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Chromosome 15q11-13 duplication syndrome brain reveals epigenetic alterations in gene expression not predicted from copy number

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Abstract

Background—Chromosome 15q11-13 contains a cluster of imprinted genes essential for normal mammalian neurodevelopment. Deficiencies in paternal or maternal 15q11-13 alleles result in Prader-Willi or Angelman syndromes, respectively, and maternal duplications lead to a distinct condition that often includes autism. Overexpression of maternally expressed imprinted genes is predicted to cause 15q11-13-associated autism, but a link between gene dosage and expression has not been experimentally determined in brain.

Methods—Post-mortem brain tissue was obtained from a male with 15q11-13 hexasomy and a female with 15q11-13 tetrasomy. Quantitative RT-PCR was used to measure ten 15q11-13 transcripts in maternal 15q11-13 duplication, Prader-Willi syndrome, and control brain samples. Southern blot, bisulfite sequencing and fluorescence *in situ* hybridization were used to investigate epigenetic mechanisms of gene regulation.

Results—Gene expression and DNA methylation correlated with parental gene dosage in the male 15q11-13 duplication sample with severe cognitive impairment and seizures. Strikingly, the female with autism and milder Prader-Willi-like characteristics demonstrated unexpected deficiencies in the paternally expressed transcripts *SNRPN*, *NDN*, *HBI185*, and *HBI52* and unchanged levels of maternally expressed *UBE3A* compared to controls. Paternal expression abnormalities in the female duplication sample were consistent with elevated DNA methylation of the 15q11-13 imprinting control region (ICR). Expression of nonimprinted 15q11-13 GABA receptor subunit genes was significantly reduced specifically in the female 15q11-13 duplication brain without detectable *GABRB3* methylation differences.

Conclusion—Our findings suggest that genetic copy number changes combined with additional genetic or environmental influences on epigenetic mechanisms impact outcome and clinical heterogeneity of 15q11-13 duplication syndromes.

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Keywords

epigenetics; imprinting; autism; neurodevelopmental; DNA methylation

Low copy repeats at five common breakpoints (BP1-BP5) predispose chromosome 15 to genomic rearrangements including both deletions and duplications [1] (Figure 1A). The 4 MB genomic region between BP2 and BP3 contains multiple imprinted genes, so the parental origin of the rearrangement influences phenotype. Paternal deletions of 15q11-13 result in Prader-Willi syndrome [MIM 175270], a neurodevelopmental disorder characterized by mild cognitive impairment, hyperphagia-mediated obesity, small hands and feet, and compulsive behaviors such as hoarding and skin picking [2]. Maternal deletions result in Angelman syndrome [MIM 105830], a distinct neurodevelopmental disorder with severe mental retardation, lack of speech, ataxia, and stereotypic smiling and laughing behaviors [3].

Maternal duplications occurring as interstitial duplications and as supernumerary isodicentric chromosomes called idic(15) lead to a variable neurodevelopmental disorder with many autistic features [4]. Despite incomplete penetrance of autism in 15q11-13 duplication syndrome, this duplication is the leading cytogenetic cause of autism, occurring in 1-3% of autism cases [5]. The parent of origin effect observed in 15q11-13 duplication syndromes, together with the elevated risk for autism in PWS maternal uniparental disomy cases [6] has led to the hypothesis that overexpression of maternally expressed imprinted genes causes the autistic features in these individuals [7]. Consistent with this hypothesis, fibroblasts and lymphoblasts containing maternal 15q11-13 duplications have been shown by Northern blot and gene expression profiling to have increased expression of the imprinted gene *UBE3A* [8,9,10]. Despite this result, many of the transcripts in 15q11-13 are expressed exclusively in the central nervous system [11,12] therefore investigation of gene expression in brain is required to fully understand the implications of excess 15q11-13 dosage on gene expression and clinical outcome.

In order to investigate the effect of chromosome 15q11-13 duplication on epigenetic and gene expression patterns, we performed fluorescence in situ hybridization (FISH), quantitative reverse-transcriptase PCR (qRT-PCR), and DNA methylation analyses on post-mortem cerebral cortex samples from two individuals with increased 15q11-13 dosage. Our findings demonstrate different epigenetic outcomes of the two brain samples and suggest that the imbalance of 15q11-13 dosage can disrupt normal parental homolog pairing, DNA methylation patterns, and gene expression patterns within 15q11-13.

