Supplementary material 2: Methylation-sensitive Southern analysis

Probe preparation:

The L32702 sequence containing exon alpha SNPRN was amplified using the forward primer TCTAGAGGCCCCCTCTCATT and the reverse primer GGCCCAGAATTCCGTTTAT yielding a predicted 394 bp fragment (chr15:22,751,022-22,751,415 Human Genome build 36). The PCR fragment was subsequently cloned using the TOPO® TA Cloning® kit (Invitrogen, Carlsbad, CA, USA) and sequenced for verification. Verified clones were digested with EcoRI and the insert extracted through the Whatman-paper method.

Sample preparation:

Icelandic samples
DNA was isolated from 9ml of frozen blood (in EDTA vacutainers), using MasterPure reagents (Epicentre® Biotechnologies) and following the manufacturer's guidelines for reagent volumes. Blood was thawed in a 37°C water bath and transferred to 50ml centrifuge tubes. DNA was isolated following the manufacturer's protocol for the most part. However, after precipitation using isopropanol (Sigma-Aldrich), the DNA was spooled onto a sterile inoculation loop (NUNC), washed in 70% ethanol and transferred to a tube containing 800ul TE-buffer (10mM Tris HCl pH8.0; 1mM EDTA).

Danish sample
DNA was sent to deCODE Genetics where it was diluted and subjected to restriction digests.

UK samples
Two DNA samples were sent to deCODE Genetics, where DNA concentration was measured and the sample diluted prior to restriction digests.

German sample
Cultured cells for the patient in freezing medium (62.3% RPMI 1640, 26.3% FCS, 10% DMSO, 0.7% Gentamycin, 0.7% Sodium Pyruvate) and sent to deCODE Genetics on dry ice. Cells were transferred to a 50ml centrifuge tube and washed twice with PBS, prior to DNA isolation using MasterPure reagents (Epicentre® Biotechnologies), following the manufacturers protocol. However, reagent volumes and centrifuge times were adjusted to accommodate larger sample size, and the DNA spooled as described for Icelandic samples.

Digestion and electrophoresis
An aliquot of Icelandic DNA samples was diluted into a 96-well UV plate (Costar) and DNA concentration measured on a SpectraMax190 (Molecular Devices) using SoftMax® Pro software. DNA concentration of other samples was measured using a NanoDrop Spectrophotometer (NanoDrop Technologies). Subsequently, a 60ul dilution of 300ng/ul DNA was made for each sample prior restriction enzyme digests. DNA from all duplication carriers was digested with XbaI and NotI; 17 µL DNA (~5 µg), 0.5 µL XbaI (NEB 20,000 U/mL), 1 µL NotI (NEB 10,000 U/mL), 0.25 µL BSA (NEB), 2.5 µL NEBuffer3, 3.75 µL water, incubated at 37°C overnight. After adding 6 µL loading buffer the digested sample was run on 0.8% Q-Bio agarose at 55 V (40 A) in x 0.5 TBE buffer for 16 hrs. We used 6 µL of Marker II (50 ng/µL) for size standard.

Southern hybridization:

After the electrophoresis the gel was denatured in 0.5M NaOH, 1.5M NaCl for 20 min. DNA in the gel was transferred to a nylon filter (Hybond N+). The membrane was then hybridized with radiolabeled [α-32 P]dCTP (specific activity 6000Ci/mmol) L32702 Topo TA sequenced verified insert using the Megaprime DNA Labeling Kit (Amersham, Cat no. RPN 1607). Unincorporated nucleotides were removed from the reaction using ProbeQuant G-50 microcolumns (Amersham Cat. 27-5335-01). Membranes were pre-hybridized in Rapid-hyb buffer (Amersham Cat. RPN 1635) for at least 30 minutes, and subsequently hybridized with the radiolabeled probe. Hybridizations were performed in Rapid-hyb buffer at 65°C overnight. The labeled probes were heated for 5 minutes at 95°C before addition to the filters in the pre-hybridization solution. After hybridization, the membranes were washed at low stringency in 2x SSC, 0.1% SDS in 65°C waterbath for 10 minutes followed by two high stringency washes in 0.1x SSC, 0.1% SDS at 65°C for 20 minutes. While the blots were moist they were sealed and exposed to film (Amersham Hyperfilm MP, cat. 28-9068-48) from 1-7 days at -80°C.

