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LRRTM1 on chromosome 2p12 is a maternally suppressed gene that is associated paternally with handedness and schizophrenia

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Abstract

Left–right asymmetrical brain function underlies much of human cognition, behavior and emotion. Abnormalities of cerebral asymmetry are associated with schizophrenia and other neuropsychiatric disorders. The molecular, developmental and evolutionary origins of human brain asymmetry are unknown. We found significant association of a haplotype upstream of the gene LRRTM1 (Leucine-rich repeat transmembrane neuronal 1) with a quantitative measure of human handedness in a set of dyslexic siblings, when the haplotype was inherited paternally ($P = 0.00002$). While we

were unable to find this effect in an epidemiological set of twin-based sibships, we did find that the same haplotype is overtransmitted paternally to individuals with schizophrenia/schizoaffective disorder in a study of 1002 affected families ($P = 0.0014$). We then found direct confirmatory evidence that LRRTM1 is an imprinted gene in humans that shows a variable pattern of maternal downregulation. We also showed that LRRTM1 is expressed during the development of specific forebrain structures, and thus could influence neuronal differentiation and connectivity. This is the first potential genetic influence on human handedness to be identified, and the first putative genetic effect on variability in human brain asymmetry. LRRTM1 is a candidate gene for involvement in several common neurodevelopmental disorders, and may have played a role in human cognitive and behavioral evolution.

Keywords

handedness; schizophrenia; association; imprinted gene; brain asymmetry

Introduction

Left–right asymmetrical function is a conserved feature of vertebrate central nervous systems.[1] Asymmetrical brain function is pronounced in humans, and underlies much of our behavior, cognition and emotion.[2] Approximately 90% of humans are right-handed.[2] This is the strongest population-level bias in handedness for any primate.[1,2] Left-handedness in humans is associated with reductions or reversals of normal brain asymmetries,[3] particularly of cerebral cortical areas related to language perception and production. This association is likely to be partly genetic in etiology.[4] Handedness and complex cognition in humans may, therefore, be related developmentally and evolutionarily, although the nature and extent of these relationships remain uncertain.

We performed previously the first genome-wide linkage scan for a quantitative measure of human handedness[5] (relative hand skill; assessed with timed peg moving[5]), and found linkage on 2p12–q11.[5] This analysis was performed in 222 reading-disabled siblings,[6] although there was no relationship detectable between reading disability (RD) and relative hand skill in this sample.[5,7] We then replicated the 2p12–q11 linkage,[8] with the same measure of relative hand skill, in 105 pairs of left-handed brothers ($P = 0.0009$).[8] We found that the linkage to relative hand skill, in the RD sibs, was derived entirely from paternal inheritance of 2p12–q11 ($P = 0.0000037$),[9] whereas the maternally inherited locus was not linked.[9] This suggested that the underlying gene was imprinted, and inactivated or downregulated on the maternally inherited chromosome.[9] We did not have parental genotypes to assign parent-of-origin for analysis of the left-handed brother sample.[9] However, under standard linkage analysis, the peak location of linkage to relative hand skill in the left-handed brothers was the same as the peak paternal-specific location in the dyslexic siblings,[9] and was within the gene CTNNA2 (α-N-catenin) on 2p12.

Schizophrenia is a heterogeneous neuropsychiatric disorder that affects roughly 1% of the adult human population. Schizophrenia is associated with reductions or reversals of normal cerebral asymmetries, including the medial temporal lobe, superior temporal gyrus, planum temporale and the overall brain anterior–posterior torque.[10,11] Schizophrenia is also associated with a slightly elevated rate of mixed or left-handedness.[12,13] Crow drew attention to the association of abnormal brain asymmetry with schizophrenia-like psychoses,[14] and was drawn to a human-specific rearrangement of the sex chromosomes and a candidate gene there,[15] Protocadherin X-Y. However, this gene has not so far been shown to be linked to asymmetry, language or psychosis.

In a recent meta-analysis of 20 linkage screens for schizophrenia, 2p12–q22 was the only location to reach significance when adjusted for genome-wide testing.[16] This linkage was derived primarily, but not exclusively, from a sample of affected sibling pairs of European descent that were recruited in New York and Oxford.[17] We showed previously that the 2p12–q11 linkage in this sample was derived predominantly from paternal sharing (log of the odds, LOD = 4.72), not maternal sharing (LOD = 0.6).[9] As parent-of-origin effects are unusual in the genome, we proposed in 2003 that a single paternally expressed gene was responsible for the linkages of 2p12–q11 to handedness and schizophrenia.[9]

In the present study we have used genetic association mapping and gene-functional analysis to identify a novel imprinted gene on 2p12, LRRTM1, that we propose is responsible for causing the linkages of this chromosomal region to human handedness and schizophrenia.

Materials and methods

All work involving human samples and materials was approved by the appropriate institutional review boards, and appropriate informed consent was obtained from all human subjects.

Details of the Materials and methods are given in Supplementary information online.

Results

We genotyped 87 single-nucleotide polymorphisms (SNPs) in 222 RD siblings and their parents, within a region of paternal-specific linkage to relative hand skill that we had detected previously in this sample (Figure 1). The SNPs were targeted within four positional and functional candidate genes on 2p12–p11 (LRRTM4, CTNNA2, LRRTM1 and DNAH6) (Figure 1 and Table S1). Following our parent-of-origin linkage data (Figure 1), we tested for quantitative association of paternally inherited SNP alleles with relative hand skill (using QTDT[18]). Four SNPs in distinct locations (rs1517771, rs290015, rs2063436 and rs723524; Table S1), which were not in significant linkage disequilibrium (LD) with one another, showed nominally significant paternal-specific association with relative hand skill ($0.05 > P > 0.01$). This was no more than the expected false-positive rate.

