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MOLECULAR CYTOGENETICS

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Assessment of copy number variations in the brain genome of schizophrenia patients

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Abstract

Background: Cytogenomic mutations and chromosomal abnormality are implicated in the neuropathology of several brain diseases. Cell mosaicism of brain tissues makes their detection and validation difficult, however. In the present study, we analyzed gene dosage alterations in brain DNA of schizophrenia patients and compared those with the copy number variations (CNVs) identified in schizophrenia patients as well as with those in Asian lymphocyte DNA and attempted to obtain hints at the pathological contribution of cytogenomic instability in schizophrenia.

Results: Brain DNA was extracted from postmortem striatum of schizophrenia patients and control subjects (n = 48 each) and subjected to the direct two color microarray analysis that limits technical data variations. Disease-associated biases of relative DNA doses were statistically analyzed with Bonferroni's compensation on the premise of brain cell mosaicism. We found that the relative gene dosage of 85 regions significantly varied among a million of probe sites. In the candidate CNV regions, 26 regions had no overlaps with the common CNVs found in Asian populations and included the genes (i.e., ANTXRL, CHST9, DNM3, NDST3, SDK1, STRC, SKY) that are associated with schizophrenia and/or other psychiatric diseases. The majority of these candidate CNVs exhibited high statistical probabilities but their signal differences in gene dosage were less than 1.5-fold. For test evaluation, we rather selected the other 10 CNV regions that exhibited higher aberration scores or larger global effects and are thus confirmable by PCR. Test PCR verified the loss of gene dosage at two loci (1p36.21 and 1p13.3) and confirmed the global variation of the copy number distributions at two loci (11p15.4 and 13q21.1), both indicating the utility of the present strategy. These test loci, however, exhibited the same somatic CNV patterns in the other brain region.

Conclusions: The present study lists the candidate regions potentially representing cytogenomic CNVs in the brain of schizophrenia patients, although the significant but modest alterations in their brain genome doses largely remain to be characterized further.

Keywords: CNV, Caudate, Genome instability, Schizophrenia, Somatic mutation

Background

Copy number variation (CNV) is defined as a deletion or duplication/multiplication of a genomic fragment spanning more than 1 kb when compared to a reference genome [1–3]. Approximately 37,000 sites of common CNVs have been identified in the human genome and they occupy 12 % of the entire genome [4, 5]. The genome-wide association studies (GWAS) on schizophrenia analyzed DNA which was isolated from peripheral lymphocytes and have identified risk CNV sites, some of which are not present in the patients' parents [6–9].

Somatic mosaicism of genome sequences and structures have recently drawn particular attention [10–12]. Nearly 30 % of developing brain cells in human are reported to harbor aberrant chromosomal compositions [13, 14]. In addition, there are significant genomic differences in somatic cells between monozygotic twins and among tissues [15–18]. Accordingly, aberrant cytogenomic variations in

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human brain are implicated in neurodegenerative and neurodevelopmental disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's diseases [19–25]. It is an open question whether the brain-specific somatic mutation or CNV might also contribute to the etiology or neuropathology of schizophrenia [26–28].

To obtain hints at the above question, we prepared DNA from the brain tissue of 48 schizophrenia patients and 48 control subjects. Labeling brain DNA samples, we directly applied those to Agilent 1 M comparative genomic hybridization (CGH) arrays to measure relative gene doses without the use of reference genome. This direct comparison through the case–control pairing reduces technical data deviations and enhances the statistical power of detection [29, 30]. With the potential genomic mosaicism of heterogeneous brain cell mixtures, we expected that the target genome could be diluted with normal DNA from the off-target cells and thus assumed non-integer values of CNVs in this analysis [31]. Technical limitations of this approach are further discussed below.

Results

The striatum contain neural stem cells that proliferate throughout human life and carries somatic mutation in its mitochondrial genome [32, 33]. Therefore, we hypothesized that the striatum may be a potential candidate region that would exhibit somatic mosaicism in brain genome structures. DNA was extracted from postmortem striatum of patients with chronic schizophrenia (n = 48) and age-matched controls who had no history of neuropsychiatric disorders (n = 48) (Additional file 1: Table S1). Although there were significant differences in postmortem intervals (PMIs) between groups, there was no detectable difference in DNA quality (data not shown). All other indices were indistinguishable between schizophrenia patients and control subjects. A DNA sample was randomly picked from each group, paired to a sample in the other group, and subjected to two-color competitive CGH analysis with 1 M SurePrint G3 Human CGH Microarrays.

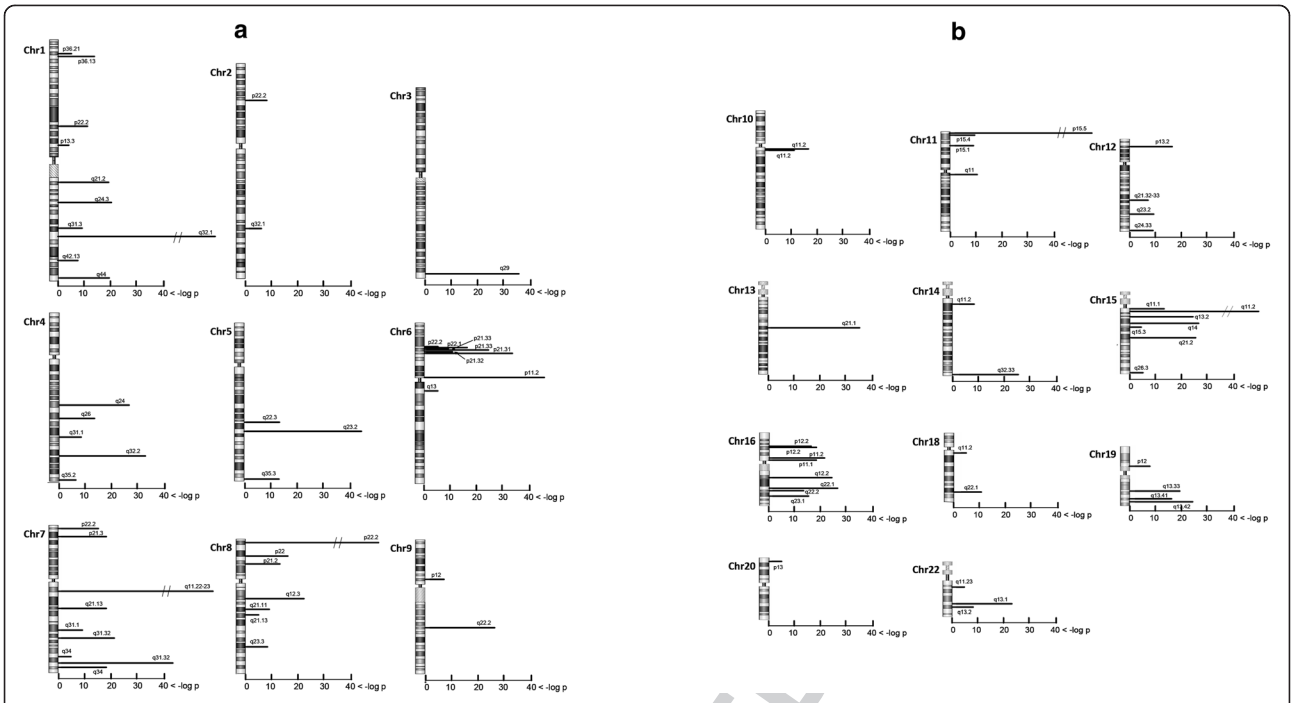
We applied the ADM-2 algorithm to the CGH signals of individual microarray probes (nearly 1 million) and searched for the primary candidate CNV loci associated with schizophrenia. A flowchart of the present study design is shown in Additional file 1: Figure S1. We chose1381 chromosomal loci that exhibited large group differences in gain/loss calls (Selection 1). In each probe site located on the primary candidate loci, we plotted the distribution of log2 signal ratios from 48 sets of microarray analyses and tested the null hypothesis that the mean log2 signal ratios was zero, indicating that the two groups were indistinguishable (Selection 2). We calculated total probabilities and averaged log2 signal ratios for individual candidate loci and judged their statistical significance with Bonferroni's correction. The number of the candidate loci maintaining the statistical significance through Selection 2 was reduced to 85 (Details in Additional file 1: Table S2).