CLINICAL REPORTS

Case 7014

A detailed clinical description of Case 7014 has been reported by Mann *et al* [13] (see Case 2). In brief, this child suffered from severe intractable epilepsy beginning with infantile spasms at age 3-4 months and evolving into myoclonic and grand mal seizures that persisted throughout his life. He had marked hypotonia in infancy and severe developmental delays and cognitive impairment with a mental age of 4 months based on the Mullen Scales of Early Learning, performed at age 5 year 5 months. He did not have head control, was nonverbal and nonambulatory. The severity of his cognitive impairment precluded diagnosis of autism using the Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule-Generic. At the time of his death at 11 years, his seizures were poorly controlled with a combination of phenobarbital, Keppra, valproic acid, and a vagal nerve stimulator.

Case 6856

Case 6856 was a 26 year old female with a history of developmental delays, autism, cognitive impairment, and seizures. She was described as a hypotonic infant who had difficulties nursing and had delayed acquisition of gross and fine motor milestones. The only dysmorphic features described were bilateral epicanthic folds, hypotonia and lower limb spasticity (Figure 1B). Her speech and language also were particularly delayed with the onset of phrase speech at 60 months of age, with the content of her early speech frequently consisting of memorized movie dialogue. In childhood, she developed hoarding behaviors directed towards toys and other nonfood items. She also began having unusual behaviors toward food including episodes of binge eating and taking food from other people's plates. This resulted in excessive weight gain in adolescence and young adulthood (maximum weight 84.5 kg, height 1.63m; BMI 32.1). She was also noted to pick at skin lesions and had increased tolerance to pain and temperature. She had a number of compulsive and insistence on sameness behaviors, such as keeping doors closed and strict preferences for specific items of clothing. She was described as a happy and relatively confident young woman who did not have significant problems with anxiety, mood, or sleep and had no aggressive behaviors. She had generalized epilepsy that began at age 16 years, treated with carbamazepine. On medication, seizures were infrequent but occurred at night or in the early morning. She was evaluated formally at age 19y 7 mos using the Autism Diagnostic Interview-Revised and Autism Diagnostics Observation Scales-Generic and met strict criteria for autism on both measures. Her IQ was 36 based on the Stanford-Binet Intelligence Scale: Fourth Edition and using the Clinical Evaluation of Language Fundamental-Revised (CELF-R), she achieved age equivalent of 7 years 6 mos on both receptive and expressive language.

METHODS

Cytogenetic and Molecular Diagnostics

Detailed clinical characterization of Case 7014 reported previously [13] revealed the presence of a trisomic derivative chromosome 15 of maternal origin. Schematically shown in Figure 2A, this chromosome contains four copies of 15q11-13 with BP3 distal boundaries. A karyotype done on Case 6856 at age 4 years identified the presence of her supernumerary idic(15) chromosome. Subsequent DNA fluorescence *in situ* hybridization (DNA FISH) studies performed clinically revealed the karyotype 47,XX,+idic(15)(q13;q13).ish(15)(D15Z++;D15S10++). Genotyping of 27 markers in the proband and both parents by methods described previously [14] demonstrated that the derivative chromosome is maternal in origin (Table 1).

Array comparative genomic hybridization using both BAC [15] and NimbleGen oligonucleotide arrays, and additional DNA FISH with probes from the 15q11-13 interval were performed as described [13] and revealed her idic(15) contains a BP4:BP5 duplication (Figure 1C and 2B).

Human Sample Preparation

Cerebral cortex samples (Brodmann Area 9) were obtained frozen from the Autism Tissue Program. Detailed descriptions of samples with cause of death and post-mortem intervals are listed in Supplementary Table 1. Cause of death in patients with 15q11-13 duplications was sudden, unexpected and likely resulted from complications with seizures. All brain tissue was stored at -80°C until use. RNA and protein were isolated with TRIzol reagent (Invitrogen) according to manufacturer's protocol. Quality and concentration of RNA was verified using the Nanodrop® D-1000 spectrophotometer. Protein concentrations were measured with the BCA™ protein assay kit (Pierce).