We then aimed to interrogate the underlying haplotype structure by testing haplotypes for association, that were constructed in 'sliding windows' representing all sets of three consecutive SNPs (disregarding any instances where SNPs were not in significant LD, that is, $P > 0.05$ based on χ^2-test of pairwise LD). We used the total, paternal and multi-allelic options of QTDT together for the association tests. Significance levels for paternal-specific association were all $P > 0.05$, except for haplotypes derived from the SNPs rs1446109-rs1007371-rs723524 ($P = 0.00002$), and the overlapping window rs1007371-rs723524-rs1025947 ($P = 0.0007$). These SNPs are on 2p12 within a region of strong intermarker LD that spans at least the first exon and 137 kb upstream of the gene LRRTM1, which is located within an intron of CTNNA2 (Figure 1). There was no significant maternal-specific haplotype association at any location (all $P > 0.05$).

The rs1446109-rs1007371-rs723524 haplotype 2-2-2 (where 2 is the minor allele of each SNP) was responsible primarily for the paternal-specific association observed with haplotypes derived from these three SNPs. The 2-2-2 haplotype had 9% frequency, and was associated with a mean shift of 1.1 s.d. toward left-handedness in the relative hand skill distribution[5,7] when inherited paternally, compared to all other haplotypes. Genotyping of 32 more SNPs, including 12 within 137 kb upstream of LRRTM1, confirmed that rs1446109-rs1007371-rs723524 haplotype 2-2-2 represents a distinct haplotype clade for at

least 76 kb upstream of, and including, the predicted promoter of LRRTM1 (Figure S1 and Table S1).

We screened the first two exons and predicted promoter of LRRTM1 for polymorphisms in 26 left-handers from the RD sample by denaturing high-performance liquid chromatography and sequencing, but we did not detect any polymorphisms that tagged rs1446109-rs1007371-rs723524 haplotype 2-2-2, or that had overt disruptive effects on the predicted LRRTM1 protein (entirely coded within exon 2). The only novel SNP that we identified was upstream of the first LRRTM1 exon (Table S1).

We analyzed the SNPs rs1007371 and rs723524, which together can be used to construct a close proxy to the 'risk' haplotype described above (see Figure S1), in a sample of normal twin-based sibships from Brisbane, Australia, that was derived from 215 independent families. We found no significant evidence for paternal association of the risk haplotype with our quantitative measure of human handedness ($P > 0.1$).

We genotyped the SNPs rs1446109 and either rs723524 or rs718466 (the latter are equivalent tagging SNPs according to international HapMap data) in four family samples of white European descent that included individuals with schizophrenia or poor-outcome schizoaffective disorder. These comprised a subset of the New York/Oxford sample[17] (226 families), an Irish 'High Density' sample[19] (236 families), a sample collected in Montreal[20] (124 families) and an Afrikaner sample[21] (416 families) (Table 1). The 2-2 haplotype defined by the rare alleles of these SNPs is equivalent to the risk haplotype for left-handedness defined above (Figure S1). The 2-2 haplotype varied in frequency between 7.6 and 12.1% in the four sample sets. We found that haplotype 2-2 was overtransmitted paternally to affected individuals in a combined analysis of the four samples, $P = 0.0014$ (one-tailed test, 38 transmissions to 16 nontransmissions; Table 1). This was a specific hypothesis test that requires no statistical adjustment. There was no significant paternal overtransmission of any other haplotype, nor was there maternal overtransmission of any haplotype. The paternal 2-2 result was derived roughly equally from three of the four samples (Table 1).

We genotyped rs1446109 (almost tagging for the risk haplotype; Figure S1), in two case–control collections of European descent (461 cases and 459 controls from Munich, Germany, and 429 cases and 428 controls from Scotland (all cases had *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition diagnoses of schizophrenia, and both sample sets were recruited according to the same protocol)).[22] As the parental origin of the alleles could not be established and the paternal and maternal alleles were confounded, we expected this analysis to be low-powered to detect an imprinted effect. Nonetheless, this SNP showed a trend toward association ($P = 0.09$) when tested under a genotypic 2-df logistic regression model using covariates for gender and the collection site (Munich/Aberdeen). The additive component of this model was significant at $P = 0.036$, and the direction of allelic effect was the same as in the family samples. When we repeated the analysis using only those cases (151) who reported a positive family history of schizophrenia or bipolar disorder, with the aim of removing sporadic environmental cases, rs1446109 showed significant association with $P = 0.013$, and the additive component of the model showed $P = 0.0038$, again in the expected direction. We also tested for association in a sample of 270 Han Chinese families[23] but we found no significant bias in paternal or maternal transmission of any haplotype to schizophrenic people (there were 65 paternal transmissions to 78 paternal nontransmissions of the 2-2 haplotype).

No imprinted genes were known previously on chromosome 2p. We found that human LRRTM1 is imprinted (paternal-only expression) in hybrid A9 cells[24] (mouse cell lines

containing single human chromosomes of known parental origin) (Figure 2). Despite certain limitations with hybrid cells in the context of imprinting studies,[25] mouse A9 cells have been used for the reliable identification and verification of human imprinted genes.[24] To validate the chromosome 2 hybrid cells, we analyzed the expression of four additional biallelically expressed genes, GGCX (2p11.2), BCL2L11 (2q13), TCF7L1 (2p11.2) and RALB (2q14.2). We detected the expression of all four genes in chromosome 2 hybrid cells, regardless of the parental origin of the human chromosome 2 (Figure S2). No detection of these genes was obtained in RT- controls. We also used transcribed polymorphisms to show mono-allelic LRRTM1 expression in tissue samples from a minority of unrelated postmortem human brains (3 out of 18) by RT-PCR and sequencing or restriction digestion (not shown). However, biallelic expression was found in 15 out of 18 brains, and LRRTM1 was expressed at similar levels from both alleles in the adult human cerebral cortex of five individuals showing biallelic expression (analyzed by quantitative PCR). All brain tissue was obtained from normal controls that were unaffected by major neurological or psychiatric disease. These results suggest that imprinting of LRRTM1 is either variable between individuals (as for the imprinted genes IMPT1 or IGF2[26]), and/or variable between different brain regions or cell/tissue types (as for UBE3A[27]). We also found mono-allelic paternal expression of LRRTM1 in four out of four unrelated EBV-transformed human lymphoblastoid cell lines that were heterozygous for a transcribed SNP (Figure 2).

