorbid RD and LI. This study is not the first to perform a GWAS on reading- and language- related traits. Luciano et al. (2013) recently reported a GWAS of quantitative measures of written and verbal language measures in two population-based cohorts, including ALSPAC. They found strong evidence that *ABCC13*, *BC0307918*, *DAZAP1*, among others contribute to performance on these measures, although the instant analyses did not provide strong evidence for them. The analytical strategies differed in two ways: (1) the use of dichotomous rather than quantitative measures to condition genetic associations and (2) examining reading and language together as

opposed to individually. Past association studies of RD and LI have shown differences in results depending on whether genetic data were conditioned on dichotomous or quantitative phenotypes. For instance, *KIAA0319* tends to associate more readily with quantitative measures, while *DCDC2* associates more often with dichotomized variables (Paracchini et al., 2008; Powers et al., 2013; Scerri et al., 2011). The present study, which examines comorbidity, and that of Luciano et al., which examined performance on reading and language tasks individually, conditioned genetic associations on different traits, which can lead to different statistical associations. Both analytical strategies are valid and have gleaned separate, yet related insight into the genetic underpinnings of written and verbal language. They demonstrate the importance of creative and careful examination of phenotypes when examining neurocognitive and other complex traits.

Following the primary analysis of comorbid RD and LI, RD and LI were examined individually to determine whether a single disorder was driving the association signals. *ZNF385D* did not associate with either RD or LI individually, indicating that *ZNF385D* contributes to processes related to both RD and LI, as opposed to only one of these disorders. Within the PING cohort, associations of *ZNF385D* markers were observed with performance on the Picture Vocabulary Test and not the Oral Reading Recognition Test. Measures of receptive vocabulary (e.g. the Picture Vocabulary Test) are related to both written and verbal language tasks (Scarborough 1990, Wise et al., 2007), while performance on decoding measures (e.g. the Oral Reading Recognition Test) appear to be specific to reading. Therefore, the Picture Vocabulary Test may reflect the comorbid RD and LI phenotype used for association in ALSPAC better than the Oral Reading Recognition Test and explain the association pattern of *ZNF385D* in PING. In addition to *ZNF385D*, suggestive associations of *COL4A2* with comorbid RD/LI and *NDST4* with LI were observed. Neither of these associations replicated with the measures in PING, but future studies should attempt to replicate these associations, particularly due to the known involvement of *COL4A2* in porencephaly and white matter lesions (Verbeek et al., 2012, Yoneda et al., 2011).

Gene-based analyses did not reveal any associations that survived correction for multiple testing. Nonetheless, there were intriguing gene associations that should be investigated in future studies. For instance, with LI, there were suggestive associations with several genes on chromosome 19—*IL4I1*, *ATF5*, *NUP62*, and *SIGLEC11*—which may correspond to the SLI2 linkage peak (Monaco, 2007; SLI Consortium, 2002), Luciano et al. (2013) found a similar

accumulation of suggestively associated genes approximately 5Mb away from the genes identified herein. Additionally, *MAP4*, a microtubule assembly gene, was the strongest associated gene with RD. There is evidence that microtubule function plays a key role in reading development as aberrant neuronal migration is thought to contribute to the etiology of RD and other RD candidate genes are thought to interact with microtubules (e.g. *DCDC2* and *ACOT13*) (Cheng et al., 2006). These findings can be validated in an independent cohort, using methods described herein and known methods to conclude they are involved in RD and LI.

The strongest observed associations in this study were with markers within *ZNF385D*. *ZNF385D* has previously been implicated in schizophrenia and attention deficit hyperactivity disorder (ADHD) (Poelmans et al., 2011; Xu et al., 2013). Both schizophrenia and ADHD are neurobehavioral disorders thought to have core impairments in common with RD and LI, including comprehension and semantic processing (Gilger et al., 1992; Li et al., 2009; Willcutt et al., 2005). Additionally, the observed association of *ZNF385D*, as described herein, on global brain volume may indicate that *ZNF385D* influences various neurocognitive traits through its effect on the entire brain.

There is little known regarding the function of *ZNF385D*, although its zinc finger domain suggests it is a transcriptional regulator. The importance of transcriptional regulation in written and verbal language is not a new concept. The most widely studied language gene, *FOXP2*, is a potent transcription factor that has been shown to regulate another language gene, *CNTNAP2* (Vernes et al., 2007; Vernes et al., 2011). Additionally, in the DYX2 locus, two risk variants, READ1 within *DCDC2* and the *KIAA0319* risk haplotype, appear to have the capacity to regulate gene expression (Couto et al., 2010; Dennis et al., 2009; Meng et al., 2011) and possibly interact (Ludwig et al. 2008; Example 1; Powers et al., 2013). *ZNF385D* variants now join this list of putative transcriptional variants that influence written and verbal language skills. The characterization of target genes of *ZNF385D* and of its transcriptional effects on these targets will be an important next step. Additionally, the identification of target genes may generate therapeutic candidates for treatment and remediation of RD and LI. To gain further insight into *ZNF385D*, imaging-genetics analyses of *ZNF385D* and fiber tract volumes of language-related tracts were performed. *ZNF385D* appears to modulate fiber tract and total brain volumes, which may subsequently affect the connectivity and functionality of brain regions important in the efficient, fluent integration of written and verbal language. Thus, identification of target genes and how the modulation of their expression during neural

development yields differences in fiber tract and total brain volumes will be vital for dissecting not only the mechanism of *ZNF385D*, but also for the development of core language skills in children.

Characteristics of the population. First, although the overall sample size of the ALSPAC is formidable, the number of cases for each definition is relatively small. This is expected in a cross-sectional cohort of the general population as the prevalence of these disorders ranges between 5-17% (Pennington & Bishop, 2009). The ALSPAC cohort would not be expected to be enriched for RD and/or LI cases. Small sample size could have hindered the statistical power and ability to identify risk genes with small effect size. Second, the reading and language measures performed in the ALSPAC and PING studies were not identical. Phenotypes in PING were treated as a quantitative trait rather than a dichotomous variable as in ALSPAC. Therefore, attempts to replicate associations observed in the ALSPAC cohort may have been hampered as reading/language measures in PING may have captured different skills than those in ALSPAC. However, the associations observed in the PING indicate that *ZNF385D* plays a substantial, consistent role in overall language processes. Third, atlas-derived tract volume measures, like volumes derived from manually traced fiber tracts, are likely underestimates of true fiber volume for most tracts. However, fiber tract volumes were derived consistently for all subjects and likely reflect inter-individual differences. Nonetheless, the strength and independent replication of the associations described herein and the relationship with brain imaging phenotypes strongly implicate *ZNF385D* in core language processes underlying RD and LI.

In conclusion, *ZNF385D* was identified as a novel gene contributing to both RD and LI, as well as fiber tract and overall brain volume. The implication of another transcription factor in communication disorders underscores the importance of transcriptional regulation in neural development of language domains in the brain. Future studies should aim to further characterize the molecular functionality of *ZNF385D* and replicate this association, as well as our non-replicated associations—*NDST4* and *COL4A2*—in RD, LI, and other related disorders.