Positive CNV loci were found in almost all chromosomes except chromosome 17 and 21 (Fig. 1). Individual loci covered 1–746 probe sites (3–2200 kb) and exhibited average log2 ratios of −1.46 to +0.63 (i.e., odds ratio (OR) = 0.36–1.55). A majority of the average log2 ratios were between −0.59 to +0.59 (i.e., < 1.5-fold differences) and only 4 loci showed more than 1.5-fold differences in array CGH signals. A genomic region spanning from 6p22.2 to 6p21.32 contained six CNV loci and included genes for the major histocompatibility complex that is highly associated with schizophrenia in GWAS [34]. Among the 85 CNV loci in Selection 2, 59 loci were reported and 26 loci were not reported in the CNV study on leukocyte DNA samples of Asian populations (Additional file 1: Table S2) [2].

To validate the authenticity of the present procedure, we attempted to verify the above genome dosage changes of several candidate loci using quantitative polymerase chain reaction (qPCR). According to the following two criteria, we selected the test loci whose signal differences were larger between groups and could be detectable with the given accuracy of qPCR; (i) those exhibiting the large and consistent gain/loss calls across the limited sample pairs (from Selection 1) and (ii) the loci represented larger global effects shared in the schizophrenia samples (from Selection 2).

In the former category, the gene dosage of Hs03385437 (1p13.3), CC70L1J (1p36.21), Hs03318079 (Chr18:q22.1), Hs04794356 (4q24), Hs05080419 (9q22.2), and Hs07134106 (19p12) produced exclusive gain/loss calls in not less than four sample pairs. No discrepant calls were detected in any sample pairs. Using the same DNA pairs showing the difference in the penetrance call (Selection 1), we determined and confirmed the gene dosage of those DNA samples using qPCR. ANOVA detected significant gene dose differences at two loci (Hs03385437 and CC70L1J) between patient and control groups (Fig. 2).

In this measurement, we used *RNaseP* gene as an internal DNA dose control. Measured genome doses of the above regions appeared not to be integer levels in several control samples, potentially reflecting the cell mosaicism of the original tissues. We also extracted DNA from the prefrontal cortex of the same subjects of both groups and compared the genome doses of the above loci (Hs03385437 and CC70L1J). We calculated the copy number ratio of the patient' DNA dosage to that of the control subject' dosage and compared these ratios between the brain regions. At both loci, almost all the copy number ratios were markedly lower than 1.0 except the C26:S34 pair, supporting our primary observation that the absolute gene dosages of these loci were decreased in the schizophrenia samples. However, copy number ratios did not significantly differ



Q6 **Fig. 1** Statistically positive loci for log2 signal ratios in 48 sets of CGH microarrays. The signal bias was tested at each probe site by a two-tailed *t*-test. Bars represent the chromosomal position and lengths represent the logarithmic value of the probability of rejecting the null hypothesis. As comparisons were repeated 1381 times, we applied the Bonferroni's correction and decreased a statistical threshold from $p = 0.05$ to $p = 0.05/1381$ ($-\log p = 4.44$). Within a positive candidate CNV locus, neighboring probe sites were merged and their probabilities were cumulated (Additional file 1: Table S2). Note: Data from chromosomes 17 and 21 are not shown because these did not include any positives

between these brain regions in any of the sample pairs (Fig. 2). At least at these two candidate loci, we failed to find evidence for a gene dosage difference between these brain regions.

In the latter category, Hs0358779 (6p22.1), Hs03265736 (7p21.3), Hs03765933 (11p15.4), and Hs03298358 (q21.1) exhibited higher log2 signal ratios and were thus subjected to the test evaluation. Gene dosage of these four loci were determined by qPCR using all the sample pairs in control and schizophrenia groups (n = 48 each). Differences in gene dosages were replicated by qPCR for Hs03765933 and Hs03298358 (Fig. 3). In contrast to the data distributions of Fig. 2, almost all the values of the gene doses were located at the levels of integers but with several exceptions. These candidate CNVs appear to reflect the gene dosage differences of germinal origin.

Discussion

Several recent reports have implicated the neuropathological contribution of somatic CNV or DNA instability of the brain genome [19–28, 35–39]. In accordance with these findings, a small proportion few percent of brain cells are known to exhibit aneuploidy and carry large CNVs [13, 14, 41]. Aneuploidy is detected by fluorescence *in situ* hybridization (FISH) and appears to be increased by the onset of Alzheimer's disease [20, 22]. The aneuploidy of chromosome 1, 18 and X was also identified in the brain of schizophrenia patients [21, 41]. Despite its advantages, FISH cannot be employed in exploratory investigations, unless the specific genome region of the CNV of interest is identified. Since bonafide genome structures from off-target cells could dilute the abnormal genome DNA population, more sensitive technologies remain to be developed, which detect low quantities of CNV in mosaic tissue structures [42, 43]. In the present study, we attempted to evaluate the efficacy of the CGH microarray technique to extract somatic CNVs in the postmortem brains of schizophrenia patients [42, 43].