By use of *in situ* hybridization in the mouse, we found that Lrrtm1 is expressed predominantly in the nervous system by postmitotic neurons, but also in some nonneuronal tissues (Figure 3; Figure S3). Expression is upregulated in the mouse brain during embryonic development and early postnatally. In adult brain, Lrrtm1 expression is most prominent in the forebrain, particularly in the thalamus (in most or all nuclei), and in cortical areas including hippocampus, piriform and posterior cingulate (Figure 3; Figure S3).

In northern blot analysis of adult human brain, LRRTM1 also showed predominant expression in forebrain regions including thalamus and cerebral cortex (Figure S4). By *in situ* hybridization in coronal sections of the post-mortem developing human brain (14–16 weeks gestation; Figure 4), strong expression was observed in anterior sections throughout the cortical plate and in septum, caudate and putamen. The absence of signal in the subventricular zone argues against a direct involvement in neurogenesis (Figure 4). Transcript distribution was similar in more caudal sections with the addition of signal in dorsolateral thalamus (Figure 4). More caudal still, thalamic signal shifted ventrally to a structure consistent with the lateral geniculate body (Figure 4). The striking signal in caudate and putamen in human (Figure 4) was not present in mouse, at least not at E15 and in adult. In addition, expression within the human thalamus was more restricted as compared to the mouse, with staining relatively limited to dorsomedial regions.

No consistent asymmetric expression was observed in any of three human developing brains examined by *in situ* hybridization (14–16 weeks' gestation), regardless of whether cerebral hemispheres were analyzed in aggregate or cortical sub-regions were examined in isolation (dorsolateral, temporal, ventrolateral or cingulated). Similarly to embryonic brain, LRRTM1 was expressed at similar levels (that is, symmetrically) in all analyzed regions of left and right adult human cerebral cortex (several different cortical regions from five individuals were analyzed by quantitative PCR). We also quantified left- and right-brain Lrrtm1 mRNA expression levels in rats and embryonic mice (see Materials and methods), but did not detect evidence for asymmetrical expression in rodents. In addition, we tested cerebral cortex, cerebellum, brain stem, olfactory bulb, thymus, heart, lung, liver, intestine, pancreas, spleen, kidney, muscle and testis of two reciprocal crossed F1 mice between C57BL/6J and JF1 strains for allele-specific expression. Each of the F1 mice was 30 weeks old. Expression of

Lrrtm1 was detected in cerebral cortex, cerebellum and brain stem, but it was biallelic (Figure S5).

In rodent primary sensory (DRG) and cortical neurons (Figure 3), and in cerebellar granular neurons (data not shown), overexpressed LRRTM1 localized to the cell soma, neurites and lamellipodia of growth cones, suggesting a function in axon guidance and/or synaptogenesis. Unexpectedly, in transfected MRC5, Cos-7 and Neuro-2a cells, LRRTM1 colocalized with endoplasmic reticulum (ER) markers (Figure 5 and Figure S6). Live-cell staining for overexpressed LRRTM1 in DRG neurons revealed that the protein is not accessible on the plasma membrane under conditions that allowed surface detection of a related member of the LRR protein superfamily, Lingo1 (Figure 5). These results suggest that endogenous LRRTM1 may have a role in intracellular trafficking within axons. However, it remains possible that LRRTM1 is localized to plasma membrane in cells expressing an unidentified LRRTM1 chaperone or coreceptor protein that promotes its processing and/or transport.

We analyzed methylation within 2 CpG islands that correspond to the predicted promoter and coding exon of LRRTM1, and a third island roughly 18 kb upstream of LRRTM1, in 17 lymphoblastoid cell lines and 17 human post-mortem brain samples, but we did not find evidence that these CpG islands are differentially methylated regions (DMRs) (data not shown).

We tested CTNNA2 for mono-allelic expression in 10 post-mortem brain samples from normal, unrelated individuals, by use of a transcribed SNP in exon 12, but expression was always biallelic, in contrast to LRRTM1 (data not shown). We did not detect CTNNA2 expression in the A9 hybrids, or in human lymphoblastoid cell lines, by RT-PCR. The first CTNNA2 exon is over 750 kb from LRRTM1 (Figure 1), and several LD blocks away, further suggesting that the regulation of these genes is distinct. There are no other genes within 1.13 Mb of LRRTM1, and the next closest gene expressed in the forebrain (expression data available via the UCSC genome browser) is over 2.75 Mb distally (LRRTM4; Figure 1).

In order to analyze the evolution of LRRTM1, we compared it between humans and chimpanzees. There is no fixed amino acid difference between human LRRTM1 and chimpanzee LRRTM1, and it is not among the genes that are differently expressed between adult humans and adult chimpanzees in brain, liver, kidney, heart or testis.[28] Furthermore, biallelic expression of LRRTM1 was detected in two out of two chimpanzee brain samples (see Materials and methods).