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Tables of Example 4

Table 1: Reading and language measures used to define Reading Disability (RD) and Language Impairment (LI) Cases

Reading Disability (RD) (n=527)*	**Language Impairment (LI) (n=337)****
Phoneme Deletion Age 7 Years	Phoneme Deletion Age 7 Years
Single Word Reading Age 7 Years	Verbal Comprehension Age 8 Years
Single Word Reading Age 9 Years	Nonword Repetition Age 8 Years
Nonword Reading Age 9 Years	
Reading Comprehension Age 9 Years	

*RD Cases had a z-score of less than or equal to -1 on at least 3 out of the 5 reading measures
**LI Cases had a z-score of less than or equal to -1 on at least 2 out of the 3 language measures

Table 2: Associations with comorbid RD and LI cases in ALSPAC (n=174)

Marker	Chr	Base Pair	Minor Allele	MAF Aff	MAF Unaff	Gene	Odds Ratio	P-value
rs12636438	3	22038281	G	0.3017	0.1927	*ZNF385D*	1.811	$5.45x10^{-7}$
rs1679255	3	22022938	C	0.3006	0.1923	*ZNF385D*	1.805	$6.87x10^{-7}$
rs9521789	13	109917621	C	0.5201	0.3879	*COL4A2*	1.71	$7.59x10^{-7}$
rs1983931	13	109916103	G	0.5201	0.3896	*COL4A2*	1.698	$1.06x10^{-6}$
rs9814232	3	21948179	A	0.2931	0.1886	*ZNF385D*	1.784	$1.30x10^{-6}$
rs7995158	13	109909718	A	0.5201	0.3911		1.687	$1.44x10^{-6}$
rs6573225	14	58354640	C	0.1965	0.1122		1.935	$1.56x10^{-6}$
rs4082518	10	17103032	T	0.3103	0.2049	*CUBN*	1.746	$2.17x10^{-6}$
rs442555	14	58365937	C	0.1983	0.1149		1.905	$2.38x10^{-6}$
rs259521	3	21942154	T	0.2902	0.1885	*ZNF385D*	1.761	$2.42x10^{-6}$

Chr, Chromosome; MAF Aff, Minor allele frequency in affected subjects; MAF Unaff, Minor allele frequency in unaffected subjects

Table 3: Associations with LI cases in ALSPAC, excluding comorbid RD cases (n=163)

Marker	Chr	Base Pair	Minor Allele	MAF Aff	MAF Unaff	Gene	Odds Ratio	P-value
rs482700	4	116286939	G	0.3896	0.2588	*NDST4*	1.827	$1.40x10^{-7}$
rs7695228	4	116309516	T	0.3920	0.2636	*NDST4*	1.801	$2.94x10^{-7}$
rs1940309	4	116306410	T	0.3865	0.2606	*NDST4*	1.788	$4.14x10^{-7}$
rs505277	4	116248257	T	0.3773	0.2528	*NDST4*	1.791	$4.35x10^{-7}$
rs476739	4	116248997	A	0.3773	0.2529	*NDST4*	1.79	$4.41x10^{-7}$
rs867036	4	116381578	C	0.3957	0.2696	*NDST4*	1.774	$5.31x10^{-7}$
rs867035	4	116381423	C	0.3957	0.2697	*NDST4*	1.773	$5.45x10^{-7}$

rs2071674	4	2366882	T	0.0920	0.0389	*ZFYVE28*	2.503	$1.90x10^{-6}$
rs7694946	4	116413588	C	0.3620	0.2526	*NDST4*	1.678	$8.95x10^{-6}$
rs4823324	22	44616787	C	0.2914	0.4143	*ATXN10*	0.581	$9.30x10^{-6}$

Chr, Chromosome; MAF Aff, Minor allele frequency in affected subjects; MAF Unaff, Minor allele frequency in unaffected subjects

Table 4: Associations with RD cases in ALSPAC, excluding comorbid LI cases (n=353)

Marker	**Chr**	**Base Pair**	**Minor Allele**	**MAF Aff**	**MAF Unaff**	**Gene**	**Odds Ratio**	**P-value**
rs180950	10	115697957	G	0.456	0.369		1.431	$5.16x10^{-6}$
rs2590673	8	126037337	G	0.133	0.083		1.697	$5.85x10^{-6}$
rs892100	19	50772522	C	0.228	0.162	*OPA3*	1.526	$6.92x10^{-6}$
rs1792745	18	51955991	T	0.187	0.129		1.558	$1.22x10^{-5}$
rs12546767	8	126151747	C	0.152	0.099	*KIAA0196*	1.618	$1.32x10^{-5}$
rs12634033	3	146524529	C	0.135	0.087		1.646	$1.80x10^{-5}$
rs892270	12	105002956	G	0.534	0.451	*NUAK1*	1.395	$2.16x10^{-5}$
rs10887149	10	124156994	A	0.278	0.357	*PLEKHA1*	0.069	$2.25x10^{-5}$
rs10041417	5	33218502	T	0.226	0.164		1.489	$2.58x10^{-5}$
rs6792971	3	68468217	C	0.111	0.068	*FAM19A1*	1.703	$2.59x10^{-5}$

Chr, Chromosome; MAF Aff, Minor allele frequency in affected subjects; MAF Unaff, Minor allele frequency in unaffected subjects

Table 5: Replication of associations in PING (n=440)

Marker	**Minor Allele**	**MAF**	**Gene**	**Oral Reading Test**		**Picture Vocabulary Test**	
				Beta	**P-value**	**Beta**	**P-value**
rs12636438	G	0.161	*ZNF385D*	-0.1867	0.9452	-2.88	0.004173*

rs1679255	G	0.292	*ZNF385D*	-1.84	0.5016	-3.048	0.002445**
rs9521789	G	0.4370	*COL4A2*	-0.3411	0.7332	0.8647	0.3877
rs476739	A	0.265	*NDST4*	0.5406	0.5891	0.5159	0.6062
rs505277	A	0.280	*NDST4*	0.5406	0.5891	-0.3452	0.7301
rs482700	G	0.278	*NDST4*	0.5498	0.5828	-0.05341	0.9574
rs7695228	A	0.295	*NDST4*	0.6258	0.5318	0.09991	0.9205
rs867036	G	0.378	*NDST4*	0.2605	0.7946	-0.1414	0.8876
rs867035	G	0.377	*NDST4*	0.2961	0.7673	-0.1565	0.8757
rs1940309	A	0.281	*NDST4*	0.6049	0.5456	0.1296	0.8969

*P-value less than FDR-adjusted statistical threshold (FDR-adjusted threshold = 0.05 x (2/19) = 0.00526

**P-value less than FDR-adjusted statistical threshold (FDR-adjusted threshold = 0.05 x (1/20) = 0.00250

MAF, Minor allele frequency in full PING sample

Table 6: *ZNF385D* Associations with DTI Fiber Tract Volumes in subjects with 100% European Genetic Ancestry (n=332)

Fiber Tract	**rs1679255**		**rs12636438**	
	Slope	**P-value**	**Slope**	**P-value**
All	-3329.9	0.044*	-3717.9	0.023*
Right All	-1731.4	0.039*	-1965	0.017*
Left All	-1616.3	0.055	-1775.6	0.033*
Right ILF	-251.3	0.011*	-234.4	0.016*
Left ILF	-256.9	0.0088**	-254.6	0.009**
Right IFO	-200.8	0.032*	-190	0.041*
Left IFO	-221	0.012*	-226.3	0.009**
Right SLF	-168.1	0.06	-206	0.02*

Left SLF	-199.5	0.022*	-212.9	0.013*
Right tSLF	-170.8	0.011*	-180.7	0.0068**
Left tSLF	-163.1	0.023*	-169.9	0.016*
Right pSLF	-153.1	0.079	-182.4	0.034*
Left pSLF	-112.2	0.18	-125.3	0.131
Right SIFC	-148.8	0.052	-165.6	0.029*
Left SIFC	-34.54	0.66	-54.3	0.48
CC	-977.1	0.15	-1181.6	0.081

*$p \leq 0.05$
**$p \leq 0.01$
Abbreviations: All (All Fiber Tracts), ILF (Inferior Longitudinal Fasiculus), IFO (Inferior Fronto-occipital Fasiculus), SLF (Superior Longitudinal Fasiculus), tSLF (Temporal Superior Longitudinal Fasiculus), pSLF (Parietal Superior Longitudinal Fasiculus), SIFC (Striatal Inferior Frontal Cortex), CC (Corpus Callosum)

Supplement Tables

Supplemental Table 1: Associations of markers within genes previously implicated in RD and/or LI with (a) Comorbid RD and LI, (b) LI individually, and (c) RD individually.