With given semi-quantitative nature of the microarray technique, we applied statistics to the 1 M array CGH results from 48 sample pairs. Using the high density CGH array and statistical approach, we found 85 candidate CNV loci in the present study; 59 CNV loci are overlapped with the common CNV regions and the remaining 26 loci are not reported in peripheral leukocyte-derived DNA of Asian people [2, 44]. Of note, the 26 candidate regions encode the seven genes that are associated with or implicated in schizophrenia or other psychiatric diseases; ANTXRL, CHST9, DNM3, NDST3, SDK1, STRC, and SKY (Additional file 1: Table S2). DNM3 in the candidate region of 1q24.3 is disruptively mutated in some of schizophrenia patients [45]. ANTXRL and CHST9 are located in

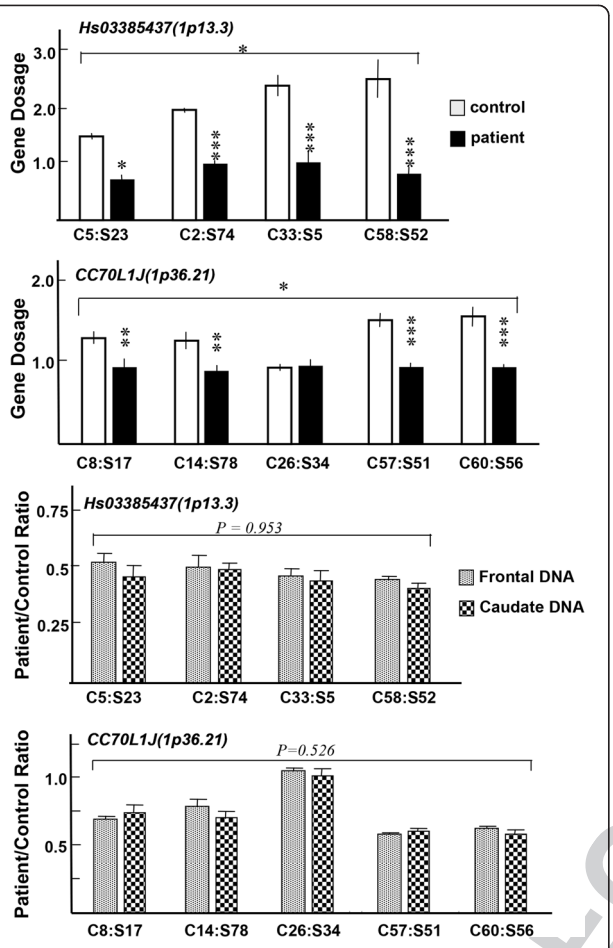


Fig. 2 Gene dosage evaluation of the individual positive pairs in CGH analysis. DNA was prepared from the striatum and prefrontal cortex of the same subjects whose pairs resulted in the exclusive penetrance calls in array CGH analysis (Selection 1). DNA was then subjected to qPCR using the *RNase P* gene as an internal control in quadruplicate. Moreover, to compare the gene dosage between the striatum and prefrontal cortex, ratios of DNA dosages of the schizophrenia patients to those of the control subjects were calculated in each brain region and plotted. Statistical comparisons of gene dosages or their ratios were conducted by two-way ANOVA with the subject factors of disease and sample pair, considering technical deviations. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

the CNV regions associated with bipolar disorder and autism [46, 47]. NDST3 and STRC are the risk genes for schizophrenia and hearing impairment that are identified by GWAS [48, 49]. SDK1 and SKY are the genes whose expression levels are markedly altered in the brain of schizophrenia patients [50, 51]. Accordingly, the present listing of the candidate brain CNVs is informative for future cytogenomic studies on schizophrenia [21, 41].

It was difficult for us to validate most of the above-mentioned 85 candidate loci with qPCR analysis with the given small signal differences between groups (i.e., less than 1.5-fold). Therefore, we selected the best 10 test loci that exhibited relatively large and/or wide effects on gene dosage. The six loci were chosen from Selection 1 as putative rare CNVs, which exhibited exclusive gain/loss calls in the limited number of samples. From Selection 2, the four loci were chosen as provisional common variants, which showed large effects and higher probability levels in the above parametric analysis. The qPCR analysis confirmed the schizophrenia-associated gene dosage differences at nearly half of the candidate CNV loci, suggesting the validity of the present strategy.

Unfortunately we had neither stored peripheral tissues nor information about these CNVs in peripheral DNA of the same subjects. To estimate the contribution of somatic CNVs to the present CNV listing, therefore, we were compelled to compare the gene dosages between the two brain regions and to search for their presence in the databases of Asian CNVs of leukocyte origin [2.44]. In the test PCR, however, we could not detect significant differences in gene dosages between the striatum and prefrontal cortex, at least, at these test CNV loci. If somatic CNVs were produced prior to neuroectodermal differentiation, there should be no difference between these two neural tissues, suggesting that the present comparison between these brain regions was inappropriate. Therefore, a comparative analysis of DNA from germinal cells of the same subjects will warrant this definitive conclusion [45].

Among the CNV candidate regions in Fig. 1, 26 candidate regions are not reported as the common CNVs of Asian populations [2, 44]. The majority of these loci exhibited high statistical significance with the probabilities of less than 10^{-100}, such as 4q35.2, 6p11.2, 7q11–12, 11p15.4–15.5, and 15q11.2. In contrast, their CGH signal differences between patients and controls were markedly smaller (OR = 0.988–1.055). As discussed above, these candidate CNV loci include the peculiar genes that are implicated in schizophrenia [45–51]. These regions, which exhibited small signal differences, might represent more promising candidates of somatic CNV sites because the genome aberration of target cells is presumably diluted in the brain and should result in smaller ORs. However, such small differences in gene dosage should make the conventional qPCR verification more challenging with the given technical deviations [53]. To avoid target DNA dilution with cell mosaicism, single cell qPCR or FISH may be more beneficial in theory [20–22, 43]. However, it would be difficult to independently perform microdissection of hundreds of cells and perform single-cell analysis unless the target cell population is identified and its sensitivity of gene detection is high enough. FISH also requires properly fixed and processed brain tissues of the same subjects. With the given technical difficulties, therefore, we have been unable to verify these small variations.