Discussion

Crow proposed that handedness, brain asymmetry and schizophrenia share an underlying genetic relationship.[14] However, no genes have previously been shown to associate across these traits. We carried out SNP-based association screening for human relative hand skill, under a parent-of-origin model, within four candidate genes close to or within 6 mb of a peak of paternal-specific linkage on 2p12, in a sample of sib pairs recruited for the presence of RD. We found strong paternal-specific association with relative hand skill implicating the gene LRRTM1 (leucine-rich repeat transmembrane neuronal 1) and a region of 80 kb upstream of this gene (which is located within an intron of CTNNA2). We did not find this association in a sample of normal twin-based sibships from Australia. However, consistent with our parent-of-origin association data, we went on to show that LRRTM1 is downregulated on the maternally inherited chromosome, and is therefore a newly identified imprinted locus in the human genome. We also found that the same haplotype that drove the paternal association with handedness was overtransmitted paternally, but not maternally, to

schizophrenic/schizoaffective individuals in 1002 affected families of European descent (P = 0.0014). This was a specific hypothesis test that requires no statistical adjustment, and indicates that LRRTM1 is a novel susceptibility factor for schizophrenia. We also found consistent evidence for association in a large case–control sample of European descent that was collected in Scotland and Germany.

We did not find that the 'risk' 2-2 haplotype that we identified in European family-based samples was associated with schizophrenia in a Han Chinese family-based sample. The frequency of the 2-2 risk haplotype was somewhat higher in the Han Chinese, at 18%. The failure to replicate may nonetheless be related to modest power in the Chinese sample of 270 families. Alternatively, the nonreplication in the Chinese may indicate that the 2-2 haplotype does not carry risk variation in this population (it may also be relevant that native Chinese speakers show some morphological differences to English speakers in language-related areas of the brain[29]). A further study in a larger Chinese sample will be required to distinguish these possibilities, together with identification of the functional variants in European populations.

Of a total of approximately 35 000 human genes, only roughly 40 were previously known to be imprinted, and the total number is unlikely to exceed this greatly.[30-32] The finding that LRRTM1 is imprinted, and maternally suppressed, was a confirmation of the parent-of-origin genetic data that led to this locus, because the a *priori* probability that LRRTM1 would be imprinted was very low (roughly 1 in 1000). Our association data suggest that allelic variation upstream of LRRTM1, that may affect the gene's expression, is relevant functionally for brain asymmetry when inherited on the active paternal chromosome, but not on the relatively inactive maternal chromosome, at certain locations and time points during human brain development. However, the 'risk' haplotype that we identified at LRRTM1 cannot explain the majority of the paternal-specific linkage across the genomic region of 2p12 (Figure 1), and therefore additional genetic or epigenetic variability at LRRTM1, or neighboring genes, may also be responsible partly for the linkages of handedness and schizophrenia to 2p12.[9] Although we found evidence that LRRTM1 is variably imprinted in the post-mortem adult human brain (as is the imprinted gene 5HT2A),[33] we cannot conclude directly, on the basis of our data, that variability in imprinting of LRRTM1 is involved in individual differences in human handedness and schizophrenia liability. Our data indicate a role for the 2-2 haplotype in handedness variability and susceptibility to schizophrenia, in European populations, while the data on imprinting are consistent with the paternal-specific nature of this effect.

Our data suggest that a subtype of schizophrenia, linked to misregulation of human LRRTM1, may have its origins in fetal neurodevelopment. Since LRRTM1 appears to underlie the strongest linkage to schizophrenia in the genome, as identified by a meta-analysis of 20 genomewide linkage scans,[16] it is possible that LRRTM1 dysfunction causes a major, common subtype of schizophrenia. Assessing the frequency of this subtype will require studies in further clinical and epidemiological samples, together with a better definition of the functional genetic and epigenetic variation at the LRRTM1 locus. This information may have a substantial impact on pharmacogenetic studies and the development of new treatments for schizophrenia, by allowing patient heterogeneity to be accounted for in clinical trial studies. The receptor-like structure of LRRTM1 also suggests that it may be a drug-tractable target in its own right.

LRRTM1 is one of a four-member family of type I transmembrane proteins containing leucine-rich repeat (LRR) domains, which are commonly involved in protein–protein interactions (LRRs are present in the Slits and Nogo-receptor,[34,35] involved in axonal pathfinding). Each LRRTM member has a specific, dynamically regulated regional brain-

expression distribution.[35] Using several gene-functional approaches, we found that LRRTM1 is likely to play a role during the development of specific forebrain structures by influencing neuronal differentiation and connectivity, with a possible role in intracellular trafficking in axons. Thus, LRRTM1 is an ideal candidate gene for having an involvement in subtle developmental abnormalities of the central nervous system.

When comparing expression between human and mouse, it seems that there is a striking absence of signal in caudate and putamen in mouse, at least at e15 and in adult. Also, expression within the fetal human thalamus is more restricted as compared to the mouse, with staining relatively limited to dorsomedial regions that have been implicated in schizophrenia.[36,37] We found no evidence for overtly asymmetrical expression of Lrrtm1 in the developing or adult brain of rodents or humans, using *in situ* hybridization and/or quantitative PCR. However, we cannot rule out a subtle asymmetry of function or expression at some restricted time point during human brain development, as for the transcription factor LMO4.[38] It remains possible that LRRTM1 is important in the establishment, consolidation or elaboration of the left–right axis during human brain growth and development, particularly before 14 weeks' gestation, which was the earliest stage that we were able to analyze in human. (Population-level morphological asymmetries of the cerebral cortex are already noticeable shortly after this time, and are visible by ultrasound in normal fetuses at 20–22 weeks' gestation.[39]) Therefore, detailed studies of the roles of LRRTM1 and the pathways in which it functions in mammalian brain development are warranted, particularly at developmental time points earlier than 14 weeks in humans, that may reveal critical new insights into the establishment and/or maintenance of normal and abnormal human brain function and asymmetry.