a) Comorbid RD and LI

Marker	Gene	Chr.	Base Pair	P-value
rs16889556	*KIAA0319*	6	24749584	0.0005177
rs1047782	*TDP2*	6	24758710	0.006515
rs1530680	*FOXP2*	7	114194632	0.0001702
rs12667130	*FOXP2*	7	114213035	0.003033
rs6965855	*CNTNAP2*	7	145348483	0.006804
rs985080	*CNTNAP2*	7	145359118	0.006157
rs4726782	*CNTNAP2*	7	145425012	0.005341
rs1718101	*CNTNAP2*	7	145753721	0.0008707
rs10487689	*CNTNAP2*	7	146835482	0.008787

rs1918296	*CNTNAP2*	7	147655135	0.00616
rs737533	*BC0307918*	10	3353137	0.001008

b) LI

Marker	Gene	Chr.	Base Pair	P-value
rs793845	*DCDC2*	6	24296970	0.005511
rs2799373	*DCDC2*	6	24303738	0.0009664
rs793862	*DCDC2*	6	24315179	0.002443
rs793834	*DCDC2*	6	24342912	0.0002679
rs2792682	*DCDC2*	6	24380363	0.006634
rs807704	*DCDC2*	6	24408825	0.001988
rs707864	*DCDC2*	6	24413827	0.001266
rs12193738	*KIAA0319*	6	24676372	0.00974
rs2817198	*KIAA0319*		24683073	0.00559
rs10456309	*KIAA0319*	6	24697541	0.002258
rs985080	*CNTNAP2*	7	145359118	0.006735
rs1554690	*CNTNAP2*	7	145377266	0.006486
rs2533096	*CNTNAP2*	7	146037312	0.004782
rs6951437	*CNTNAP2*	7	146037340	0.0000462
rs344470	*CNTNAP2*	7	146044430	0.001697
rs344468	*CNTNAP2*	7	146050259	0.003965

c) RD

Marker	Gene	Chr.	Base Pair	P-value
rs4725745	*CNTNAP2*	7	147032172	0.002407
rs12444778	*CMIP*	16	80330728	0.003148
rs1444186	*CMIP*	16	80330745	0.00482

Supplemental Table 2: Gene-based analyses of comorbid RD and LI, LI individually, and RD individually. The top ten gene-based associations for each are shown.

Gene	Ch	Start Position	Stop Position	No. SNPS in Gene	p-value
RD and LI					
OR5H2	3	99484421	99485366	16	0.000072
OR5H6	3	99465818	99466796	19	0.000127
RRAGA	9	19039371	19041021	30	0.000276
OR6B3	2	240633166	240634162	36	0.000294
UMOD	16	20251873	20271538	29	0.000307
A26C1A	2	131692393	131738886	1	0.000389
FAM29A	9	19043140	19092902	44	0.000406
CHRNA1	2	175320568	175337446	23	0.000420
IFIT5	10	91164418	91170733	27	0.000475
LOC643905	2	240629902	240631072	39	0.000562
LI					
NEK2	1	209902744	209915590	28	0.000117
DLEC1	3	38055699	38139232	20	0.000171
NARS	18	53418891	53440175	36	0.000203
IL4I1	19	55084722	55124574	22	0.000305
PKD2	4	89147843	89217953	34	0.000313
ATF5	19	55123785	55129004	18	0.000344
NUP62	19	55101893	55124598	19	0.000402
SIGLEC11	19	55144061	551556241	49	0.000578
ACAN	15	87147677	87219589	43	0.000633
PGD	1	10381671	10402788	12	0.000668
RD					
MAP4	3	47867188	48105715	18	0.000085
OR2L8	1	246178782	246179721	19	0.000139
CRYBA4	22	25347927	25356636	40	0.000219
OR2T8	1	246150942	246151881	24	0.000225
KIAA1622	14	93710401	93815825	42	0.000255

OR2AK2	1	246195256	246196264	15	0.000315
DHX30	3	47819654	47866687	11	0.000316
GEMIN6	2	38858830	38862610	8	0.000351
C20orf10	20	43435933	43440371	23	0.000450
PPIF	10	80777225	38862610	22	0.000493

CLAIMS

What is claimed is:

1. A method of determining if a sample obtained from an individual comprises nucleic acid which comprises a haplotype associated with susceptibility for developing a learning disability (LD) in humans, comprising assaying a sample that comprises nucleic acid from the individual for the presence in the DYX2 locus of at least one of the following markers: (a) CGCGAG in a DCDC2 gene; (b) CACGAG in a DCDC2 gene; (c) both CGCGAG and CACGAG in a DCDC2 gene; (d) rs4504469 in a KIAA0319 gene; (e) rs2038137 in a KIAA0319 gene; (f) rs2143340 in a KIAA0319 gene; (g) any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene; and (h) any combination of CGCGAG in a DCDC2 gene; CACGAG in a DCDC2 gene; both CGCGAG and CACGAG in a DCDC2 gene; rs4504469 in a KIAA0319 gene; rs2038137 in a KIAA03109 gene; rs2143340 in a KIAA0319 gene; and any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene, wherein if the sample comprises at least one of (a) – (h), the sample comprises a haplotype associated with susceptibility for developing a learning disability in humans.

2. The method of claim 1, wherein the sample is assayed for at least one marker of (a), (b) and (c) and at least one marker of (d), (e), (f) and (g).

3. The method of claim 1 or 2, wherein the LD is reading disability (RD) or language impairment (LI) or both RD and LI.

4. The method of any one of claims 1-3, wherein the sample is blood, cells, or tissue.

5. The method of any one of claims 1-4, further comprising assaying the sample for allele 5 of DCDC2 gene, allele 6 of DCDC2 gene, or both allele 5 of DCDC2 gene and allele 6 of DCDC2 gene.

6. The method of claim 5, wherein allele 5 of DCDC2 gene comprises SEQ ID NO:35 and allele 6 of DCDC2 comprises SEQ ID NO:36.

7. The method of any one of claims 1-6, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

8. A method of assaying a sample for a marker of a haplotype associated with susceptibility for developing a learning disability (LD) in humans, comprising: (a) obtaining a sample comprising nucleic acid from an individual; and (b) determining if the sample comprises at least one of the following: (i) CGCGAG in a DCDC2 gene in the DYX2 locus; (ii) CACGAG in a DCDC2 gene in the DYX2 locus; (iii) both CGCGAG and CACGAG in a DCDC2 gene in the DYX2 locus; (iv) rs4504469 in a KIAA0319 gene in the DYX2 locus; (v) rs2038137 in a KIAA0319 gene in the DYX2 locus; (vi) rs2143340 in a KIAA0319 gene in the DYX2 locus; (vii) any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene in the DYX2 locus; and (viii) any combination of CGCGAG in a DCDC2 gene; CACGAG in a DCDC2 gene in the DYX2 locus; both CGCGAG and CACGAG in a DCDC2 gene in the DYX2 locus; rs4504469 in a KIAA0319 gene in the DYX2 locus; rs2038137 in a KIAA03109 gene in the DYX2 locus; rs2143340 in a KIAA0319 gene in the DYX2 locus; and any combination of two or three of rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene in the DYX2 locus, wherein if the sample comprises at least one marker of (i) – (vii), the sample comprises a haplotype associated with susceptibility for developing a learning disability in humans.

9. The method of claim 8, wherein the sample is assayed for at least one marker of (i), (ii) and (iii) and at least one marker of (iv), (v), (vi) and (vii).

10. The method of claim 8 or 9, wherein the LD is reading disability (RD) or language impairment (LI) or both RD and LI.

11. The method of any one of claims 8-10, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

12. The method of any one of claims 8-11, wherein the sample is blood, cells or tissue.

13. A method of determining if a sample obtained from an individual comprises nucleic acid which comprises an allele associated with susceptibility for developing a learning disability (LD) in humans, comprising: assaying a sample that comprises nucleic acid from the individual

for the presence of allele 5 of DCDC2 gene in the DYX2 locus, allele 6 of DCDC2 gene in the DYX2 locus, or both allele 5 of DCDC2 gene and allele 6 of DCDC2 gene, wherein if the sample comprises at least one of allele 5 and allele 6, the sample comprises an allele associated with susceptibility for developing a learning disability.

14. The method of claim 13, wherein the LD is reading disability (RD) or language impairment (LI) or both RD and LI.

15. The method of claim 14 or 14, wherein allele 5 of DCDC2 gene comprises SEQ ID NO:35 and allele 6 of DCDC2 comprises SEQ ID NO:36.

16. The method of any one of claims 13-15, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

17. The method of any one of claims 13-16, wherein the sample is blood, cells or tissue.

18. A method of determining if a sample obtained from an individual comprises at least one marker associated with comorbid reading disability (RD) and language impairment (LI) in humans, comprising: (a) obtaining a sample that contains nucleic acid from the individual and (b) assaying the sample for (i) at least one marker in DCDC2 gene in the DYX2 locus for a haplotype associated with susceptibility for developing RD in humans and (ii) at least one marker in KIAA0319 gene in the DXY2 locus for a haplotype associated with susceptibility for developing LI in humans, wherein if the sample comprises a marker of (i) and a marker of (ii), the sample comprises at least one marker associated with comorbid RD and LI.