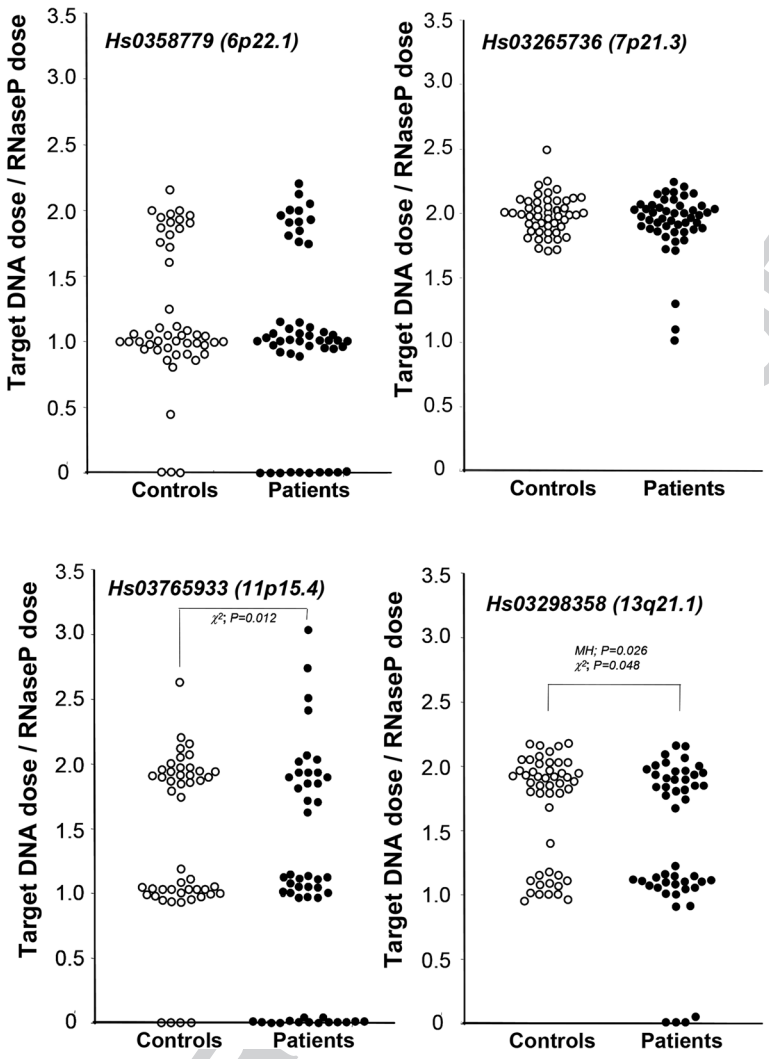


Fig. 3 qPCR evaluations of candidate CNV loci from Selection 2. Gene dosages of the candidate loci 6p22.1, 7p21.3, 11p15.4, 13q21.1, and the *RNase P* (internal control) were determined by qPCR using all sample DNAs and TaqMan probes (Additional file 1: Table S3). Individual gene dosages of 48 patients' or 48 controls' DNAs were plotted and compared between groups using the Chi-square and Mann–Whitney U tests

Conclusion

The present CGH analysis lists the potential candidate regions of somatic CNVs associated with schizophrenia, although most of those exhibited the modest but highly significant alterations in brain genome doses. Future studies aim to develop more elaborate techniques for somatic genome mosaicism and to verify the schizophrenia-associated cytogenomic instability in the above CNV candidates [53–56].

Methods

Ethical approval

The study was approved by Niigata University Medical Ethics Committee (No. 683). The use of postmortem brain tissues was authorized by the Matsuzawa Hospital Ethics Committee, Kobe University Medical Ethics Committee, Fukushima Medical University Ethics Committee, and Niigata University Medical Ethics Committee. The families of the control and schizophrenia patients provided written informed consent to allow the use of brain tissues for pathological investigations.

Brain tissue

Postmortem brain tissue was collected from patients with chronic schizophrenia (30 men, 18 women; mean age, 64.5 ± 12.5 years old) and from age-matched control subjects (30 men, 18 women; mean age, 64.2 ± 12.0 years old), with no history of neuropsychiatric disorders (Additional file 1: Table S1). The diagnosis of schizophrenia was confirmed by examining the patient's report according to DSM-III or DSM-IV categories (American Psychiatric Association). Postmortem brains of schizophrenia patients

were collected at Matsuzawa Hospital, Kobe University, Fukushima Medical University and Niigata University, while those of control subjects were collected at Niigata University. In brief, the left cerebral hemisphere was fixed in formalin for diagnostic examination and the right hemisphere was frozen at −80 °C. Tissue samples were taken from postmortem brains that did not exhibit neurodegenerative abnormalities by conventional pathological staining (data not shown). The striatum (caudate) was identified in frozen coronal slices according to a human brain atlas. All tissues were collected and stored according to the principles of the Declaration of Helsinki, and tissue use was in compliance with the Human Tissue Act 2004.

DNA extraction

High molecular weight DNA was extracted by the guanidinium – phenol procedure (Gentra Pure Gene Tissue Kit, Qiagen, Tokyo, Japan) according to the manufacturer's protocol. Extracted DNA was quantified by spectrophotometry using a Nanodrop ND-2000® (Thermo Scientific Wilmington, DE, USA). Samples with absorbance ratios of A260/280 ~ 1.80 and A260/230 > > 1.90, respectively, were regarded as sufficiently pure and suitable for CGH analysis. Some DNA samples were subjected to 1.0 % agarose gel electrophoresis for quality control. Evidence of DNA degradation was not detected in randomly-picked DNA samples from patient or control groups (data not shown).