DNA FISH

DNA fluorescence *in situ* hybridization was performed on formalin fixed 5 micron sections of cerebral cortex according to previously described methods [16]. A BAC contig probe (RP11-974L14, RP11-89E18, RP11-243J20, and RP11-688O20) mapping within the 15q11-13 GABA_A receptor gene cluster was used to verify the copy number of 15q11-13 alleles in neurons. Pairing of 15q11-13 homologues was scored as signals less than 2 microns apart. 100 neurons with all 15q11-13 alleles visible were scored per case. Parental 15q11-13 alleles were differentiated by a *SNRPN* BAC contig probe (RP11-125E1, RP11-186C7, RP11-171C8, RP11-1081A4) that displays an extended signal on the paternal allele (Leung et al *in preparation*).

Gene Expression Analysis

High quality RNA was treated with DNase I (Invitrogen) to eliminate any residual genomic DNA in the RNA preparation. cDNA was synthesized from 1.2 ug of high quality RNA using oligo-d(T) primer and the First-Strand cDNA synthesis kit (Roche). For each individual analyzed, reactions with and without Reverse Transcriptase (+/- RT) were synthesized and -RT samples were tested by PCR to ensure genomic DNA contamination was not present. Where possible, primers were designed to span at least one intron/exon boundary. Primers designed to amplify 15q11-13 transcripts and two housekeeping genes, *GAPDH* and *ACTB*, are listed in Supplementary Table 2 and primers used for assaying the HBII52 and HBII85 snoRNAs were obtained from Runte *et al* [17].

Quantitative RT-PCR was performed with FastStart DNA Master SYBR Green reagents (Roche) on the MasterCycler RealPlex thermocycler (Eppendorf). For each gene analyzed, 3-5 replicate reactions were completed per individual: controls (n = 6), PWS maternal uniparental disomy (PWS UPD, n = 2), PWS deletion (PWS Del, n = 2). Melting curve analysis was performed to ensure that a single product was amplified with each primer set. Crossing point values for 15q11-13 transcripts were normalized to *GAPDH* or *ACTB* using the comparative CT method (Applied Biosystems). The Mann-Whitney test was used to determine statistically significant differences in expression. GABRB3 immunoblots were performed according to previously described methods [18].

DNA Methylation Analysis

Methylation analysis of *SNRPN* was performed by Southern blot using methyl-sensitive restriction enzymes according to previously described methods [13]. Bisulfite sequencing of genomic brain DNA, isolated from frozen tissue by the Puregene® DNA isolation kit (Qiagen) was performed as previously described [18] with the following modifications. Briefly, 1 ug of genomic DNA was converted with the EZ DNA Methylation-Gold kit (Zymo). Primers spanning the imprinting control region (ICR) in the 5' end of *SNRPN* were designed using Methprimer (www.urogene.org/methprimer/index1.htm) and are as follows: Forward: GGTGGTTTTTTTAAAGAGATAGTTTGGG, Reverse: CATCCCCCTAATCCACTACCATAAC. Methylation specific PCR was performed on 1-2.5 ug of bisulfite converted genomic DNA from brain as described [19] and normalized using the average ratio of methylated to unmethylated products obtained in two normal control lymphocyte samples.

RESULTS

Since clinical diagnostics were performed on peripheral tissue, DNA fluorescence *in situ* hybridization was used to confirm excess 15q11-13 dosage in brain tissue. Neurons containing normal maternal and paternal alleles and the supernumerary derivative chromosomes

confirmed the 15q11-13 hexasomy and tetrasomy as shown in Figure 2A and 2B. Homologous associations of maternal and paternal 15q11-13 alleles have been previously described in lymphoblasts [20] as well as neurons [16] therefore we investigated the impact of isodicentric and isotricentric chromosomes on normal maternal to paternal 15q11-13 interactions. Figures 2C and 2D represent the distribution of paired 15q11-13 alleles for 100 neurons in each individual. Interestingly, the derivative chromosomes paired nonselectively with the normal maternal and paternal alleles in both cases.