19. The method of claim 18, wherein the marker of (b)(i) and the marker of (b)(ii) are the same marker or are two different markers.

20. The method of claim 18 or 19, wherein the at least one marker of (b)(i) is CGCGAG or GACGAG and the at least one marker of (b)(ii) is rs4504469; rs2038137; or rs2143340.

21. The method of any one of claims 18-20, further comprising assaying the sample for allele 5 of DCDC2 gene in the DYX2 locus, allele 6 of DCDC2 gene of DYX2 locus, or both allele 5 of DCDC2 gene in the DYX2 locus and allele 6 of DCDC2 gene in the DYX2 locus.

22. The method of claim 18 or 19, wherein the at least one marker of (b)(i) and the at least one marker of (b)(ii) are selected from: rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs4504469; rs2038137; rs2143340; rs9295626; rs7763790 rs6935076; rs2817201; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs6456624; rs6935076; rs2038137; rs3756821; rs1883593; rs3212236; rs6456621; rs12193738; rs2817198; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; and rs807694.

23. The method of any one of claims 18-22, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

24. The method of any one of claims 18 to 23, wherein the sample is blood, cells or tissue.

25. A method of determining if a sample obtained from an individual comprises a marker associated with language impairment (LI) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: CACGAG in a DCDC2 gene in the DYX2 locus; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs807694; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; and a marker for at least one of the following genes: NEK2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; and PGD.

26. The method of claim 25, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

27. The method of claim 25 or 26, wherein the sample is blood, cells or tissue.

28. A method of determining if a sample obtained from an individual comprises a marker associated with reading disability (RD) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: CGCGAG in a DCDC2 gene in the DYX2 locus; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs4725745; rs12444778; rs1444186; rs2294691;

rs10456309; rs1562422; and a marker for at least one of the following genes: MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

29. The method of claim 28, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

30. The method of claim 28 or 29, wherein the sample is blood, cells, or tissue.

31. A method of determining if nucleic acids (DNA, RNA) in an individual comprise markers of haplotypes that interact in a synergistic manner in resulting in a learning disorder (LD) in humans comprising: (a) obtaining a sample that comprises nucleic acids from an individual and (b) assaying the sample for at least one DCDC2 risk haplotype or DCDC2 risk allele and at least one KIAA0319 risk haplotype, wherein the at least one DCDC2 risk haplotype is CGCGAG or GACGAG, the at least one DCDC2 risk allele is allele 5 of DCDC2 gene in the DYX2 locus (SEQ ID NO:35) or allele 6 of DCDC2 gene in the DYX2 locus (SEQ ID NO:36) and the at least one KIAA0319 risk haplotype is a variant KIAA0319 haplotype comprising a snp which is rs4504469; rs2038137; or rs2143340 and wherein if the sample comprises at least one DCDC2 risk haplotype or at least one DCDC2 risk allele and at least one KIAA0319 risk haplotype, the nucleic acids comprise markers of haplotypes that interact in a synergistic manner in resulting in a LD in humans.

32. The method of claim 31, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

33. The method of claim 31 or 32, wherein the sample is blood, cells or tissue.

34. A method of identifying or aiding in identifying an individual at risk for developing at least one learning disability (LD), comprising assaying a sample obtained from the individual for the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans, wherein the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans indicates that the individual is at risk for developing a LD.

35. The method of claim 34, wherein the at least one LD is a reading disability (RD) or language impairment (LI).

36. The method of claim 34 or 35, wherein the at least one haplotype is located in the DCDC2 gene within the DYX2 locus or in the KIAA0319 gene within the DYX2 locus.

37. The method of any one of claims 34-36, wherein the at least one haplotype comprises (a) CGCGAG or CACGAG in a DCDC2 gene within the DYX2 locus; or (b) rs4504469, rs2038137, rs2143340, or any combination thereof in a KIAA0319 gene within the DYX2 locus; or (c) any combination of the haplotypes in (a) and (b).

38. The method of any one of claims 34-37, wherein the assay involves hybridization-mediated methods, sequencing, or combinations thereof.

39. The method of any one of claims 34-38, wherein the sample is blood, cells, or tissue.

40. A method of determining if a sample obtained from an individual comprises a marker for susceptibility for developing a learning disability (LD) that is reading disability (RD) or language impairment (LI), comprising: obtaining a sample that comprises nucleic acid from the individual and determining if the sample comprises at least one marker selected from the group consisting of: rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs12636438; rs1679255; rs9521789; rs476739; rs505277; rs482700; rs7695228; rs867036; rs867035; rs1940309; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs4725745; rs12444778; rs1444186; rs2294691; rs10456309; rs1562422; rs807694; rs3756814; rs3777663; rs9295626; rs7763790; rs6935076; rs9348646; rs2328791; rs2328791; rs2817201, rs9295626; rs4576240; rs17307478, rs9356939, rs7763790, rs6456621; rs6456624, rs6935076, rs2038137, rs3756821, rs1883593, rs3212236; rs3777663, rs3756814, rs6931809, rs6916186, rs6933328, rs17491647; rs2328791; rs33914824a; rs807694a; rs707864a; rs10456301a; rs16889066a; rs9379651a; rs2817201;

rs9295626; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs3756821; rs1883593; rs3212236; rs2294691; rs3777663; rs3756814; rs6931809; rs6916186; rs6933328; rs17491647; rs9348646; rs1562422 and a marker for each of the following genes:R5H2; OR5H6; RRAGA; OR6B3; UMOD; A26C1A; FAM29A; CHRNA1; IFIT5; LOC643905; K2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; PGD; MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

41. The method of claim 40, wherein the assay comprises a hybridization-mediated method, nucleic acid sequencing, or both a hybridization-mediated method and nucleic acid sequencing .

42. The method of claim 40 or 41, wherein the sample is blood, cells, or tissue.

43. A method of identifying or aiding in identifying an individual at risk for developing at least one learning disability (LD), comprising assaying a sample obtained from the individual for the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans, wherein the presence in the DYX2 locus of at least one haplotype that is associated with susceptibility for developing a LD in humans indicates that the individual is at risk for developing a LD.

44. The method of claim 43, wherein at least one LD is a reading disability (RD) or language impairment (LI).

45. The method of claim 43 or 44, wherein the at least one haplotype is located in the DCDC2 gene within the DYX2 locus or in the KIAA0319 gene within the DYX2 locus.

46. The method of any one of claims 43-45, wherein the at least one haplotype comprises (a) CGCGAG, CACGAG, or both CGCGAG and CACGAG in a DCDC2 gene within the DYX2 locus; or (b) rs4504469, rs2038137, rs2143340, or any combination thereof in a KIAA0319 gene within the DYX2 locus; or (c) any combination of the haplotypes in (a) and (b).

47. The method of anyone of claims 43-46, wherein the assay comprises a hybridization-mediated method, nucleic acid sequencing, or both a hybridization-mediated method and nucleic acid sequencing.

48. The method of any one of claims 43-47, wherein the sample is blood, cells, or tissue.

49. A method of identifying an individual as having, or being susceptible to developing, a learning disability (LD), comprising obtaining a sample comprising nucleic acid from an individual; determining whether nucleic acid in the sample comprises a DCDC2 gene haplotype in the DYX2 locus associated with susceptibility for developing reading disability (RD) and a KIAA0319 gene haplotype associated with susceptibility for developing language impairment (LI), wherein the DCDC2 gene haplotype and the KIAA0319 gene haplotype interact synergistically in decreasing performance in phoneme deletion and in resulting in a learning disorder (LD) in humans, wherein if the sample comprises both haplotypes, the individual is identified as having or being susceptible to developing a LD.

50. The method of claim 49, wherein the determining comprises a hybridization-mediated method, nucleic acid sequencing, or both a hybridization-mediated method and nucleic acid sequencing.

51. The method of claim 49 or 50, wherein the sample is blood, cells, or tissue.

52. An array (microarray) comprising a support having a plurality of discrete regions, each discrete region having at least one (one or more) nucleic acid fragment spotted thereon, wherein each region comprises sequences that are complementary to nucleic acids (DNA, RNA, genomic DNA) that encompass (contain within) a (at least one) marker (e.g., snp) associated with susceptibility for developing a learning disability (LD) in humans, wherein the at least one marker is in a variant DCDC2 gene or in a variant KIAA0319 gene.