Humans have the strongest population-level bias in handedness of any primate,[1,2] and LRRTM1 is a candidate for having had a role in the evolution of this trait. As the human and mouse predicted LRRTM1 proteins are 96% identical, and the human and chimpanzee proteins are 100% identical, any potential human-specific properties of LRRTM1 may involve the spatiotemporal control of its imprinted expression. (It is interesting that recent analysis of conserved noncoding elements demonstrated that a sequence element 130 kb downstream of LRRTM1 displays accelerated evolution in the human lineage, which may be important for the gene's regulation).[40] We detected only biallelic, nonimprinted expression of Lrrtm1 in cerebral cortex, cerebellum, and brain stem from two 30-week-old reciprocal crossed F1 mice between C57BL/6J and JF1 strains (data not shown), and only biallelic expression in the postmortem brains of two adult chimpanzees. Larger sample sizes will be needed to test whether imprinting of LRRTM1 is found only in humans. (There is a high level of discordance in imprinting between humans and mice;[32] the gene DLX5 is an example.[41]) It will also be interesting to compare the regulation of LRRTM1 longitudinally during the development of humans and other species, as the imprinted regulation may be restricted to certain developmental periods.[38] In addition, human genetic variation at this locus in nonclinical populations could be analyzed with dense marker genotyping, to test for evidence of selection, particularly with regard to the risk haplotype.

Genomic imprinting can arise when the optimal level of maternal resource investment in offspring differs between the two parental sexes, in polygamous mating systems.[31,42] Paternally inherited alleles of imprinted genes often sequester more maternal resources than do maternally inherited alleles, especially when influencing growth *in utero*.[31,42] The imprinting of LRRTM1 in humans, therefore, suggests an intriguing evolutionary scenario, in which the parental sexes have conflicting interests in relation to the outcome of lateralized brain development in their offspring, which underlies much of human cognition and behavior. Further study of the neural systems and behaviors that LRRTM1 influences will be required to understand the selective forces that drove LRRTM1 to be imprinted.

We did not detect evidence for differential methylation of the paternal and maternal alleles at the CpG islands associated directly with LRRTM1, and therefore the mechanism underlying imprinted expression of LRRTM1 remains unclear. However, many other imprinted genes are not regulated by differential methylation within their own promoters, but are regulated instead by imprinted control centers which may be located many kilobases distant.[31] Also, allele-specific histone modifications are emerging currently as equally important determinants of imprinted gene expression.[43] The lack of methylation does not, therefore, refute the imprinted expression of LRRTM1, which we observed in three different tissues and experimental settings. We recommend that the mechanism of imprinted regulation at this locus should be investigated as a priority, as epigenetic misregulation of LRRTM1 may have clinical relevance and could underlie much of the paternal linkage of 2p12 to schizophrenia.

We analyzed schizophrenia and schizoaffective disorder jointly in the New York/Oxford sample to maximize the numbers of informative paternal transmissions for the risk haplotype (Table 1). However, this sample was not sufficiently large to test for heterogeneity of the association effect between these two patient groups. Our previous linkage analysis[9] of 2p12 had indicated that the linkage was stronger for the subset of sibling pairs with schizophrenia only (paternal LOD 4.72), but this remains to be investigated more thoroughly by targeted association analysis in additional samples.

LRRTM1 is the first genetic influence on human handedness to be identified. Handedness, brain asymmetry and schizophrenia are likely to be etiologically complex traits with several, or many, genetic and environmental influences. As we detected an effect of LRRTM1 on handedness in dyslexic siblings, but not in normal twin-based sibships, it is possible that LRRTM1 influences behavioral lateralization more strongly in clinically selected populations for psychiatric/neurodevelopmental dysfunctions, than in the general population. This would suggest an interactive, nonadditive effect of LRRTM1 with other genetic and/or environmental effects that predispose to neuropsychiatric dysfunction, such that the effect of LRRTM1 depends on other risk factors in order to manifest fully. However, it is also possible that the failure to replicate the association with handedness was due to low power and a relatively small replication sample in our study. It is a well-described statistical feature of genetic association analysis that follow-up samples should usually be much larger than initial samples to provide adequate power, as the genetic effect in the initial sample is typically inflated.[44] Unfortunately, we are unaware of other family-based collections with accurate quantitative assessments of handedness, that are suitable for genetic studies.