Quantification of bands:

The band intensity was quantified using Kodak 1D Image Analysis Software v.3.5 and ratio between intensities of the paternal allele (0.9 kb band) and maternal allele (4.2 kb band) calculated after correcting for background emission.

Results:

The parental origin of duplications in all identified carriers was assayed in a total of three hybridizations, with most of the samples being assayed in all instances, while some only once or twice. The results of the three experiments are summarized in supplementary table 1.

Supplementary table 2: Result summary for parental origin of duplications.

Sample[1]	**Exp 1 (ratio**[2]**)**	**Exp 2 (ratio**[2]**)**	**Exp 3 (ratio**[2]**)**	**Duplication origin**	**Comment**
Case 1	0.06	-	-	Maternal	
Case 2	0.05	0.16	0.22	Maternal	
Case 3	-	0.31	-	Maternal	
Case 4	-	0.27	-	Maternal	
Case 5[3]	-	-	0.37	Maternal	
Case 6	0.06	0.16	0.30	Maternal	
Case 7[3]	0.08	0.20	0.30	Maternal	
Ctrl 1[4]	0.37	0.65	1.13	Paternal	
Father 1[4]	-	0.51	1.74	Paternal	Father of Ctrl 1
Ctrl 2	0.29	0.52	1.30	Paternal	
Ctrl 3	0.05	0.20	0.22	Maternal	
Ctrl 4	0.05	0.15	0.29	Maternal	
Ctrl 5	0.09	0.16	0.39	Maternal	

[1]All five controls, the father of Ctrl 1, and the autism and bipolar cases are from the Icelandic SGENE+ sample.
[2]The ratio between optical densities of the paternal (0.9 kb) and maternal (4.2 kb) bands, adjusted for background.
[3]Maternal origin of the duplication was independently determined through clinical genetics analysis (Case 5) and SNP microarray genotype analysis in parents (Case 7).
[4]Paternal origin of the duplication was independently determined by microsatellite genotypes available for Ctrl 1 and her family.

First hybridization
This includes the Danish and German schizophrenia cases (Case 1 and Case 2), the Icelandic bipolar disorder and autism cases (Case 6 and Case 7) as well as the five Icelandic controls (Ctrl 1-5). Negative controls (neg-ctrl 1-3) are samples with normal copy number in the region (one maternal and one paternal allele). For Ctrl 1 the duplication was independently determined to be paternally inherited through analysis of microsatellite genotypes (see supplementary material 3). Through analysis of SNP microarray genotypes available for both parents of Case 7, the duplication was determined to be carried *de novo* in Case 7 deriving from an unequal crossover in her mother's germline. Thus, Ctrl 1 and Case 7 provide positive controls for the Southern analysis of paternally and maternally derived duplications, respectively. The negative control in lane one failed to produce reliably measureable bands. The remaining samples cluster in two clearly distinct clusters with respect to paternal to maternal band ratio; one includes the known maternal origin duplication in Case 7, the negative controls, Cases 1,2, and 6, and Ctrls 3-5 (ratio range 0.05-0.11), while the other includes only the known paternal origin duplication in Ctrl 1 as well as Ctrl 2 (ratio range 0.29-0.37). We conclude that the assay may distinguish between paternally and maternally originated duplications, but not between normal copy number and maternally derived duplications. The results are shown in supplementary table 2a and supplementary figure 1a.