53. The array (microarray) of claim 52 which sequences complementary to at least two different markers associated with susceptibility for developing a learning disability (LD).

54. The array (microarray) of claim 53 which comprises, in at least one region, sequences that are complementary to a marker in a variant DCDC2 gene associated with susceptibility for developing a learning disability (LD) and, in at least one different region, sequences that are complementary to a marker in a variant KIAA0319 gene associated with susceptibility for developing a LD.

55. The microarray of any one of claims 52- 54, wherein the one or more markers in a DCDC2 gene are selected from CGCGAG, CACGAG, READ1 allele 5 (SEQ ID NO:35), READ1 allele 6 (SEQ ID NO:36), or any combination of two, three or four of CGCGAG, CACGAG, READ1 allele 5 (SEQ ID NO:35), and READ1 allele 6 (SEQ ID NO:36); and wherein the one or more markers in a KIAA0319 gene are selected from rs4504469, rs2038137, rs2143340, or any combination of two or three of rs4504469, rs2038137 and rs2143340.

56. The microarray of claim 52-54, wherein the LD is language impairment (LI) in humans, wherein the one or markers in a DCDC2 gene are selected from CACGAG, READ1 allele 6 (SEQ ID NO:36), rs793845, rs2799373, rs793862, rs793834, rs2792682, rs807704, rs707864, rs807694, or any combination thereof; and the one or more markers in a KIAA0319 gene are selected from rs12193738, rs2817198, rs10456309, or any combination thereof.

57. The microarray of claim 56, further comprising one or more nucleic acid fragments on the support that comprise sequences that are complementary to genomic sequences that flank one or more markers associated with susceptibility for developing LI in humans, wherein the one or more markers are selected from rs985080, rs1554690, rs2533096, rs6951437, rs344470, rs344468, rs482700, rs7695228, rs1940309, rs505277, rs476739, rs867036, rs867035, rs2071674, rs7694946, rs4823324, and markers for the following genes: NEK2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; and PGD.

58. The microarray of claim 52-54, wherein the LD is reading disability (RD) in humans, wherein the one or markers in a DCDC2 gene are selected from CGCGAG and READ1 allele 5 (SEQ ID NO:35), or both; and the one or more markers in KIAA0319 is rs10456309.

59. The array (microarray) of claim 56, further comprising one or more nucleic acid fragments on the support that comprise sequences that are complementary to genomic sequences that flank one or more markers associated with susceptibility for developing RD in humans, wherein the one or more markers are selected from rs180950, rs2590673, rs892100, rs1792745, rs12546767, rs12634033, rs892270, rs10887149, rs10041417, rs6792971, rs4725745, rs12444778, rs1444186, rs2294691, rs10456309, rs1562422, and at least one marker for each of the following genes: MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

60. The microarray of claim 52-54, further comprising one or more nucleic acid fragments spotted on the support that comprise sequences that are complementary to genomic sequences that flank one or more markers associated with susceptibility for developing a LD in humans, wherein the one or more markers are selected from rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324; rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rs10887149; rs10041417; rs6792971; rs12636438; rs1679255; rs9521789; rs476739; rs505277; rs482700; rs7695228; rs867036; rs867035; rs1940309; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs4725745; rs12444778; rs1444186; rs2294691; rs10456309; rs1562422; rs807694; rs3756814; rs3777663; rs9295626; rs7763790; rs6935076; rs9348646; rs2328791; rs2328791; rs2817201, rs9295626; rs4576240; rs17307478, rs9356939, rs7763790, rs6456621; rs6456624, rs6935076, rs2038137, rs3756821, rs1883593, rs3212236; rs3777663, rs3756814, rs6931809, rs6916186, rs6933328, rs17491647; rs2328791; rs33914824a; rs807694a; rs707864a; rs10456301a; rs16889066a; rs9379651a; rs2817201; rs9295626; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs3756821; rs1883593; rs3212236; rs2294691; rs3777663; rs3756814; rs6931809; rs6916186; rs6933328; rs17491647; rs9348646; rs1562422 and markers of the following genes:R5H2; OR5H6; RRAGA; OR6B3; UMOD; A26C1A; FAM29A; CHRNA1; IFIT5; LOC643905; K2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; PGD; MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPIF.

61. A method of treating an individual for a learning disability (LD) comprising inhibiting ETV6 in the individual.

62. The method of claim 61, wherein the individual has RD, LI, or both.

63. The method of any one of claims 49-51, further comprising treating an individual identified as having or susceptible for developing a learning disability (LD), wherein treating comprises providing interventions, including services and materials, including but not limited to: using special teaching techniques; making classroom modifications, such as providing extra time to complete tasks and taped tests to permit the individual to hear, rather than read the tests; using books on tape; using word-processing programs with spell-check features; helping the individual

learn through multisensory experiences; teaching coping tools; and providing services to strengthen the individual's ability to recognize and pronounce words.

64. The method of any one of claims 43-48, further comprising monitoring the individual identified as at risk for developing a learning disability to assess whether development of a learning disability occurs and, if development occurs, treating the individual, wherein treating comprises providing intervention, including services and materials, including but not limited to: using special teaching techniques; making classroom modifications, such as providing extra time to complete tasks and taped tests to permit the individual to hear, rather than read the tests; using books on tape; using word-processing programs with spell-check features; helping the individual learn through multisensory experiences; teaching coping tools; and providing services to strengthen the individual's ability to recognize and pronounce words.

FIG. 1

A.

Unit #	Repeat unit 1	Repeat unit 2	Repeat unit 3	Repeat unit 4	Constant Region	Repeat unit 5	Constant Region
Sequence	GAGAGGAAGGAAA	GGAA	GAAA	GGAA	GGAAAGAATGAA	GGAA	GGGA
Number Present	1-2	4-10	0-2	0-2	Constant	3-4	Constant

B.

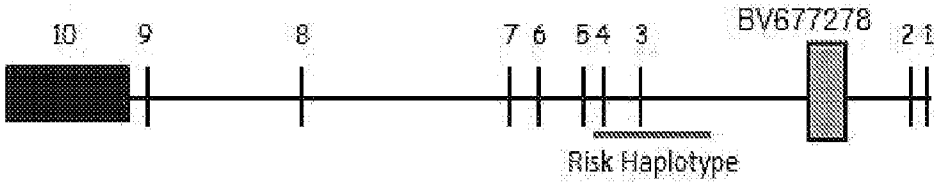
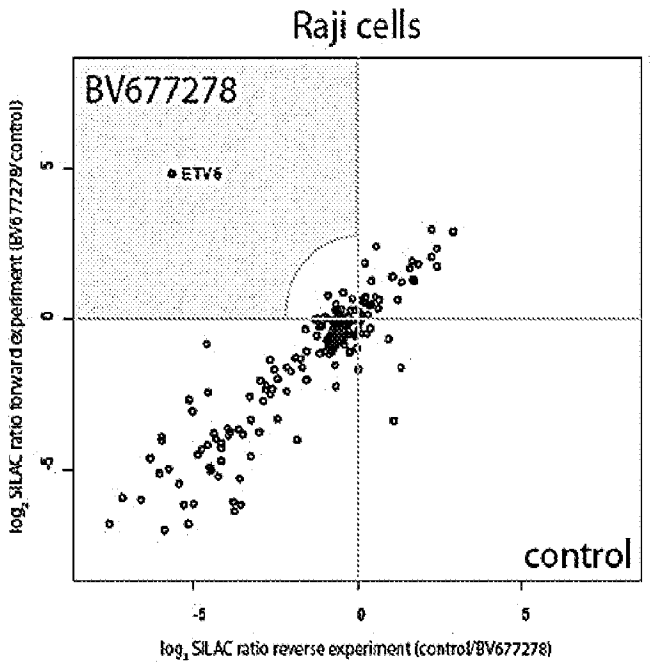


FIG. 2

A.


Raji cells
BV677278
ETV6
control
log2 SILAC ratio forward experiment (BV677278/control)
log2 SILAC ratio reverse experiment (control/BV677278)
5
0
-5

B.