Maternally Expressed Genes

To determine the effect of increased 15q11-13 dosage on gene expression in brain, quantitative RT-PCR was used to measure the levels of 10 transcripts in the critical duplication region between BP2 and BP3 and two non-15q11-13 housekeeping gene controls, *GAPDH* and *ACTB*. In addition to age and gender-matched controls with normal biparental 15q11-13 dosage, Prader-Willi syndrome samples with deletions (PWS Del) and maternal uniparental disomy (PWS UPD) were used to assess expected gene expression levels for a single maternal allele and two maternal alleles. Gene expression analysis of the maternally expressed imprinted gene *UBE3A* revealed the expected 4-5-fold increase in transcript abundance in Case 7014, but no difference in *UBE3A* expression was observed in Case 6856 compared to controls (Figure 3B). *ATP10A* has been described as exhibiting similar imprinted gene expression to *UBE3A* [21, 22], however imprinting of the mouse orthologue of *ATP10A* has been disputed, and may be dependent on genetic background [23, 24, 25]. Surprisingly, reduced *ATP10A* transcript levels in PWS UPD samples (Figure 3C) and biallelic expression in control brain samples (Hogart et al *in preparation*) is consistent with incomplete imprinting of *ATP10A* in humans. *ATP10A* levels were increased by 3-fold as expected based on maternal dosage in Case 6856, but comparable to control levels in Case 7014, suggesting influences other than maternal origin contribute to expression levels.

Paternally Expressed Genes

Gene expression analysis of paternally expressed imprinted genes revealed that both normal and abnormal paternal gene expression can arise from excess maternal 15q11-13 dosage (Figure 3 D-G). With the exception of subtly elevated expression of *NDN* (Figure 3D), Case 7014 paternal transcript levels were similar to control levels. In contrast, Case 6856 demonstrated significant deficiencies in paternal gene expression for all genes analyzed. While unexpected based on parental copy number, the paternal gene expression deficiencies of Case 6856 were consistent with her Prader-Willi-like features, such as hypotonia, obesity, binge eating, skin picking, and hoarding behaviors.

The imprinted genes of 15q11-13 are under the control of a common regulatory sequence, the imprinting control region (ICR), which is a differentially methylated CpG island at the 5' end of *SNRPN* (shown schematically in Figure 3A) that is heavily methylated on the silent maternal allele and unmethylated on the active paternal allele [26]. To investigate the mechanism for paternal gene dysregulation in Case 6856, DNA methylation of the PWS ICR was examined. While previously published quantitative Southern blot analysis of the ICR in Case 7014 revealed the expected maternal:paternal ratio of 4.8:1 [13], the ratio obtained for idic 6856 was 4.35:1, higher than the expected value of 3:1 (Figure 4A). Similarly, methylation-sensitive PCR analysis also hypermethylation compared to the expected 3:1 methylated:unmethylated alleles in Case 6856 brain DNA (data not shown).

Bisulfite sequencing was performed to determine the extent of methylation on individual strands of DNA. Sequencing results of multiple genomic brain DNA clones for Case 6856 and Case 7014 are shown in figures 4B and 4C, respectively. Bisulfite sequencing of PWS brain DNA revealed that silent maternal alleles have a range from 82%-100% methylation (Figure

4D). Interestingly, all methylated DNA strands sampled from Case 6856 are within the range of fully silenced maternal alleles (Figure 4D), suggesting that aberrant complete methylation of the paternal ICR in some cells may explain the paternal gene expression deficiencies.

GABA_A Receptor Genes

The three nonimprinted biallelically expressed 15q11-13 GABA_A receptor subunit genes, *GABRB3*, *GABRA5*, and *GABRG3* are attractive candidate genes for idiopathic autism as *Gabrb3* null mice exhibit behaviors consistent with autism [27], and multiple genetic studies have found significant evidence for association [28]. Furthermore, significantly reduced *GABRB3* protein levels in some autistic brain samples suggests that dysregulation of the GABA inhibitory pathway may play a major role in a subset of autism cases [29]. While these genes are normally expressed equally from both maternal and paternal alleles in control brain, *trans* effects that were described previously [16] affect normal biallelic expression [18], providing the potential for extra maternal alleles to influence gene expression from normal *GABRB3* alleles. Consistent with previous observations, both PWS UPD and PWS deletion brain samples had significantly reduced *GABRB3* expression compared to control samples with biparental 15q11-13 alleles (Figure 3H) [18].