Comparative genomic hybridization (CGH)

Array-based CGH was performed by the manufacturer Takara Bio Dragon Genomics Center (Seta, Shiga, Japan). In brief, DNA (2 g) was fluorescent-labeled by random priming DNA synthesis in the presence of Cy3-dUTP (control group) or Cy5-dUTP (patient group) (Genomic DNA Enzymatic Labeling Kit; Agilent Technologies, Hachioji, Tokyo, Japan). DNA labeling efficiency was estimated by spectrophotometry (Nanodrop ND-2000®) measuring optical absorbance at 260 nm for DNA, at 550 nm for Cy5, and at 649 nm for Cy3. Cy5- and Cy3-labeled DNAs were randomly paired, mixed, and hybridized to SurePrint G3 Human CGH Microarrays (1 M) in the presence of human Cot-1 DNA (Oligo aCGH/ChIP-on-chip Hybridization Kit, Agilent Technologies). Following hybridization for 24 h, microarray slides were washed according to the manufacturer's instructions and immediately scanned on a DNA Microarray Scanner (Agilent Technologies). With the given limitation of the sample number, we took an advantage of the above direct comparison between case and control samples [57]. This approach allowed us to determine relative ratios of their gene dosages but not their absolute gene dosages. However this procedure decreased data deviations, compared with the CGH analysis utilizing two microarrays and reference genome DNA [30].

Quantitative polymerase chain reaction (qPCR)

To validate the results from the microarray experiments, we performed qPCR using TaqMan probes (Applied Biosystems, Foster City, CA) as described previously [32]. Gene dosages of the following genomic regions of interest were measured for the sample pair sets that exhibited the exclusive positive penetrance call with the Aberration Detection Method 2 (ADM-2) algorithm; CC70L1J (1p13.3), Hs03385437 (1p36.21), Hs04794356 (4q24), Hs05080419 (9q22.2), Hs03318079 (18q21.1), and Hs07134106 (19p12). Using all the samples, we also determined the gene dosages of the candidate CNV loci that exhibited lower probability scores by the global *t*-test analysis; Hs03587795 (6p22.1), Hs03265736 (7p21.3), Hs03765933 (11p15.4), and Hs03298358 (13q21.1). DNA sequences of TaqMan probes and PCR primers are shown in the Additional file 1. We obtained cycle threshold (CT) values for the region of interest for each sample with FAM™-labeled probes, simultaneously monitoring those for *RNaseP* gene (an internal control) with its VIC®-labeled probe (ABI PRISM 7900HT Sequence Detection System and SDS v2.3 software, both Applied Biosystems). These CT values of the target gene and *RNaseP* gene were obtained for all the DNA samples. Copy number of the target gene was estimated from CT values by CopyCaller v1.0 software (Applied Biosystems).

Statistics

The ADM-2 algorithm prompted by Genomic Workbench software (edition 5.0.14, Agilent Technologies, 2010) was used to identify individual and common aberrations for 48 microarray data sets. This algorithm identifies all aberrant intervals with consistently high or low log ratios based on the statistical score. The algorithm searches for intervals where a statistical score based on the average quality-weighted log ratio of the sample and reference channels exceed a user-specified threshold. For the primary screening (Selection 1), we applied the following filtering options to the human genome assembly hg19 (excluding sex chromosomes): sensitivity threshold = 6, fuzzy zero = On, bin size = 10, and centralization threshold = 6. We then selected the primary candidate loci of somatic CNVs which exhibited > =4 difference in gain/loss calls in the whole penetrance summary.

To calculate mean signal OR and the probability of CNVs between groups, we plotted individual log2 signal ratios at all the probe sites within the above candidate loci. The Kolmogorov – Smirnov test revealed that log2 signal ratios were judged to fit into the Gaussian distribution at more than 80 % of probe sites. Assuming their Gaussian distribution, we analyzed their statistical biases

against log2 = 0 (i.e., the null hypothesis of equal signal intensities between patients and controls) by two tailed *t*-test at each probe position. Within a candidate CNV locus containing multiple probe sites, their log2 signal ratios were averaged and probabilities were summed and then subjected to Bonferroni's correction (Selection 2). Statistical difference of qPCR results between individual sample pairs was determined with ANOVA or two tailed *t*-test, incorporating technical errors into account. Alternatively, group differences of qPCR results from individual samples were estimated by the chi-square and Mann–Whitney U tests. Statistical analyses were performed using SPSS software (IBM Japan., Tokyo, Japan).

Additional file

Additional file 1: Figure S1. A flowchart of the study design. **Table S1.** Autopsy and clinical information of the subjects used. **Table S2.** List of candidate CNV regions and their statistical details. **Table S3.** Custom Taqman PCR primers and probes used.

Abbreviations

ADM: Aberration detection method; ANOVA: Analysis of variance; CGH: Comparative genomic hybridization; CNV: Copy number variation; CT: Cycle threshold; FISH: Fluorescent in situ hybridization; OR: Odds ratio; PMI: Postmortem interval; qPCR: Quantitative polymerase chain reaction.

Competing interests

The authors have no conflicts of interest to declare except OS; The author received honoraria from Otsuka Pharmaceutical; Shionogi; GlaxoSmithKline; Eli Lilly; and Tanabe Mitsubishi Pharma, and donations for research from the Otsuka Pharmaceutical Corporation.

Authors' contributions

TS, RK, and HN designed the experiments and wrote the manuscript; M. Sakai, YW, KA, M. Shibuya, AM, and HN performed the experiments; KN, KO, YK, HY, JM, AW, MH, TH, AH NK, SI, OS, KM, SN, HT, and AK collected and examined postmortem tissues. All authors read and approved the final manuscript.

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Supplemental information

M Sakai et al;

Assessment of Copy Number Variations in The Brain Genome of Schizophrenia Patients

Figure S1.

A flowchart of the study design

Table S1.

Autopsy and clinical information of the subjects used

Table S2.