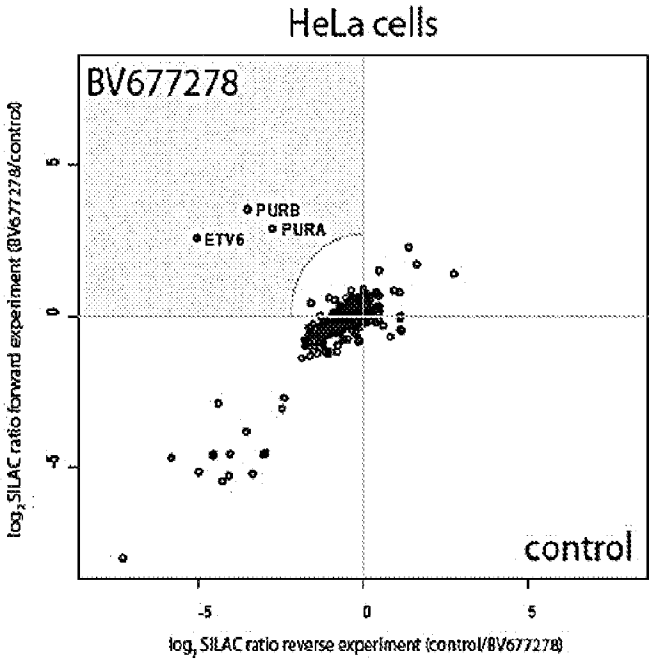

HeLa cells
BV677278
PURB
PURA
ETV6
control
log2 SILAC ratio forward experiment (BV677278/control)
log2 SILAC ratio reverse experiment (control/BV677278)
5
0
-5

FIG. 2 Cont.

C.

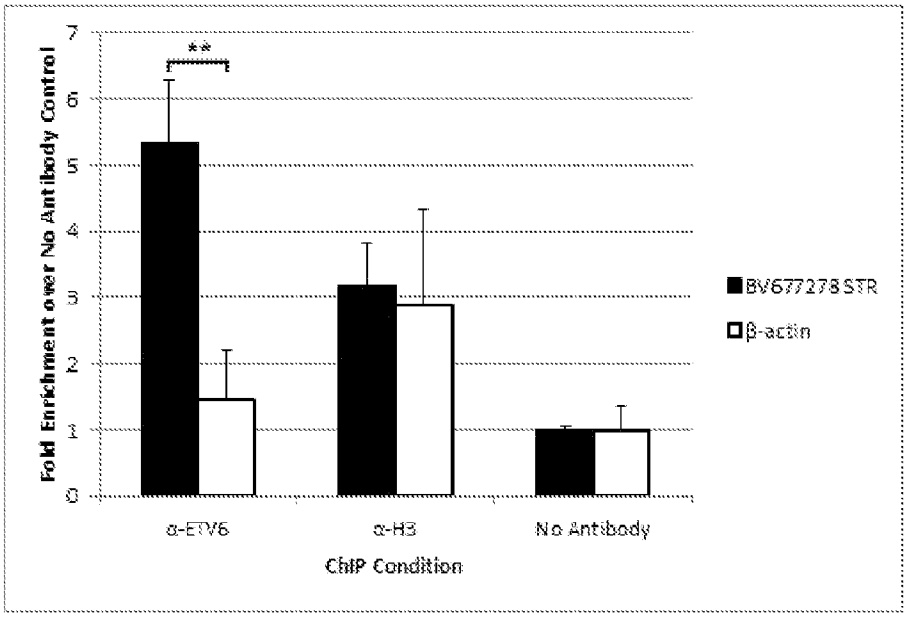
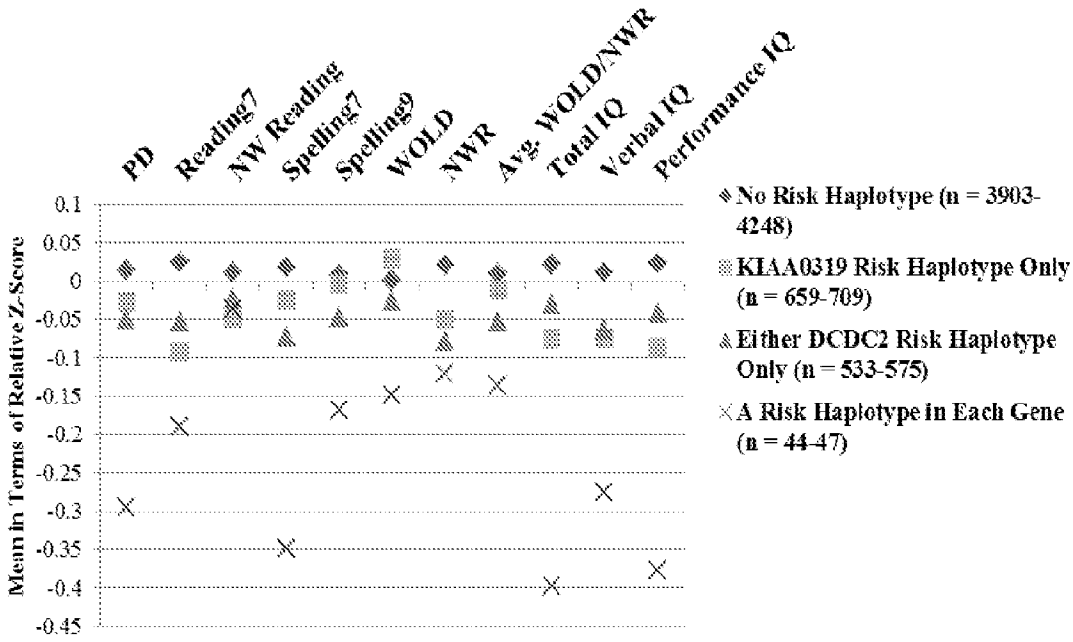

Fold Enrichment over No Antibody Control
7
6
5
4
3
2
1
0
**
α-ETV6
α-H3
No Antibody
ChIP Condition
BV677278STR
β-actin

FIG. 3

A.


PD
Reading7
NW Reading
Spelling7
Spelling9
WOLD
NWR
Avg. WOLD/NWR
Total IQ
Verbal IQ
Performance IQ
Mean in Terms of Relative Z-Score
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
-0.4
-0.45
No Risk Haplotype (n = 3903-4248)
KIAA0319 Risk Haplotype Only (n = 659-709)
Either DCDC2 Risk Haplotype Only (n = 533-575)
A Risk Haplotype in Each Gene (n = 44-47)

B.

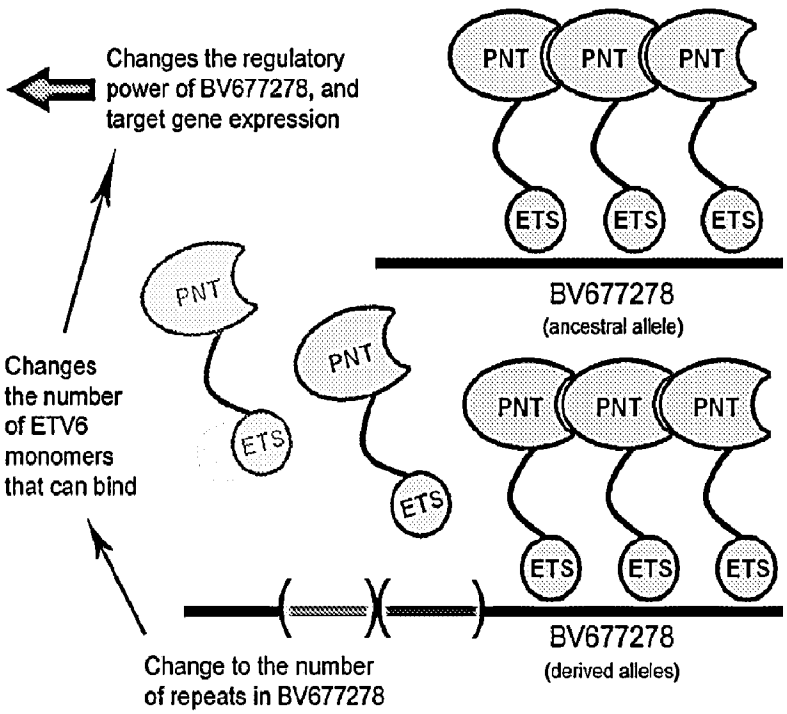

Changes the regulatory power of BV677278, and target gene expression
Changes the number of ETV6 monomers that can bind
Change to the number of repeats in BV677278
PNT
ETS
BV677278
(ancestral allele)
BV677278
(derived alleles)