Surprisingly, the two 15q11-13 maternal duplication samples showed opposite directional changes in GABA receptor genes, although gene expression changes were consistent between all three GABA receptor genes for each individual. As expected for nonimprinted genes, *GABRB3*, *GABRA5*, and *GABRG3* levels were significantly elevated in Case 7014 compared to controls and PWS samples (Figure 3H-K). In striking contrast, transcript levels for each of the three GABA receptor subunit genes were significantly decreased in Case 6856 to 10% of control levels (Figure 3H-K) despite genetic tetrasomy at these loci. To determine if the transcriptional dysregulation has functional consequences at the level of *GABRB3* protein, we performed semi-quantitative immunoblot analysis of cerebral cortex samples. Consistent with qRT-PCR results, *GABRB3* protein was reduced in Case 6856 compared to controls, while Case 7014 had elevated *GABRB3* (Figure 5A). The protein data suggest that transcriptional dysregulation in both cases was moderately attenuated at the translational level, as the differences compared to controls were much less profound than was observed by qRT-PCR. Interestingly, while DNA methylation abnormalities occurred in the ICR and correlated with abnormal paternal gene expression, methylation patterns in the 5' end of *GABRB3* in Case 6856 were consistent with control patterns (Figure 5B).

DISCUSSION

Increased dosage of the PWS/AS critical gene region between BP2 and BP3 positively correlates with phenotypic severity in patients with 15q11-13 duplications, however clinical heterogeneity in patients is not explained by variations in breakpoints [30], suggesting that additional factors contribute to clinical complexity. In this study we have quantitatively measured expression of 10 transcripts located in the critical region from two different post-mortem 15q11-13 duplication syndrome brain samples. Our gene expression findings revealed striking differences in expression outcomes, with Case 7014 exhibiting expected alterations in gene expression based on dosage and Case 6856 demonstrating significantly decreased expression of *SNRPN*, *NDN*, snoRNAs, and GABA_A receptor transcripts despite increased maternal dosage. While lack of expression of the GABA_A receptor genes, *NDN*, *HBII85*, and *HBII52* in lymphoblasts precluded detection in previous gene expression profiling studies of 15q11-13 duplication samples, these studies also failed to detect significant alterations in *SNRPN* [9,10]. Differences in the sensitivity of the techniques, expression differences between brain and blood, or individual heterogeneity (neither Case 7014 nor Case 6856 were analyzed in previous studies) may explain the different outcomes. Defects in paternal gene expression

in Case 6856 correlate with increased methylation at the ICR, suggesting that alterations in epigenetic regulation contribute to gene expression abnormalities. Interestingly, PWS-like features were observed in Case 6856 and have been documented in clinical descriptions of other patients with increased dosage of maternal and paternal 15q11-13 alleles [30,31]. As additional brain samples become available for research, additional studies will be necessary to determine the frequency of deviations from expected gene expression patterns in individuals with 15q11-13 duplications as well as potential brain regional differences.

The three 15q11-13 GABA_A receptor subunit genes were significantly dysregulated in both Case 7014 and Case 6856. Notably, both cases had histories of epilepsy although their clinical courses and management were remarkably different, with Case 7014 having numerous daily myoclonic seizures and Case 6856 having infrequent generalized tonic clonic seizures. As functional GABA_A receptors are formed through heteropentameric assemblies of alpha, beta, and gamma subunits with distinct spatial and temporal expression, overexpression or underexpression of subunits likely impairs the function of this important inhibitory pathway [32]. Although we did not find evidence for aberrant promoter DNA methylation causing the significantly reduced levels of *GABRB3* in Case 6856, it is possible that other regulatory epigenetic alterations, such as long-range chromatin organization or homologous 15q11-13 pairing, led to transcriptional down regulation of the GABA_A receptor subunit genes in this individual. Future studies focused on elucidating the factors that regulate 15q11-13 GABA_A receptor subunit genes are needed as dysregulation of these genes likely contributes to the pathogenesis of the 15q11-13 deletions and duplications, as well as idiopathic autism.