Second hybridization
In addition to the samples from the first hybridization (except Case 1 with not enough DNA remaining), this one includes the two British cases (Case 3 and Case 4). Also we have added here the father of Ctrl 1; the paternal origin of the duplication in him had been determined from family microsatellite genotypes (see supplementary material 3). Again the negative control in lane one failed to produce reliably measurable bands although we use here a different sample than in the first hybridization. Again we can divide the samples in two clusters; the maternally derived duplications and normal copy number (ratio range 0.15-0.31), and the paternally derived duplications (ratio range 0.51-0.65). The results are shown in supplementary table 2b and supplementary figure 1b.

Third hybridization
This hybridization includes all samples from the second hybridization as well as the Icelandic schizophrenia case (Case 5, duplication identified after recalling a part of the Icelandic sample with PennCNV and verifying calls in an improved genomic viewer). A review of medical records revealed that a previously performed clinical genetics analysis had already determined the duplication in the Icelandic schizophrenia case to be transmitted from her mother. Again the negative control in lane one, but also Case 3 and Case 4, failed to produce reliably measurable bands in this experiment, most likely because we had little DNA remaining for these samples after the first two experiments and/or because of poor DNA digestion. As before, the samples produce two clusters with respect to paternal to maternal band intensity ratios; the maternally derived duplications and normal copy number (ratio range 0.22-0.44), and the paternally derived duplications (ratio range 1.13-1.74). l. The results are shown in supplementary table 2c and supplementary figure 1c.

Supplementary table 3a: Parental origin of duplications from first hybridization.

Lane	Sample	Ratio	Call	Comment
1	neg-ctrl 1	-		Failed
2	neg-ctrl 2	0.11		
3	Case 1	0.06	Maternal	
4	Case 2	0.05	Maternal	Possible extra band
5	Ctrl 1	0.37	Paternal	Paternal origin known from other source
6	Ctrl 2	0.29	Paternal	
7	Ctrl 3	0.05	Maternal	
8	Case 7	0.08	Maternal	Maternal origin known from other source

9	Ctrl 4	0.05	Maternal	
10	Ctrl 5	0.09	Maternal	
11	Case 6	0.06	Maternal	
12	neg-ctrl 3	0.09		

Supplementary Table 3b: Parental origin of duplications from second hybridization.

Lane	**Sample**	**Ratio**	**Call**	**Comment**
1	neg-ctrl 1	-		Failed
2	neg-ctrl 2	0.21		
3	Case 2	0.16	Maternal	
4	Case 3	0.31	Maternal	
5	Case 4	0.27	Maternal	
6	Ctrl 1	0.65	Paternal	Paternal origin known from other source
7	Father of Ctrl1	0.51	Paternal	Paternal origin known from other source
8	Ctrl 2	0.52	Paternal	
9	Ctrl 3	0.20	Maternal	
10	Case 7	0.20	Maternal	Maternal origin known from other source
11	Ctrl 4	0.15	Maternal	
12	Ctrl 5	0.16	Maternal	
13	Case 6	0.16	Maternal	
14	neg-ctrl 3	0.26		

Supplementary Table 3c: Parental origin of duplications from third hybridization.

Lane	**Sample**	**Ratio**	**Call**	**Comment**
1	neg-ctrl 1	-		Failed
2	neg-ctrl 2	0.30		
3	Case 2	0.22	Maternal	
4	Case 3	-	-	Failed
5	Case 4	-	-	Failed
6	Case 5	0.37	Maternal	Maternal origin known from other source
7	Ctrl 1	1.13	Paternal	Paternal origin known from other source
8	Father of Ctrl 1	1.74	Paternal	Paternal origin known from other source
9	Ctrl 2	1.30	Paternal	
10	Ctrl 3	0.22	Maternal	
11	Case 7	0.30	Maternal	Maternal origin known from other source
12	Ctrl 4	0.29	Maternal	
13	Ctrl 5	0.39	Maternal	
14	Case 6	0.30	Maternal	
15	neg-ctrl 3	0.44		

Supplementary figure 1: First (a), second (b), and third (c) hybridization.