FIG. 4

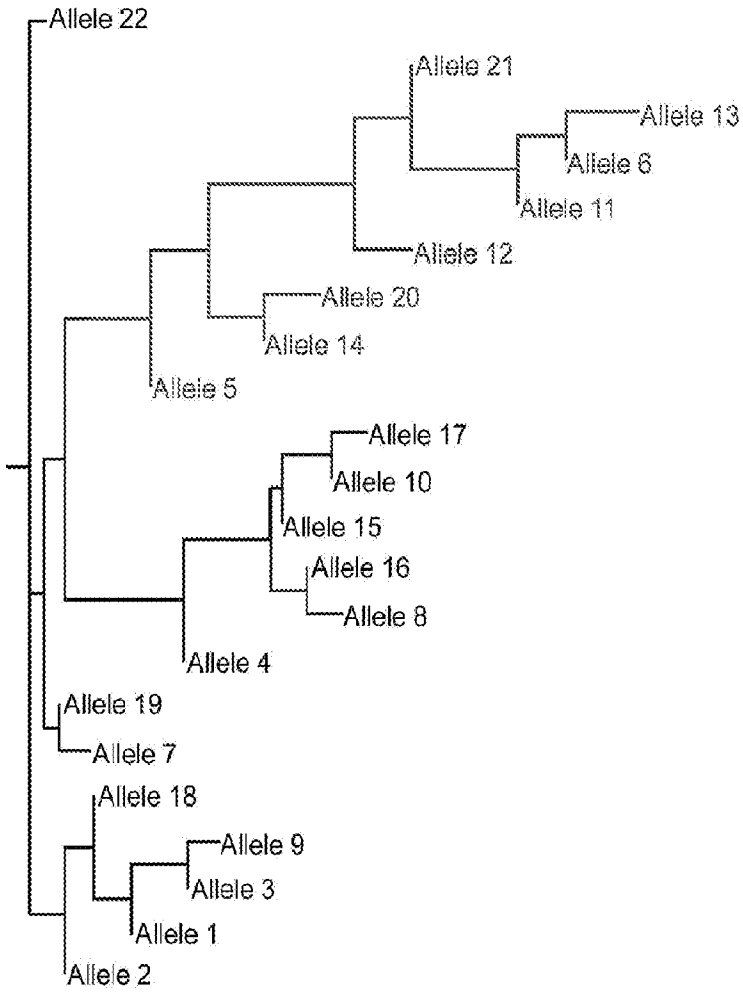
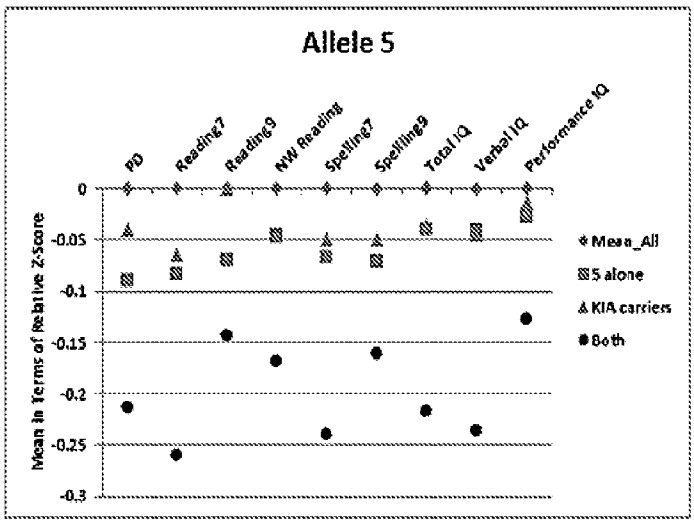

Allele 22
Allele 21
Allele 13
Allele 6
Allele 11
Allele 12
Allele 20
Allele 14
Allele 5
Allele 17
Allele 10
Allele 15
Allele 16
Allele 8
Allele 4
Allele 19
Allele 7
Allele 18
Allele 9
Allele 3
Allele 1
Allele 2

FIG. 5

A.


Allele 5
PD
Reading7
Reading9
NW Reading
Spelling7
Spelling9
Total IQ
Verbal IQ
Performance IQ
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
Mean in Terms of Relative Z-Score
Mean_All
5 alone
KIA carriers
Both

B.

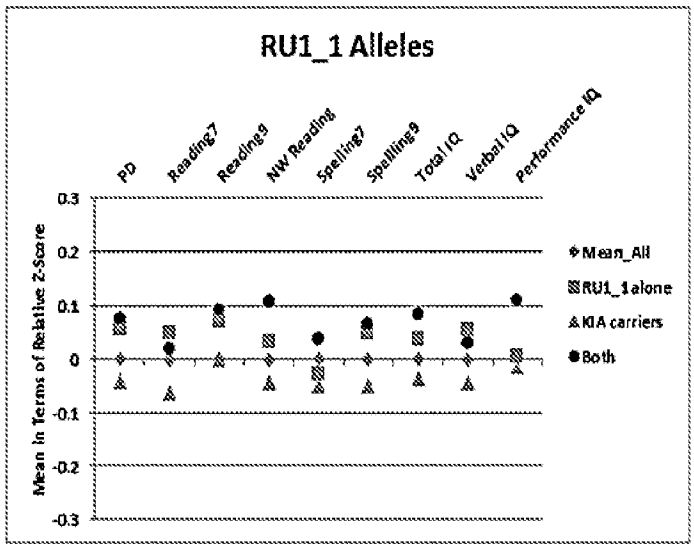
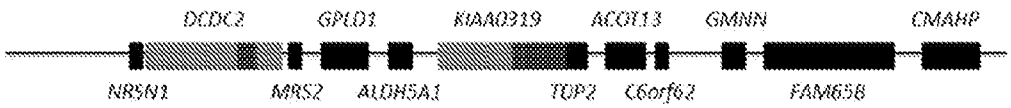
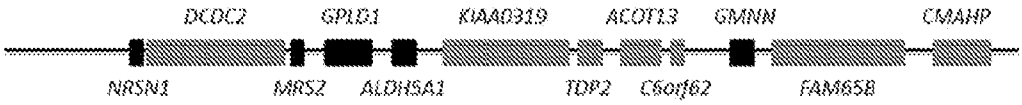

RU1_1 Alleles
PD
Reading7
Reading9
NW Reading
Spelling7
Spelling9
Total IQ
Verbal IQ
Performance IQ
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
Mean in Terms of Relative Z-Score
Mean_All
RU1_1 alone
KIA carriers
Both

FIG. 6

A.


DCDC2
GPLD1
KIAA0319
ACOT13
GMNN
CMAHP
NR5N1
MRS2
ALDH5A1
TDP2
C6orf62
FAM65B

B.


DCDC2
GPLD1
KIAA0319
ACOT13
GMNN
CMAHP
NR5N1
MRS2
ALDH5A1
TDP2
C6orf62
FAM65B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/069015

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/032021 A2 (UNIV YALE [US]; GRUEN JEFFREY R [US]; MENG HAIYING [US]) 23 March 2006 (2006-03-23)	34-36, 38,39, 43-45, 47,48, 52,53
Y	claims 1, 31, 34; example 2; table S1	49-51, 54,63,64

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2013	20/02/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Santagati, Fabio