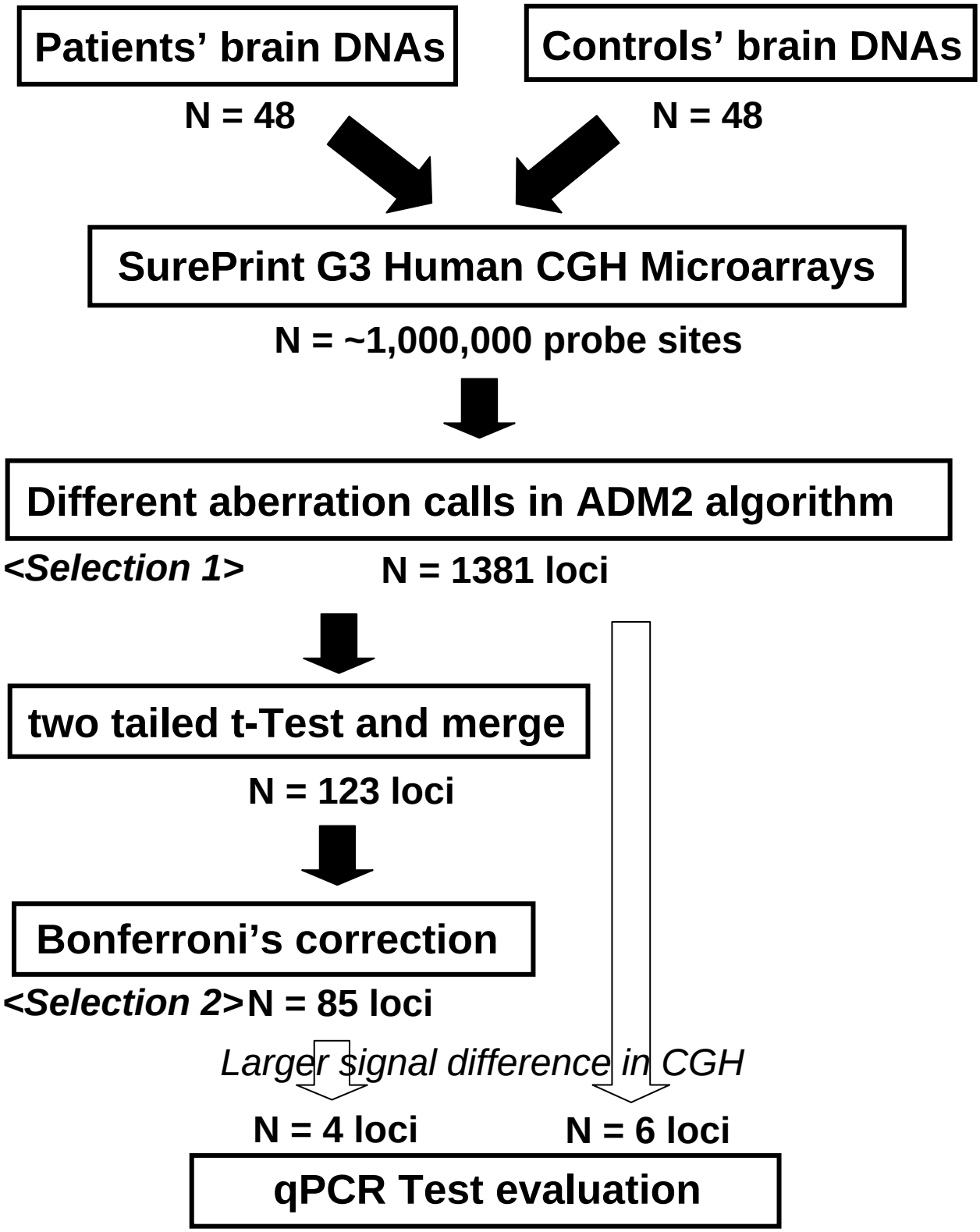
Statistical details of array CGH signals

Table S3.

Custom Taqman PCR primers and probes designed by the authors.

Note; Illumina Co Ltd does not disclose the sequences of the other primers and probes

Figure S1. A flowchart of the study design



Autopsy and clinical information of the subjects used

schizo	gender	age	cause of death	PMI	brain weight
1	M	68	Bleeding	2	UN
2	M	59	Myotonic dystrophy	12	UN
3	F	63	unidentified	2	UN
4	M	72	unidentified	4	UN
5	M	49	gastric cancer	11	UN
6	F	64	decbihypoglicemia	21	1380
7	M	50	renal failure	2	1155
8	F	56	acute heart failure	2	1220
9	M	49	gastric cancer	UN	UN
10	M	59	Myotonic dystrophy	12	1120
11	M	72	unidentified	UN	1310
12	M	51	unidentified	5	1210
13	F	72	liver failure	UN	UN
14	F	62	unidentified	4	1220
15	M	36	unidentified	16	1740
16	M	88	unidentified	UN	1340
17	M	72	ileus	UN	UN
18	F	83	unidentified	3	930
19	F	85	unidentified	UN	UN
20	M	51	renal failure	UN	1500
21	F	72	unidentified	10	1405
22	F	83	unidentified	13	1135
23	M	48	gastric cancer	UN	UN
24	M	70	Bile duct cancer	31	1334
25	M	75	pneumonia	19	1157
26	M	64	pneumonia	6.5	1310
27	F	68	Chronic renal failure	16	1297
28	M	60	acute myocardinal infarction	46	1316
29	F	77	pneumonia	26	1301
30	M	74	pneumonia/heart failure	20.5	1114
31	M	71	pneumonia	17.5	1185
32	M	39	suicide	35	1260
33	M	58	stomach cancer	10	1320
34	M	56	pancreatic cancer	1.5	1280
35	F	53	Cardiac Failure	5	1090
36	F	66	ascending cancer	3.5	1110
37	M	77	Chronic renal failure	5	1305
38	F	76	Pneumonia	8.5	1100
39	F	59	Pneumonia	2.5	1350
40	M	57	pancreatic cancer	45.5	1340
41	M	78	gall stone , pneumonia	13	1355
42	F	70	Pick disease	16.5	1210
43	M	59	unidentified	18.43	1290
44	M	76	unidentified	26.5	1280
45	M	63	colonal cancer	UN	1300
46	M	65	gastric cancer	20	UN
47	F	38	ovarian tumor	UN	UN
48	F	83	unidentified	3	930
	M 30	Mean 64.2	Mean	13.6	1256
	F 18	SD 12.0	SD	11.7	148
			Note; UN represents "unknown"		

