3 Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R *et al. Proc Natl Acad Sci USA* 2006; **103**: 6747–6752.
4 Tan W, Wang Y, Gold B, Chen J, Dean M, Harrison PJ *et al. J Biol Chem* 2007; **282**: 24343–24351.
5 Law AJ, Shannon Weickert C, Hyde TM, Kleinman JE, Harrison PJ. *Neuroscience* 2004; **127**: 125–136.
6 Hahn C-G, Wang H-Y, Cho D-S, Talbot K, Gur RE, Berrittini WH *et al. Nat Med* 2006; **12**: 824–828.
7 Roy K, Murtie JC, El-Khodor BF, Edgar N, Sardi SP, Hooks BM *et al. Proc Natl Acad Sci USA* 2007; **104**: 8131–8136.
8 Chen YJ, Johnson MA, Lieberman MD, Goodchild RE, Schobel S, Lewandowski N *et al. J Neurosci* 2008; **28**: 6872–6883.
9 Flynn SW, Lang DJ, Mackay AL, Goghari V, Vavasour IM, Whittall KP *et al. Mol Psychiatry* 2003; **8**: 811–820.
10 Georgieva L, Dimitrova A, Ivanov D, Nikolov I, Williams NM, Grozeva D *et al. Biol Psychiatry* 2008; **64**: 419–427.
11 Hall J, Whalley HC, Job DE, Baig BJ, McIntosh AM, Evans KL *et al. Nat Neurosci* 2006; **9**: 1477–1478.

Supporting evidence for *LRRTM1* imprinting effects in schizophrenia

Molecular Psychiatry (2009) **14,** 743–745;
doi:10.1038/mp.2009.28

A recent paper in this Journal reported evidence for the involvement of a novel imprinted gene on chromosome 2p12, the *leucine-rich repeat transmembrane neuronal 1* (*LRRTM1*) gene, in the development of schizophrenia/schizoaffective disorder and human handedness. Francks *et al.*[1] found a three-marker haplotype upstream of *LRRTM1* to be associated with schizophrenia when inherited paternally. It is interesting that the same haplotype was also found to be paternally associated with handedness in a sample of reading-disabled sibships, implying the existence of specific imprinting effects on human brain asymmetry. We tested Francks *et al.*'s hypotheses in large independent samples of (i) schizophrenia and (ii) dyslexia patients with handedness information.

We genotyped the three markers that define the paternally overtransmitted haplotype in the Francks *et al.* study, rs1446109–rs1007371–rs723524, in 180 parent–offspring trios with schizophrenia. Assuming the hypothesis that true parent-of-origin effects detected in trios should also result in allelic association in case–control studies,[1] we additionally genotyped these markers in 673 patients with schizophrenia (358 males/315 females) and 1060 controls (566 males/494 females). Individuals, who were recruited from consecutive admissions to the Department of Psychiatry at the University of Bonn, Germany, were of German descent, and had given written informed consent to participate in this study. Diagnoses were made according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria[2] on the basis of a Structured Clinical Interview for DSM-Disorders (SCID-I) interview,[3] review of medical records and use of the family history method. The Operational Criteria for Psychotic and Affective Illness (OPCRIT) system was also used.[4] Genotyping on DNA samples was carried out using Sequenom's iPlex assay (Sequenom Inc., San Diego, CA, USA), according to the manufacturer's protocol.

Assessment of haplotype frequencies in both, parents of probands and unrelated control samples, showed similar frequencies to those observed by Francks *et al.* (data not shown), who only tested a two-marker proxy haplotype when they studied association for schizophrenia. In the trio sample, the number of paternally and maternally transmitted haplotypes was estimated using FAMHAP v18.[5] Subsequently, a transmission disequilibrium test was performed. The 2-2-2 haplotype (with '2' representing the minor allele of each single nucleotide polymorphism (SNP)) showed a tendency towards overtransmission for the paternal alleles (12 transmissions (T) vs 6 non-transmissions (NT, Table 1)). However, this tendency did not reach statistical significance ($P = 0.153$). The complementary 1-1-1-haplotype was observed to be paternally undertransmitted to the affected probands (21 T vs 27 NT). When analysing the case–control sample, no differences in haplotype frequencies between affected and unaffected individuals could be detected.

In order to maximize the power to detect genetic effects at this locus, we combined our and Francks *et al.*'s family-based samples and conducted a meta-analysis. As shown in Table 1, the combined analysis revealed a highly significant effect of paternal overtransmission of the 2-X-2 haplotype ($P = 0.00097$; that is, the proxy haplotype to 2-2-2) and paternal undertransmission of the complementary 1-X-1 haplotype ($P = 0.0374$; proxy haplotype to 1-1-1).

In addition to the markers forming the specific haplotype upstream of *LRRTM1*, we genotyped an additional set of 11 tagSNPs to systematically cover variation at this locus (rs13019601, rs1930, rs1446110, rs10170020, rs6718055, rs2862286, rs6712681, rs6733871, rs11126755, rs6755232 and rs767587). We identified the minor allele of rs6733871 to be significantly maternally undertransmitted to affected probands ($P = 0.025$, Table 1). This marker is a non-synonymous SNP (asparagine to serine at position 330). Although this *P*-value does not withstand correction for multiple testing, additional support for a parent-of-origin effect is obtained from the observed tendency of paternal overtransmission for the same allele (21 T vs 14 NT). Hence, we also observe paternal overtransmission/maternal suppression for a coding SNP, which was not analyzed by Francks *et al.*

Francks *et al.* further hypothesized that the observed association might be more generally related to human brain asymmetry. They found the three-marker haplotype to be paternally associated with human handedness in a set of reading-disabled sibships. We have access to a