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	D HAROLD ET AL: "Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia", MOLECULAR PSYCHIATRY, vol. 11, no. 12, 10 October 2006 (2006-10-10), pages 1085-1091, XP055093117, ISSN: 1359-4184, DOI: 10.1038/sj.mp.4001904 cited in the application page 1088, right-hand column, last paragraph; table 2	18,19, 23,24, 49-51, 54,63,64
Y	TOM S SCERRI ET AL: "DCDC2, KIAA0319 and CMIP Are Associated with Reading-Related Traits", BIOLOGICAL PSYCHIATRY, ELSEVIER SCIENCE, NEW YORK, NY; US, vol. 70, no. 3, 5 February 2011 (2011-02-05), pages 237-245, XP028379153, ISSN: 0006-3223, DOI: 10.1016/J.BIOPSYCH.2011.02.005 [retrieved on 2011-02-12]	18,19, 23,24
A	tables 3-5	1-12, 25-39, 43-59, 63,64
X,P	NATALIE R. POWERS ET AL: "Alleles of a Polymorphic ETV6 Binding Site in DCDC2 Confer Risk of Reading and Language Impairment", THE AMERICAN JOURNAL OF HUMAN GENETICS, vol. 93, no. 1, 1 July 2013 (2013-07-01), pages 19-28, XP055092702, ISSN: 0002-9297, DOI: 10.1016/j.ajhg.2013.05.008 the whole document	1-12, 18-21, 23-39, 43-59, 63,64
A	NATALIE COPE ET AL: "Variants in the DYX2 locus are associated with altered brain activation in reading-related brain regions in subjects with reading disability", NEUROIMAGE, vol. 63, no. 1, 1 October 2012 (2012-10-01), pages 148-156, XP055092703, ISSN: 1053-8119, DOI: 10.1016/j.neuroimage.2012.06.037 figure 1; table 3	1-12, 18-21, 23-39, 43-59, 63,64

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/069015

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LUCIANO ET AL: "A Haplotype Spanning KIAA0319 and TTRAP Is Associated with Normal Variation in Reading and Spelling Ability", BIOLOGICAL PSYCHIATRY, ELSEVIER SCIENCE, NEW YORK, NY; US, vol. 62, no. 7, 11 September 2007 (2007-09-11), pages 811-817, XP022242432, ISSN: 0006-3223, DOI: 10.1016/J.BIOPSYCH.2007.03.007 table 3	1-12, 18-21, 23-39, 43-59, 63,64
A	PENELOPE A LIND ET AL: "Dyslexia and DCDC2: normal variation in reading and spelling is associated with DCDC2 polymorphisms in an Australian population sample", EUROPEAN JOURNAL OF HUMAN GENETICS, vol. 18, no. 6, 1 June 2010 (2010-06-01), pages 668-673, XP055092969, ISSN: 1018-4813, DOI: 10.1038/ejhg.2009.237 figure 1; table 2	1-12, 18-21, 23-39, 43-59, 63,64

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/069015

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-12, 18-21, 23-39, 43-59, 63, 64(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12, 18-21, 23-39, 43-59, 63, 64(all partially)

Methods of determining if a sample obtained from an individual comprises nucleic acid which comprises a haplotype associated with susceptibility for developing a learning disability (LD), reading disability (RD), language impairment (LI) or comorbid reading disability and language impairment in humans, comprising assaying a sample that comprises nucleic acid from the individual for the presence in the DYX2 locus of at least one of the following markers: CGCGAG in a DCDC2 gene; CACGAG in a DCDC2 gene; both CGCGAG and CACGAG in a DCDC2 gene; and any combination comprising CGCGAG; CACGAG; or both CGCGAG and CACGAG. An array (microarray) comprising a support having a plurality of discrete regions, each discrete region having at least one (one or more) nucleic acid fragment spotted thereon, wherein each region comprises sequences that are complementary to nucleic acids (DNA, RNA, genomic DNA) that encompass (contain within) a (at least one) marker (e.g., snp) associated with susceptibility for developing a learning disability (LD) in humans, wherein the at least one marker is in a variant DCDC2 gene, wherein the one or more markers in a DCDC2 gene are selected from CGCGAG and CACGAG.

2-4. claims: 1-12, 18-24, 31-39, 43-59, 63, 64(all partially)

Methods of determining if a sample obtained from an individual comprises nucleic acid which comprises a haplotype associated with susceptibility for developing a learning disability (LD) or comorbid reading disability (RD) and language impairment (LI) in humans, comprising assaying a sample that comprises nucleic acid from the individual for the presence in the DYX2 locus of at least rs4504469, rs2038137 or rs2143340 in a KIAA0319 gene; and any combination comprising rs4504469, rs2038137 and rs2143340 in a KIAA0319 gene. An array (microarray) comprising a support having a plurality of discrete regions, each discrete region having at least one (one or more) nucleic acid fragment spotted thereon, wherein each region comprises sequences that are complementary to nucleic acids (DNA, RNA, genomic DNA) that encompass (contain within) a (at least one) marker (e.g., snp) associated with susceptibility for developing a learning disability (LD) in humans, wherein the at least one marker is in a variant KIAA0319 gene, wherein the one marker in a KIAA0319 gene is rs4504469, rs2038137 or rs2143340; wherein each invention corresponds to one marker.

5-6. claims: 13-19, 21, 31-33, 52-59(all partially)

A method of detennining if a sample obtained from an

individual comprises nucleic acid which comprises an allele associated with susceptibility for developing a learning disability (LD) in humans, comprising: assaying a sample that comprises nucleic acid from the individual for the presence of allele 5 or allele 6 of DCDC2 gene in the DYX2 locus. An array (microarray) comprising a support having a plurality of discrete regions, each discrete region having at least one (one or more) nucleic acid fragment spotted thereon, wherein each region comprises sequences that are complementary to nucleic acids (DNA, RNA, genomic DNA) that encompass (contain within) a (at least one) marker (e.g., snp) associated with susceptibility for developing a learning disability (LD) in humans, wherein the at least one marker is in a variant DCDC2 gene, wherein the one marker in a DCDC2 gene is allele 5 or allele 6.

7-55. claims: 18, 19, 22-24, 52-54, 60(all partially)

A method of determining if a sample obtained from an individual comprises at least one marker associated with comorbid reading disability (RD) and language impairment (LI) in humans, comprising: (a) obtaining a sample that contains nucleic acid from the individual and (b) assaying the sample for (i) at least one marker in DCDC2 gene in the DYX2 locus for a haplotype associated with susceptibility for developing RD in humans and (ii) at least one marker in KIAA0319 gene in the DXY2 locus for a haplotype associated with susceptibility for developing LI in humans wherein the at least one marker selected from rs12636438; rs1679255; rs9521789; rs1983931; rs9814232; rs7995158; rs6573225; rs4082518; rs442555; rs259521; rs16889556; rs1047782; rs1530680; rs12667130; rs6965855; rs985080; rs4726782; rs1718101; rs10487689; rs1918296; rs737533; rs9295626; rs7763790 rs6935076; rs2817201; rs10456309; rs4576240; rs17307478; rs9356939; rs7763790; rs6456621; rs6456624; rs6935076; rs2038137; rs3756821; rs1883593; rs3212236; rs6456621; rs12193738; rs2817198; rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; and rs807694. (48 SNPs); wherein each invention corresponds to one marker.

56-82. claims: 25-27, 40-42, 52-54, 60(all partially)

A method of determining if a sample obtained from an individual comprises a marker associated with language impairment (LI) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: rs793845; rs2799373; rs793862; rs793834; rs2792682; rs807704; rs707864; rs12193738; rs2817198; rs10456309; rs985080; rs1554690; rs2533096; rs6951437; rs344470; rs344468; rs807694; rs482700; rs7695228; rs1940309; rs505277; rs476739; rs867036; rs867035; rs2071674; rs7694946; rs4823324 (27 SNPs); and a marker for

at least one of the following genes: NEK2; DLEC1; NARS; IL4I1; PKD2; ATF5; NUP62; SIGLEC11; ACAN; and PGD, wherein each invention corresponds to one marker.

83-98. claims: 28-30, 40-42, 52-54, 60(all partially)

A method of determining if a sample obtained from an individual comprises a marker associated with reading disability (RD) in humans, comprising assaying a sample obtained from the individual for at least one of the following markers: rs180950; rs2590673; rs892100; rs1792745; rs12546767; rs12634033; rs892270; rsl0887149; rsl0041417; rs6792971; rs4725745; rs12444778; rs1444186; rs2294691; rs10456309; rs1562422 (16 SNPs); and a marker for at least one of the following genes: MAP4; OR2L8; CRYBA4; OR2T8; KIAA1622; OR2AK2; DHX30; GEMIN6; C20orf10; and PPTF; wherein each invention corresponds to one marker.

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99. claims: 61, 62

A method of treating an individual for a learning disability (LD) comprising inhibiting ETV6 in the individual.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/069015

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006032021 A2	23-03-2006	US 2008318217 A1	25-12-2008
		WO 2006032021 A2	23-03-2006