Molecular investigation of gene expression in brain samples with extra copies of 15q11-13 provides insight into the potential complexities of other copy number variations in autism [33,34]. Extra copies of genes are predicted to lead to increased expression, however our study revealed that gene expression can be altered in unexpected ways through epigenetic changes. Epigenetic differences between individuals with the same genetic copy number variation could be stochastic, environmentally determined, or influenced by genetic background. Although more samples are needed before broader conclusions can be made, we speculate that compensatory epigenetic alterations led to gene expression changes and distinct clinical features in Case 6856. These findings bring to light the possibility that gene expression changes beyond the expected maternally expressed imprinted genes contribute to the variability in phenotypes in 15q11-13 duplication syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Robinson WP, Dutly F, Nicholls RD, et al. The mechanisms involved in formation of deletions and duplications of 15q11-q13. *J Med Genet* 1998;35(2):130–6. [PubMed: 9580159]
2. Bittel DC, Butler MG. Prader-Willi syndrome: clinical genetics, cytogenetics and molecular biology. *Expert Rev Mol Med* 2005;7(14):1–20. [PubMed: 16038620]
3. Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. *J Med Genet* 2003;40(2):87–95. [PubMed: 12566516]
4. Cook EH Jr, Lindgren V, Leventhal BL, et al. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet* 1997;60(4):928–34. [PubMed: 9106540]
5. Schroer RJ, Phelan MC, Michaelis RC, et al. Autism and maternally derived aberrations of chromosome 15q. *Am J Med Genet* 1998;76(4):327–36. [PubMed: 9545097]
6. Milner KM, Craig EE, Thompson RJ, et al. Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. *J Child Psychol Psychiatry* 2005;46(10):1089–96. [PubMed: 16178933]
7. Bolton PF, Dennis NR, Browne CE, et al. The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. *Am J Med Genet* 2001;105(8):675–85. [PubMed: 11803514]
8. Herzing LBK, Cook EH, Ledbetter DH. Allele-specific expression analysis by RNA-FISH demonstrates preferential maternal expression of UBE3A and imprint maintenance within 15q11-q13 duplications. *Hum Mol Genet* 2002;11(15):1707–18. [PubMed: 12095913]
9. Baron CA, Tepper CG, Liu SY, et al. Genomic and functional profiling of duplicated chromosome 15 cell lines reveal regulatory alterations in UBE3A-associated ubiquitin-proteasome pathway processes. *Hum Mol Genet* 2006;15(6):853–69. [PubMed: 16446308]
10. Nishimura Y, Martin CL, Vazquez-Lopez A, et al. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. *Hum Mol Genet* 2007;16(14):1682–98. [PubMed: 17519220]
11. Jay P, Rougeulle C, Massacrier A, et al. The human necdin gene, NDN, is maternally imprinted and located in the Prader-Willi syndrome chromosomal region. *Nat Genet* 1997;17(3):357–61. [PubMed: 9354807]
12. Cavaille J, Buiting K, Kieffmann M, et al. Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. *Proc Natl Acad Sci U S A* 2000;97(26):14311–6. [PubMed: 11106375]
13. Mann SM, Wang NJ, Liu DH, et al. Supernumerary trisomic derivative chromosome 15 in two boys with intractable epilepsy: another mechanism for partial hexasomy. *Hum Genet* 2004;115(2):104–11. [PubMed: 15141347]
14. Wang NJ, Parokony AS, Thatcher KN, et al. Multiple forms of atypical rearrangements generating supernumerary derivative chromosome 15. *BMC Genet* 2008;9(2)
15. Wang NJ, Liu D, Parokony AS, et al. High-resolution molecular characterization of 15q11-q13 rearrangements by array comparative genomic hybridization (array CGH) with detection of gene dosage. *Am J Hum Genet* 2004;75(2):267–81. [PubMed: 15197683]
16. Thatcher KN, Peddada S, Yasui DH, et al. Homologous pairing of 15q11-13 imprinted domains in brain is developmentally regulated but deficient in Rett and autism samples. *Hum Mol Genet* 2005;14(6):785–97. [PubMed: 15689352]
17. Runte M, Huttenhofer A, Gross S, et al. The IC-SNURF-SNRPN transcript serves as a host for multiple small nucleolar RNA species and as an antisense RNA for UBE3A. *Hum Mol Genet* 2001;10(23):2687–700. [PubMed: 11726556]
18. Hogart A, Nagarajan RP, Patzel KA, et al. 15q11-13 GABAA receptor genes are normally biallelically expressed in brain yet are subject to epigenetic dysregulation in autism-spectrum disorders. *Hum Mol Genet* 2007;16(6):691–703. [PubMed: 17339270]
19. Kubota T, Das S, Christian SL, et al. Methylation-specific PCR simplifies imprinting analysis [letter]. *Nat Genet* 1997;16(1):16–7. [PubMed: 9140389]
20. LaSalle J, Lalande M. Homologous association of oppositely imprinted chromosomal domains. *Science* 1996;272:725–8. [PubMed: 8614834]