Control	gender	age	cause of death	PMI	Brain Weight
1	F	35	Chronic myeloblastic leukemia	2	1255
2	M	67	Hemophagocytosis	1	1220
3	F	49	Sudden death	1	1075
4	M	82	Mixed connective tissue disease	2	1150
5	F	61	Pneumonia	3	1335
6	M	77	Myelodysplastic syndrome	22	1175
7	F	75	Cushing's disease	15	1140
8	F	55	Adult T-cell leukemia	4	1230
9	M	64	Renal failure	3.5	1240
10	M	51	Acute abdomen	5.5	1320
11	M	41	Ducchene muscular dystrophy	4	1380
12	M	48	Myotonic dystrophy	7	1450
13	F	53	Myotonic dystrophy	4	1100
14	M	76	Herpes encephalitis, chronic stage	1.5	1230
15	F	73	Myositis	4	1010
16	M	74	Esophageal cancer	2	1270
17	M	56	Marinesco-Sjagren syndrome	20	1180
18	M	68	Myopathy	4	1220
19	M	71	Multiple small infarcts	3	1280
20	F	44	Gorham's disease	6	1240
21	M	51	pontine hemorrhage	5	1550
22	F	64	polymyositis	2	1150
23	F	48	Myastenia gravis	2	1150
24	M	75	pathymeningitis	2	1105
25	F	70	Multiple cerebral infarcts	2	1105
26	M	68	Cbr.infarction	3	1205
27	M	84	Decrepitude	13	UN
28	M	70	gastric cancer	5	UN
29	M	77	gastric cancer	6	UN
30	M	65	pulmonary infarction	6	UN
31	F	49	Crow-Fukase syndrome	2	1135
32	M	55	myotonic distrophy	4.5	1150
33	F	86	SCA6	2	990
34	F	64	polymiositis	2	1150
35	F	79	Foix-Alajpurnine	2.5	1100
36	M	71	infarcts	UN	1365
37	M	59	Myeloma	2	1280
38	F	60	Myopathy	3	1145
39	M	76	GI bleeding	3.5	1270
40	F	49	Myopathy	3	1070
41	F	42	Fresh muscle necrosis, tetanus	6.5	1115
42	M	70	Multiple fresh infarcts	3.5	1070
43	M	71	Crow-Fukase symdrome	3.5	1065
44	M	72	Myasthenia gravis	2.5	1420
45	M	68	Lung cancer	3	1205
46	M	60	small infarcts	2	1390
47	M	82	Multiple infarcts, gastric cancer	6	1195
48	M	75	pathymeningitis	2	1105
	M 30	Mean 64.2	Mean	4.5	1204
	F 18	SD 12.6	SD	4.4	120

Supple Table S1

Statistical details of array CGH signals

Column Definitions
1) locus; chromosomal region with positive aberrations in aCGH
2) start/stop; nucleotide region showing the positive aberrations in hg 19
3) r; reported, n; not reported; the CNV region is reported or not reported in the Database of Genomic Variant
4) prove; the number of probes showing positive aberrations in the above region
5) (-logp) AVE; the mean of logarithmic probabilities of individual aberrations
6) (-logp) AVE; the standard deviation of logarithmic probabilities of individual aberrations
7) total -logp; cumulative logarithmic probability in which the mean of individual signal ratios is indistinguishable from 1.000
8) log2ratioAVR; averaged logarithmic signal odd ratios for the chromosomal region

	locus	start	stop	reported/not reported	probe	total -logp	(-logp) AVR	(-logp) SD	Log_2 ratio AVR	
Chr1										
1	p36.21	13178469	13178528	r	1	5.02	—	—	-0.34	
2	p36.13	17207777	17241809	r	5	13.10	2.66	1.47	-0.21	
3	p22.2	89263381	89302718	r	11	11.24	3.36	1.44	-0.04	
4	p13.3	109578043	109578102	r	1	4.87	—	—	-0.23	
5	q21.2	149144534	149205091	r	7	18.79	2.68	1.92	0.17	
6	q24.3	172114109	172115477	n	3	20.04	6.68	3.91	0.21	DNM3
7	q31.3	196747506	196796229	r	4	9.22	2.31	0.40	-0.20	
8	q32.1	202346831	202534440	r	19	76.81	4.43	2.27	0.13	
9	q42.13	229812529	229816231	r	4	7.67	1.92	0.32	0.63	
10	q44	248624618	248745861	r	8	19.35	2.42	1.13	-0.20	
Chr2										
1	p22.1	41239485	41240851	r	2	8.05	4.02	3.28	-0.26	
2	q32.1	185754844	185772343	r	3	6.83	4.02	1.90	-0.15	
Chr3										
1	q29	195419168	195478558	r	10	35.76	3.58	1.47	0.25	
Chr4										
1	q24	107065443	107065502	n	1	26.63	—	—	0.33	
2	q26	114345582	119127415	n	4	13.26	3.32	1.35	-0.23	NDST3
3	q31.1	140949472	140955970	n	2	7.68	3.84	3.26	-0.03	
4	q32.2	161861549	161931883	r	10	32.23	3.22	1.30	-0.26	
5	q35.2	190471322	190659488	r	23	6.28	4.48	3.25	-0.004	
Chr5										
1	q22.3	114711767	114721036	n	3	12.63	4.21	2.12	0.18	
2	q23.2	126595033	126595033	n	6	44.09	9.33	2.73	0.25	
3	q35.3	180410254	180424663	r	4	12.65	3.16	3.43	0.32	
Chr6										
1	p22.2	26745191	26753264	r	2	4.66	2.33	0.93	-0.28	
2	p22.1	29854870	29896710	r	7	16.07	2.30	1.43	-0.46	
3	P21.33	31117019	31188599	n	2	8.94	4.47	0.43	0.18	
4	p21.33	31281393	31451139	r	17	24.58	3.96	3.57	-0.09	
5	p21.32	32525713	32634848	r	9	11.01	2.08	0.62	0.26	
6	p21.31	35323974	35767193	r	9	33.12	3.68	2.05	0.24	
7	p11.2	57436765	57581950	n	31	44.95	6.54	6.69	0.03	
8	q13	74709760	74711681	r	2	4.97	2.48	0.70	0.21	