In addition to schizophrenia, other neurodevelopmental disorders including bipolar disorder, autism and language impairment have shown evidence for associations with left-handedness and/or abnormal asymmetrical brain structure/function.[2,10,11,45-49] LRRTM1 is, therefore, also a candidate for involvement in these traits. The LRRTMs are a four-member gene family first described in 2003, and it is interesting to note that LRRTM3 has recently been proposed as a susceptibility factor for late-onset Alzheimer's disease.[50] We recommend, therefore, that the whole LRRTM gene family is investigated in relation to psychiatric and neurological disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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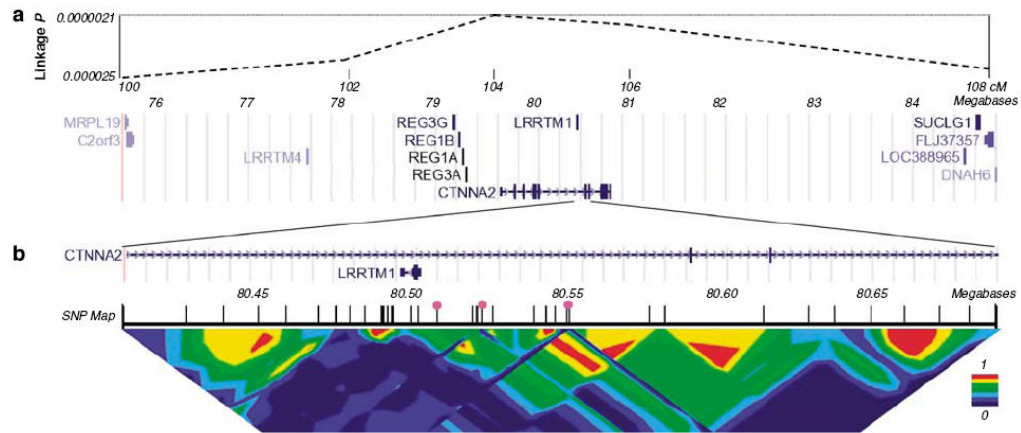
Figure 1.
Mapping of an imprinted quantitative-trait-locus (QTL) for human handedness. (**a**) Top: pointwise significance of linkage derived from paternal sharing across 8 cM of chromosome 2p12-p11.[9] This corresponds roughly to a 1-LOD unit support interval for the QTL. Significance of linkage is shown on a logarithmic scale. Bottom: known genes within the region (adapted from UCSC Genome Browser). Note LRRTM1 (transcribed proximal to distal (right to left)) sited within an intron of CTNNA2 (transcribed distal to proximal). (**b**) Close up of 286 kb around LRRTM1. Two exons of CTNNA2 are also visible. The final SNP map that we used in this region is shown (see also Table S1), and the three SNPs that showed the initial paternal-specific haplotype associations are highlighted by pink circles (rs1446109-rs1007371-rs723524). Pairwise intermarker LD (Cramer's V) is shown at the bottom.
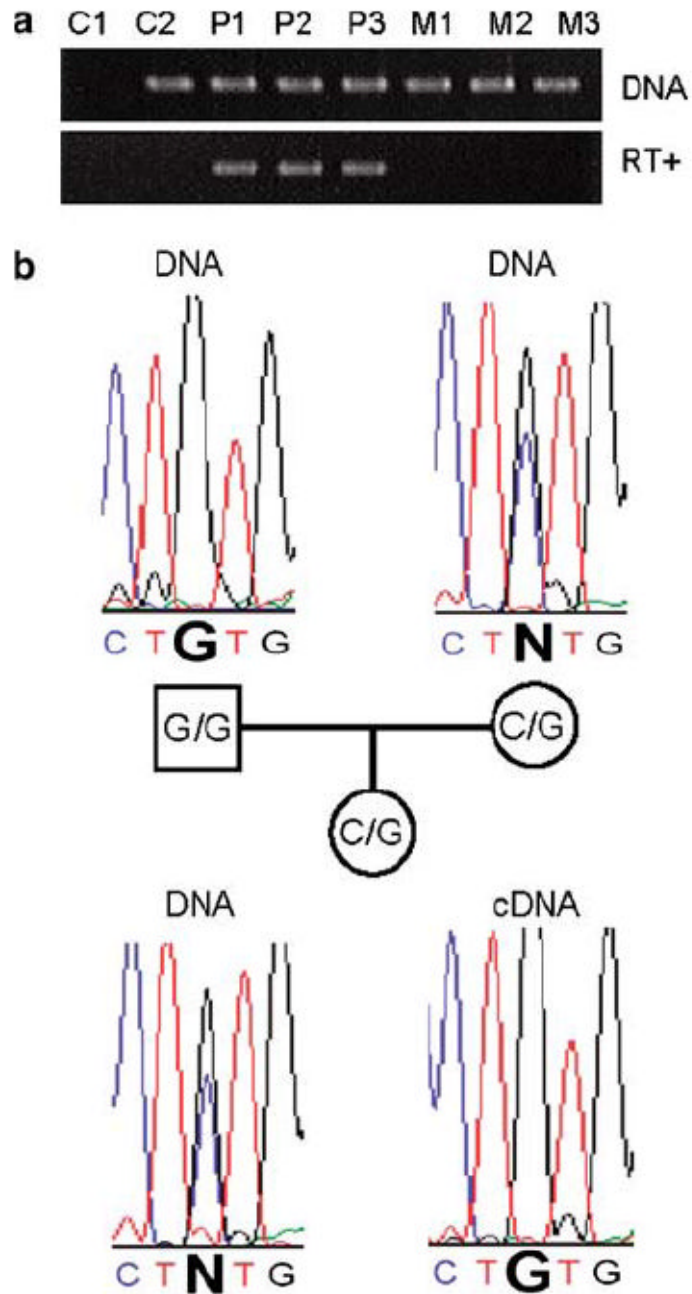
Figure 2.
LRRTM1 expression is downregulated maternally in humans. (**a**) Data are shown from A9 cells that each contains a single human chromosome 2 of known parental origin. Products are shown from PCR using primers specific for human LRRTM1. Upper panel: the human gene is present in genomic DNA (labeled 'DNA') from all cell lines tested, apart from C1 (mouse A9 cell line containing no human chromosome). Lower panel: the human gene is only expressed (detected by RT-PCR) in three cell lines containing paternally derived human chromosome 2s (P1, P2, P3), and not in three cell lines containing maternally derived human chromosome 2s (M1, M2, M3). (C2 is a human fibroblast cell line). (**b**) Monoallelic paternal expression of LRRTM1 in a human EBV-transformed lymphoblastoid cell line.