Table 1 Test statistics for German schizophrenia sample

Marker	*Allele*	*Data*	*Maternal transmissions*				*Paternal transmissions*				*Case control*			
			T	*NT*	χ^2	P-*value*	*T*	*NT*	χ^2	P-*value*	Freq Ca	*Freq Co*	χ^2	P-*value*
(A)														
rs6733871	2	Own	15	30	5.00	**0.025**	21	14	1.46	0.237	0.180	0.183	0.046	0.830
(B)														
G A T[a]	2-2-2	Own	11	10	0.05	0.827	12	6	2.04	0.153	0.084	0.091	0.543	0.461
	2-X-2	Francks	28	41	2.45	0.118	38	16	8.96	**0.0028**				
		Meta	39	51	1.60	0.206	50	22	11.0	**0.00097**				
A T G	1-1-1	Own	13	22	2.34	0.126	21	27	0.75	0.386	0.805	0.805	0.003	0.957
	1-X-1	Francks	68	63	0.19	0.663	44	64	3.70	0.054				
		Meta	81	85	0.10	0.756	65	91	4.33	**0.0374**				
A T T	1-1-2	Own	16	7	3.62	0.057	17	17	0.00	1	0.101	0.095	0.327	0.568
	1-X-2	Francks	42	32	1.35	0.245	31	29	0.07	0.791				
		Meta	58	39	3.72	0.054	48	46	0.04	0.837				

Abbreviations: Freq Ca, frequency in case; Freq Co, frequency in control; NT, non-transmitted; T, transmitted; 1, major allele; 2, minor allele.[a]Haplotype showing paternal overtransmission in Francks *et al*.
(A) Single-marker analysis within genomic locus of *leucine-rich repeat transmembrane neuronal 1* (*LRRTM1*). Only single nucleotide polymorphisms (SNPs) with nominally significant results are shown.
(B) Haplotype analysis for own data set (rs1446109–rs1007371–rs723524). For the Francks *et al*. results, the 2-marker proxy (rs1446109–rs723524) is presented, together with a combined meta-analysis. Only common haplotypes (frequency > 1%) are depicted. *P*-values for Francks *et al*. have been generated based on the χ^2-statistics given in the original study and are two-tailed.

well-characterized dyslexia sample of German descent for which information on handedness is available (398 sib pairs with parents),[6] and analyzed the aforementioned 14 markers. Using the quantitative trait disequilibrium test QTDT,[7] seven markers out of 14 had nominally significant *P*-values <0.05. The significant SNPs included rs6733871, but none of the haplotype-forming markers (lowest *P*-value = 0.0055 for rs2862286). This is more than what would be expected by chance alone, suggesting that *LRRTM1* might make a general contribution to handedness. However, parental effects in the hypothesized direction were not observed. A sufficiently powerful haplotype analysis was not possible because of the limited number of left-handers in our sample ($n=63$).

Our data show a statistical trend supporting earlier findings of imprinting effects within *LRRTM1*, and their association with schizophrenia. Larger samples, however, may be necessary to further strengthen this trend. It is interesting that a combined analysis of both data sets provides strong support for a true imprinting effect in schizophrenia at this locus. In addition to the replication of paternal overtransmission of the three-marker haplotype upstream of *LRRTM1*, we showed such an effect for a non-synonymous variant within the gene. Evidence in our schizophrenia sample comes primarily from the family-based data sets. The fact that this effect is not observed in the case–control data might be because of the differences in recruitment strategies, power issues, or the fact that the effects of paternal transmission and maternal suppression are equally strong.

In summary, our results offer a degree of support for Francks *et al*.'s findings and strengthen the evidence for an association of imprinted alleles of *LRRTM1* with schizophrenia. Weaker supportive evidence was also obtained for a possible association of *LRTTM1* with human brain asymmetry.

KU Ludwig[1], M Mattheisen[1,2], TW Mühleisen[1], D Roeske[3], C Schmäl[4], R Breuer[4], G Schulte-Körne[5], B Müller-Myhsok[3], MM Nöthen[1,6], P Hoffmann[1,6], M Rietschel[4] and S Cichon[1,6]
[1]*Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany;*
[2]*Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany;*
[3]*Max-Planck Institute of Psychiatry, Munich, Germany;*
[4]*Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany;*
[5]*Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital Munich, Munich, Germany and*
[6]*Institute of Human Genetics, University of Bonn, Bonn, Germany*
E-mail: sven.cichon@uni-bonn.de

References

1 Francks C, Maegawa S, Lauren J, Abrahams BS, Velayos-Baeza A, Medland SE *et al. Mol Psychiatry* 2007; **12**: 1129–1139, 1057. (e-pub ahead of print 31 July 2007).
2 American Psychiatric Association CoNaS. American Psychiatric Association: Washington, DC, USA, 1994.
3 First MB, Spitzer RL, Gibbon M, Williams JBW. *Clinical Interview for DSM-IV Axis I Disorders*. Patient edition. Biometrics Research Department, New York State Psychiatric Institute: New York, NY, USA, 1997.
4 Farmer AE, Wessely S, Castle D, McGuffin P. *Br J Psychiatry* 1992; **161**: 824–830.
5 Becker T, Knapp M. *Genet Epidemiol* 2004; **27**: 21–32.
6 Schulte-Körne G, Ziegler A, Deimel W, Schumacher J, Plume E, Bachmann C *et al. Ann Hum Genet* 2007; **71**(Part 2): 160–175.
7 Abecasis GR, Cardon LR, Cookson WO. *Am J Hum Genet* 2000; **66**: 279–292.