Supple Table S2-2

	locus	start	stop	reported/not reported	probe	total -logp	(-logp) AVR	(-logp) SD	Log_2 ratio AVR	
Chr7										
1	p22.2	3440049	3441459	n	2	15.48	7.74	8.00	-0.16	
2	p21.3	11313242	11342156	n	8	16.51	2.91	2.53	-0.06	SDK1
3	q11.22 q11.23	72026466	72315358	r	29	77.81	4.01	2.09	-0.08	
4	q21.13	90186224	90220600	r	11	18.30	4.06	1.84	0.05	
5	q31.1	111035795	111037250	r	2	8.73	4.37	4.11	-0.21	
6	q31.32	121165229	121175668	r	4	21.07	5.27	2.52	-0.20	
7	q34	141766883	141786368	r	2	4.71	2.36	1.06	-0.74	
8	q36.1	149779296	149859892	n	9	43.09	5.09	4.54	-0.16	
9	q36.2	153672608	153742314	r	6	17.26	3.81	1.11		
Chr8										
1	p23.2	3734470	3816456	r	26	79.40	3.23	2.01	-0.14	
2	p22	15403439	15413466	r	3	15.73	5.24	0.58	-0.31	
3	p21.2	25411694	25414740	r	3	13.14	4.38	2.75	-0.29	
4	q12.3	63352266	63397172	n	11	21.40	2.49	1.02	0.10	
5	q21.11	77161734	77188957	n	2	9.68	4.84	1.07	0.30	
6	q21.13	83918000	83929768	n	2	5.99	3.00	2.52	-0.15	
7	q23.3	112323159	112340290	n	3	8.18	2.73	1.88	0.23	
Chr9										
1	p12	43590080	43686974	r	3	6.40	2.13	1.27	0.25	
2	q22.2	93564261	93564318	n	4	27.64	6.48	0.33	-0.23	SKY
Chr10										
1	q11.2	46969728	46971695	r	2	16.03	8.01	3.16	0.20	
2	q11.2	47610149	47697197	n	12	10.58	3.55	1.36	0.02	ANTXRL
Chr11										
1	p15.5 p15.4	262349	3167131	r	746	3470.71	6.59	5.93	0.08	
2	p15.4	4970007	4975697	r	5	9.49	1.90	0.36	-0.98	
3	p15.1	18949929	18960666	r	4	9.02	2.25	0.55	0.22	
4	q11	55433570	55444227	r	2	10.42	5.21	1.95	-0.12	
Chr12										
1	p13.2	10574520	11249210	r	6	15.76	3.15	2.30	-0.18	
2	q21.31 q21.32	86695679	86703030	r	3	7.19	2.40	0.79	-0.16	
3	q23.2	102477092	102477151	n	4	8.86	2.22	0.50	-0.18	
4	q24.33	131797898	131802404	r	2	8.87	4.44	4.10	0.16	
Chr13										
1	q21.1	57759370	57783723	r	13	34.53	2.66	0.77	-0.62	
Chr14										
1	q11.2	24267085	24271075	r	3	7.70	2.57	0.94	-0.14	
2	q32.33	106602112	106727130	r	5	24.90	4.98	4.35	-0.37	

Supple Table S2-3

	locus	start	stop	reported/not reported	probe	total -logp	(-logp) AVR	(-logp) SD	Log_2 ratio AVR	
Chr15										
1	q11.1	20549990	20616722	r	5	13.02	2.60	1.57	0.27	
2	q11.2	20863313	22551703	r	61	122.55	2.54	2.01	0.25	
3	q13.2	30652489	31043038	r	14	22.63	2.87	1.30	-1.46	
4	q14	34730547	34794497	r	10	25.88	2.59	1.06	-0.32	
5	q15.3	43895633	43895690	n	1	4.84	—	—	0.34	
6	q21.2	49652586	49684919	n	10	25.11	3.79	2.18	-0.05	
7	q26.3	102480829	102480888	r	1	4.79	—	—	-0.20	
Chr16										
1	p12.2	21475039	21593631	r	4	15.96	3.99	3.68	-0.18	
2	p12.2	22645706	22697409	r	5	17.59	3.52	1.89	-0.21	
3	p11.2	32471625	33923140	r	10	21.26	2.13	0.46	0.07	
4	p11.1	35024449	35055525	n	6	18.51	3.08	1.36	0.06	
5	q12.2	55806212	55811275	r	3	24.34	8.11	3.31	0.23	
6	q22.1	70052979	70280942	r	7	26.90	3.84	2.25	-0.13	
7	q22.2	72083919	72097048	r	3	13.15	4.38	1.72	0.19	
8	q23.1	77206239	77224028	r	4	15.35	3.84	2.07	-0.15	
Chr17										
none										
Chr18										
1	q11.2	24756700	24758882	n	2	4.45	2.23	1.01	0.13	CHST9
2	q22.1	66742452	66742511	n	1	10.50	—	—	-0.19	
Chr19										
1	p12	20718947	20719006	n	1	7.48	—	—	-0.19	
2	q13.33	49183320	49206305	n	4	19.14	6.20	8.17	-0.19	
3	q13.41	53328041	53352736	r	5	16.04	3.71	1.54	-0.15	
4	q13.42	54732917	54742404	n	3	23.68	9.59	6.80	0.17	
Chr20										
1	p13	1573410	1583221	r	1	4.77	1.59	0.06	-0.77	
Chr21										
none										
Chr22										
1	q11.23	25664618	25667114	r	2	4.63	2.31	1.23	0.18	
2	q13.1	39291820	39385485	r	11	21.87	2.31	1.24	0.33	
3	q13.2	42896699	42907693	r	2	7.74	7.29	5.47	0.10	

Supple Table S3

Custom Taqman PCR primers and probes used

Oligo DNA sequences in Figure 2

chromosome		start	stop	Assay number	RT-PCR assay	DNA sequence
chr6	p22.1	29854870	29896710	Hs03587795_cn	recepter	CAGGAGAATGTTCCTGCTGAGGACA
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr7	p21.3	11313242	11342156	Hs03265736_cn	recepter	GATGAGAAATTTTGCCGCTGTTTGA
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr11	p15.4	4970007	4975697	Hs03765933_cn	recepter	TACTGCCTATTTGTCTGCCCACGAT
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr13	q21.1	57759370	57783723	Hs03298358_cn	recepter	GGCAGACAAGTATGGGTGCTAAATC
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished

Oligo DNA sequences in Figure 3

chromosome		start	stop	Assay number	RT-PCR assay	DNA sequence
chr1	p13.3	109578043	109578102	Hs03385437_cn	recepter	GGTAAGCCTTCCTACTCCAGTCAGG
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr1	p36.21	13178469	13178528	CC70L1J	recepter	CCTAGATAGTGTTCCAGACTCCC
					F primer	CCATCTACATAATGCATGTCAAACCAA
					R primer	GCCTAGAAAACATTAGTGTAACTGGGT
chr4	q24	107065443	107065502	Hs04794356_cn	recepter	TGTCCCAAATTTGGAACTGATATAC
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr9	q22.2	93564261	93564318	Hs05080419_cn	recepter	AAAATACAGGTGGGTTCCGCCAGCT
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr18	q22.1	66742452	66742511	Hs03318079_cn	recepter	GAACTGGGAATACGACTCCTTGAGG
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished
chr19	p12	20718947	20719006	Hs07134106_cn	recepter	TTTTATCCGCTTCACTTCCATTCAC
					F primer	AppliedBiosystems designed but unpublished
					R primer	AppliedBiosystems designed but unpublished