Sequence traces derived from genomic DNA (labeled 'DNA') surrounding a C/G SNP are shown for a father, mother and child. The child is heterozygous, but the cDNA prepared from mRNA showed expression of only the paternally inherited G allele of LRRTM1.
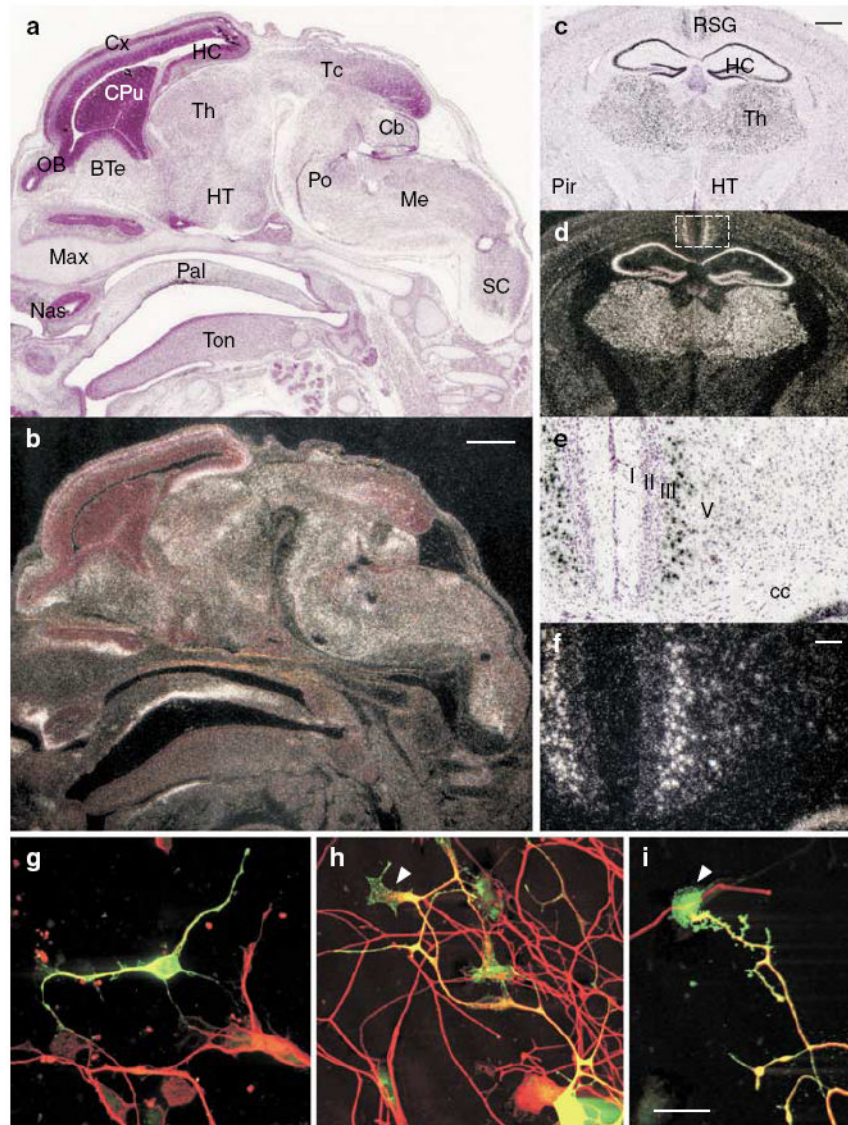
Figure 3.
Developmental increase of Lrrtm1 mRNA expression in mouse thalamus, hippocampus and retrosplenial cortex, and localization of LRRTM1 in neurons. *In situ* hybridization analysis in sagittal sections of E15 mouse embryos (**a** and **b**) and coronal sections of adult mouse brain (**c–f**). Bright-field images counterstained with hematoxylin (**a, c** and **e**) and corresponding dark-field autoradiographs are shown (**b, d** and **f**). The white signal in the dark-field images indicates Lrrtm1 expression. (**e** and **f**) Higher magnification of the retrosplenial granular cortex (boxed area in **d**) shows a high level of Lrrtm1 expression in layer III–IV neurons. (**g–i**) Myc-LRRTM1 was transfected into E17 mouse cortical neurons (**g**) and nucleofected into rat P18 dorsal root ganglion neurons (**h** and **i**) and detected 40 h later with anti-myc antibody. Neurons were visualized with anti-*β*-tubulin antibody. LRRTM1 is localized to the cell soma and in the neurites; in neurites it is also localized to lamellipodia of the growth cones (arrowheads in **h** and **i**). BTe, basal telencephalon; Cb, cerebellum; cc, corpus callosum; CPu, caudate-putamen; Cx, cerebral cortex; HC, hippocampus; HT, hypothalamus; Max, maxilla, Me, medulla; Nas, nasal cavity; OB, olfactory bulb; Pal, palatine; Pir, piriform cortex; Po, pons; RSG, retrosplenial granular

cortex; Sc, Spinal cord; Tc, tectum; Th, thalamus; Ton, tongue. Scale bar is 500 μm in (**a–d**), 100 μm in (**e–f**) and 20 μm in (**g–i**).
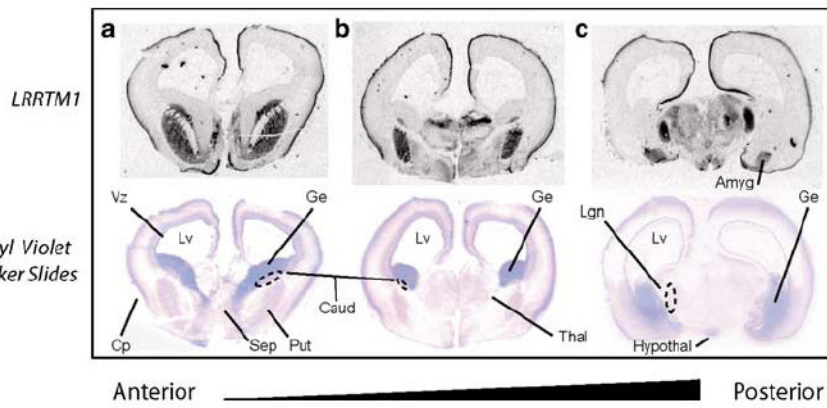
Figure 4.
In situ hybridization analysis of *LRRTM1* expression in the developing human brain at 15 weeks' gestation. In coronal sections of anterior brain (**a**), expression is strong throughout the cortical plate and otherwise restricted to septum, caudate, and putamen. Transcript distribution is similar in more caudal sections (**b**) with the addition of signal in dorsolateral thalamus. More caudal still (**c**), thalamic signal shifts ventrally to a structure consistent with the lateral geniculate body. Abbreviations are as follows: Amyg (Amygdala), Caud (Caudate), Cp (Cortical), Ge (Ganglionic Eminence), Hypothal (Hypothalamus), Lgn (Lateral Geniculate Nucleus), Lv (Lateral Ventricle), Thal (Thalamus), Vz (Ventricular Zone).