21. Herzing LBK, Kim S-J, Cook EH, et al. The human aminophospholipid-transporting ATPase gene ATP10C maps adjacent to UBE3A and exhibits similar imprinted expression. *American Journal of Human Genetics* 2001;68(6):1501–5. [PubMed: 11353404]
22. Meguro M, Kashiwagi A, Mitsuya K, et al. A novel maternally expressed gene, ATP10C, encodes a putative aminophospholipid translocase associated with Angelman syndrome. *Nat Genet* 2001;28(1):19–20. [PubMed: 11326269]
23. Kayashima T, Ohta T, Niikawa N, et al. On the conflicting reports of imprinting status of mouse ATP10a in the adult brain: strain-background-dependent imprinting? *J Hum Genet* 2003;48(9):492–3. [PubMed: 12955587]author reply 4
24. Kayashima T, Yamasaki K, Joh K, et al. Atp10a, the mouse ortholog of the human imprinted ATP10A gene, escapes genomic imprinting. *Genomics* 2003;81(6):644–7. [PubMed: 12782135]
25. Kashiwagi A, Meguro M, Hoshiya H, et al. Predominant maternal expression of the mouse Atp10c in hippocampus and olfactory bulb. *J Hum Genet* 2003;48(4):194–8. [PubMed: 12730723]
26. Sutcliffe JS, Nakao M, Christian S, et al. Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. *Nat Genet* 1994;8:52–8. [PubMed: 7987392]
27. Delorey TM, Sahbaie P, Hashemi E, et al. Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: A potential model of autism spectrum disorder. *Behav Brain Res* 2008;187(2):207–20. [PubMed: 17983671]
28. Freitag CM. The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry* 2007;12(1):2–22. [PubMed: 17033636]
29. Samaco RC, Hogart A, LaSalle JM. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. *Hum Mol Genet* 2005;14:483–92. [PubMed: 15615769]
30. Dennis NR, Veltman MW, Thompson R, et al. Clinical findings in 33 subjects with large supernumerary marker(15) chromosomes and 3 subjects with triplication of 15q11-q13. *Am J Med Genet A* 2006;140(5):434–41. [PubMed: 16470730]
31. Ungaro P, Christian SL, Fantes JA, et al. Molecular characterisation of four cases of intrachromosomal triplication of chromosome 15q11-q14. *J Med Genet* 2001;38(1):26–34. [PubMed: 11134237]
32. Steiger JL, Russek SJ. GABAA receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol Ther* 2004;101(3):259–81. [PubMed: 15031002]
33. Sebat J, Lakshmi B, Malhotra D, et al. Strong association of de novo copy number mutations with autism. *Science* 2007;316(5823):445–9. [PubMed: 17363630]
34. Marshall CR, Noor A, Vincent JB, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008;82(2):477–88. [PubMed: 18252227]

Figure 1.

Ideograms and DNA fluorescence *in situ* hybridization (FISH) showing chromosome 15 duplications. A) Ideogram of the acrocentric normal chromosome 15 with relative positions of five common breakpoints (BP) indicated on the right. Position of the DNA FISH probe, located within the GABA_A receptor gene cluster, is indicated with a red line in between BP2 and BP3. B) Photo of Case 6856 showing epicanthal folds, exotropia, and moderate obesity. C) Array comparative genomic hybridization of Case 6856 genomic DNA to an oligonucleotide array with chromosome 15 probes shows tetrasomy for genomic sequences through BP4 and trisomy for genomic sequences between BP4 and BP5.