Figure 5.
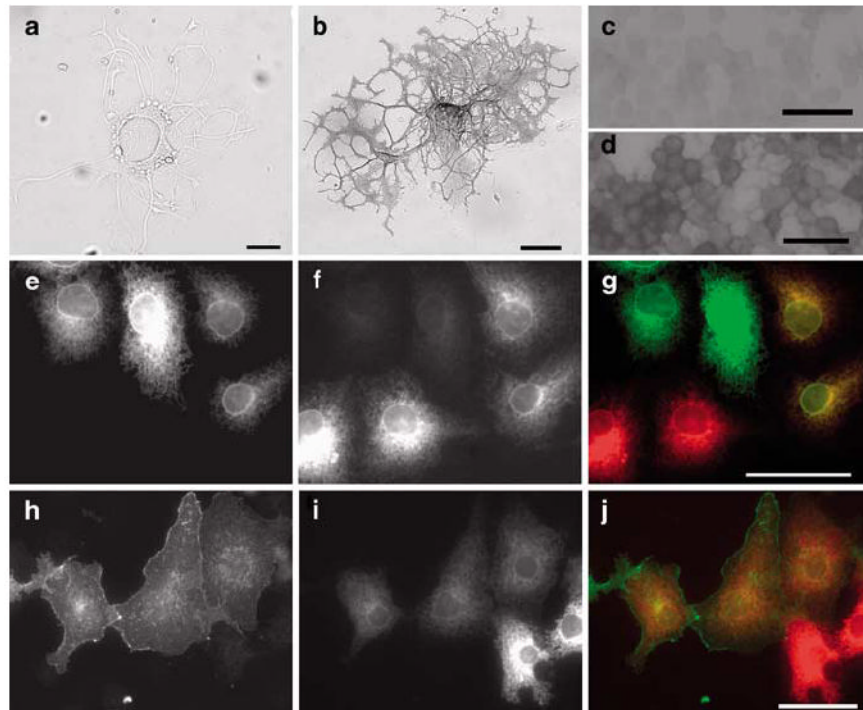LRRTM1 is not localized on the plasma membrane. No cell-surface expression of LRRTM1 is seen in primary sensory neurons electroporated with myc-LRRTM1 construct and detected by live-cell staining with alkaline phosphatase-conjugated anti-myc antibody (**a**); strong cell-surface staining of myc-Lingo1 expressing neuron is seen (**b**). Live-cell staining resulted in 20–30 strongly stained DRG neurons per well for myc-Lingo1 but none for myc-LRRTM1. Similarly, in neuro-2a neuroblastoma cells myc-LRRTM1 (**c**) is not, but myc-lingo1 is (**d**) localized to plasma membrane as detected by live-cell staining. In permeabilized Cos-7 cells LRRTM1 (anti-myc antibody; **e**) is colocalized (merge picture; **g**) with ER-marked dsRED2-ER (**f**), whereas lingo1 (anti-myc antibody; **h**) is only partially colocalized with dsRED2-ER, and it is partially localized on the plasma membrane (**i**; merge picture: **j**). Scale bars are 50 μm.

Table 1

Transmission disequilibrium statistics for two-marker haplotypes (see main text), for families affected with schizophrenia/schizoaffective disorder

	N	%	Paternal			Maternal			Both parents		
			TR	NT	χ^2	TR	NT	χ^2	TR	NT	χ^2
New York/Oxford											
Haplo.1	504	81.8	18	27	1.80	21	19	0.10	40	47	0.56
Haplo.2	**50**	**8.1**	**15**	**6**	**3.86**	**8**	**9**	**0.06**	**23**	**15**	**1.68**
Haplo.3	61	9.9	13	13	0.00	15	14	0.03	29	28	0.02
Haplo.4	1	0.2	0	0	0.00	0	2	2.00	0	2	2.0
Irish high density											
Haplo.1	336	79.6	6	14	3.20	15	17	0.12	23	33	1.79
Haplo.2	**51**	**12.1**	**10**	**1**	**7.36**	**11**	**6**	**1.47**	**22**	**8**	**6.53**
Haplo.3	30	7.1	5	5	0.00	5	8	0.69	11	14	0.36
Haplo.4	5	1.2	0	1	1.00	1	1	0.00	1	2	0.33
Montreal											
Haplo.1	327	86.5	4	4	0.00	11	9	0.20	15	13	0.14
Haplo.2	**31**	**8.2**	**3**	**3**	**0.00**	**5**	**12**	**2.88**	**8**	**15**	**2.13**
Haplo.3	19	5.0	2	2	0.00	7	2	2.78	9	4	1.92
Haplo.4	1	0.3	0	0	0.00	0	0	0.00	0	0	0.00
Afrikaner											
Haplo.1	1013	81.0	16	19	0.26	21	18	0.23	39	39	0.00
Haplo.2	**95**	**7.6**	**10**	**6**	**1.00**	**4**	**14**	**5.56**	**15**	**21**	**1.00**
Haplo.3	118	9.4	11	9	0.20	15	8	2.13	27	18	1.80
Haplo.4	24	1.9	2	5	1.29	3	3	0.00	5	8	0.69
Total sample											
Haplo.1	2180	81.8	44	64	3.70	68	63	0.19	117	132	0.90
Haplo.2	**227**	**8.5**	**38**	**16**	**8.96**	**28**	**41**	**2.45**	**68**	**59**	**0.64**
Haplo.3	228	8.6	31	29	0.07	42	32	1.35	76	64	1.03
Haplo.4	31	1.2	2	6	2.00	4	6	0.40	6	12	2.00

χ^2, χ^2 statistic; haplo.1, haplotype 1-1; haplo.2, haplotype 2-2; haplo.3, haplotype 1-2; haplo.4, haplotype 2-1; *N*, number of instances of the haplotype in the sample; NT, number of non-transmitted haplotypes from heterozygous parents; TR, number of transmitted haplotypes from heterozygous parents; %, haplotype frequency.

The left-handedness risk haplotype 2-2 is highlighted in bold.