Figure 2.

Fluorescence *in situ* hybridization (FISH) analysis of idic15 brain samples to confirm copy number and examine homologous pairing. A) Ideogram representing the tricentric derivative chromosome of Case 7014 with two sets of inverted 15q11-13 duplications with BP3 boundaries. DNA FISH signals (red spots) in neurons (DAPI nuclear stain) of Case 7014 confirm hexasomy in brain, with two closely spaced doublet observed for the derivative chromosome. B) Ideogram of the asymmetrical isodicentric chromosome 15 of Case 6856, including one BP4 and one BP5 boundary. DNA FISH of Case 6856 neurons confirm the partial tetrasomy for 15q11-13 in brain. C) Graphs for Case 7014 and D) Case 6856 reveal similar distributions of homologous pairing, with the derivative chromosomes (der) interacting nonselectively with the normal chromosome 15 alleles in equal proportions.

Figure 3.

Gene expression analysis of 15q11-13 transcripts in post-mortem brain. A) Schematic representation of the genes analyzed in the critical region between BP2 and BP3 of 15q11-13. Arrows indicating the direction of transcription and the names of genes are shown to the left of the gray line, with the imprinting control region (ICR) shown as a filled circle at the 5' end of *SNRPN*. B-K) Graphs summarizing quantitative RT-PCR measurements of 10 transcripts in the critical region normalized to *GAPDH*, with error bar representing $\pm$ SEM. Fold changes relative to control expression are indicated above individual bars. Significant differences are indicated with * $p < 0.01$, ** $p < 0.005$, and *** $p < 0.0005$. B and C are maternally expressed imprinted genes, D-G are paternally expressed imprinted genes, and H-K are nonimprinted biallelically expressed genes.

Figure 4.

Methylation analysis of the imprinting control region (ICR) in the 5' end of *SNRPN* A) Methylation-sensitive Southern blot analysis of Case 6856 reveals the ratio of methylated alleles (maternal, mat) to unmethylated alleles (paternal, pat) is higher than expected based on parental copy number, 4.35:1 observed versus 3:1 expected. B, C) Bisulfite sequencing of the ICR in Case 6856 (B) and Case 7014 (C). Circles represent the 33 CpG sites present in each clone, with filled circles representing methylated CpG sites, and unfilled circles representing unmethylated CpG sites. Each horizontal line represents the sequence of an individual clone. D) Graph of the percent methylation in individual clones from Prader-Willi syndrome samples (19 clones) compared to Case 6856 (21 clones) and Case 7014 (22 clones). All clones for Case 6856 and 7014 are either fully methylated (above 80%) or completely unmethylated.

Figure 5. Analysis of *GABRB3* protein and DNA methylation A) *GABRB3* protein level in Case 6856 is reduced compared to controls, while Case 7014 has moderately elevated *GABRB3*. GAPDH was used as a loading control. B) Schematic representation of the proximal end of *GABRB3* with the green line indicating the position of the CpG island. Numbered boxes represent exons and red lines with numbered circles represent the regions that were cloned during bisulfite sequencing of Case 6856. Bisulfite sequencing results, shown below the schematic, reveal hypomethylation of the *GABRB3* CpG island. Partial methylation of Region 3 (formally Region 1) within *GABRB3* intron 3 was compared to a matched control (1486) and determined to be within the normal range of methylation previously described [18].

Table 1

Genotyping data for Case 6856.

	Mother	Proband	Father
D15S11CA	1,3	1,3,2	2,2
D15S646	1,3	1,3,2	2,2
D15S817	3,3	3,2	2,4
D15S122	1,3	1,3,2	2,2
D15S986	2,4	2,4,3	3,3
GABRB3	1,3	1,3,2	2,5
D15S1043	1,2	1,2,3	3,3
D15S184	1,2	1,3	3,4
D15S1031	2,3	3,2	2,1
D15S144	5,1	1,2	2,3