ORIGINAL ARTICLE

Highly Penetrant Alterations of a Critical Region Including *BDNF* in Human Psychopathology and Obesity

Carl Ernst, PhD; Christian R. Marshall, PhD; Yiping Shen, PhD; Kay Metcalfe, MD; Jill Rosenfeld, PhD; Jennelle C. Hodge, PhD; Alcy Torres, MD; Ian Blumenthal, BSE; Colby Chiang, BA; Vamsee Pillalamarri, MS; Liam Crapper, BSc; Alpha B. Diallo, PhD; Douglas Ruderfer, MSc; Shahrin Pereira, BSc; Pamela Sklar, MD, PhD; Shaun Purcell, PhD; Robert S. Wildin, MD; Anne C. Spencer, MSc; Bradley F. Quade, MD, PhD; David J. Harris, MD; Emanuelle Lemyre, MD; Bai-lin Wu, PhD; Dimitri J. Stavropoulos, PhD; Michael T. Geraghty, MSc; Lisa G. Shaffer, PhD; Cynthia C. Morton, PhD; Stephen W. Scherer, PhD; James F. Gusella, PhD; Michael E. Talkowski, PhD

Context: Brain-derived neurotrophic factor (BDNF) is suspected of being a causative factor in psychiatric disorders based on case reports or studies involving large structural anomalies.

Objective: To determine the involvement of *BDNF* in human psychopathology.

Design: Case-control study.

Setting: Microarray-based comparative genomic hybridization data from 7 molecular diagnostic centers including 38 550 affected subjects and 28 705 unaffected subjects.

Patients: Subjects referred to diagnostic screening centers for microarray-based comparative genomic hybridization for physical or cognitive impairment.

Main Outcome Measures: Genomic copy number gains and losses.

Results: We report 5 individuals with psychopathology and genomic deletion of a critical region including *BDNF*. The defined critical region was never disrupted in control subjects or diagnostic cases without developmental abnormalities.

Conclusion: Hemizygosity of the *BDNF* region contributes to variable psychiatric phenotypes including anxiety, behavioral, and mood disorders.

Arch Gen Psychiatry. 2012;69(12):1238-1246.
Published online October 8, 2012.
doi:10.1001/archgenpsychiatry.2012.660

Author Affiliations are listed at the end of this article.

BRAIN-DERIVED NEUROTROPHIC factor (BDNF) is a nervous system growth factor that plays a critical role in synaptic modeling, neurodevelopment, and cell signaling.[1] It is a member of the nerve growth factor family with structural similarity to nerve growth factor and neurotrophin 3 and neurotrophin 4 and structural differences distinct from the other nervous system growth factor families, which include fibroblast growth factor, insulin-like growth factor, transforming growth factor β, and cytokine families.[2] While all nervous system growth factors support neurodevelopment, BDNF has been singularly implicated for its role in obesity, pain, and memory.[3-7] The protein is encoded by *BDNF*, located on the short arm of chromosome 11 at band p14, where a polymorphic variant at codon 66 specifies either valine or methionine and is thought to affect processing of proBDNF to BDNF. This locus has been considered as a risk factor for schizophrenia, major depression, attention-deficit/hyperactivity disorder, bipolar disorder, and many other psychopathologies,[8,9] primarily from association-based studies evaluating the nonsynonymous Val66Met variant and studies comprising cases with deletions on 11p associated with deletions in *WT1* and *PAX6*.[10,11]

BDNF sequencing studies in psychiatry and genomic copy loss studies support a link between *BDNF* with behavior and obesity. WAGR syndrome, a deletion syndrome of the short arm of chromosome 11 associated with Wilms tumor, aniridia, genitourinary anomalies, and mental retardation in which deletions include *PAX6* and *WT1*, sometimes includes larger deletions extending to *BDNF*. Two recent studies associated subjects with WAGR syndrome with deletions extend-

Table 1. Information on Cases and Controls[a]

Site	Indications for Study	Sample Size	Platform
	Cases		
SickKids	NDD, MCA only	3258	Agilent 44K/180K
Boston Children's Hospital	All	7320	Agilent 244K
SG	NDD only	14 616	Signature ChipOS 105K/135K (SG)
Mayo	All	13 135	Agilent 180K
Harvard	Balanced chromosomal rearrangement with phenotype	221	Next Generation sequencing
	Controls		
ISC	Unaffected	7878	Affymetrix 5.0/6.0
Cooper et al[22,b]	Unaffected	6113	Affymetrix 6.0
OHI	Unaffected	1234	Affymetrix 6.0
PopGen	Unaffected	1123	Affymetrix 6.0
HapMap 3	Unaffected	1056	Affymetrix 6.0
SAGE	Unaffected	1287	Illumina 1M
Shaikh et al[23]	Unaffected	2026	Affymetrix 6.0
DGV[c]	Unaffected	7988	Multiple

Abbreviations: All, all indications for study included; precise phenotypes of all individuals were not available for further delineation of NDD; DGV, Database of Genomic Variants; HapMap 3, International HapMap 3 Consortium; ISC, International Schizophrenia Consortium; Mayo, Mayo Clinic; MCA, multiple congenital anomalies; NDD, any neurodevelopmental disorder, behavioral, or neuropsychiatric disorder, including autism and autism spectrum disorder; OHI, Ottawa Hospital Institute; SAGE, Study of Addiction: Genetics and Environment; SG, Signature Genomics; SickKids, The Hospital for Sick Children.

[a] See text for references for each cohort.

[b] Publicly available control data from Cooper et al with Wellcome Trust Case-Control Consortium controls already analyzed in the ISC control set removed.

[c] Controls from DGV filtered for overlap with other control studies presented.

ing to *BDNF* with obesity, bipolar disorder, or attention-deficit/hyperactivity disorder.[10,11] In support of a psychiatric phenotype due to copy loss at the *BDNF* locus, 2 independent case reports (3 subjects in total) described obese patients who presented with complex neurobehavioral phenotypes.[12,13] Further, a deep resequencing study of *BDNF* exons and flanking regions from subjects with major depression and controls revealed several novel variants associated with major depression, suggesting that genetic variation in *BDNF* may have an impact on mood.[14]

Molecular studies in rodents have supported a role for *Bdnf* in behavior, in particular through the finding that defective neuronal release of BDNF by in vivo knockdown leads to increased anxiety-like traits in mice,[15,16] and while heterozygous *Bdnf* knockout mice do not display anxiety traits,[17] they are reported to be more aggressive and hyperphagic than wild-type mice.[18] *Bdnf* has also been shown to have a key role in mediating social defeat stress in rodents[19]; in particular, it is required for the development of experience-dependent social aversion.[15] With respect to sensory systems, homozygous *Bdnf* knockout mice show sensory deficits with decreased survival of sensory ganglia while sparing motor neuron development,[20,21] in line with data from human patients with WAGR syndrome with a *BDNF* deletion that suggest a deficit in nociception.[11] Together, data from rodents suggest that whole-organism deletion of *Bdnf* leads to behavioral, sensory, and weight alterations, while deletion of *Bdnf* specifically in brain areas associated with behavior leads to anxiety and aggression.

In view of the large number of association studies with suggestive evidence for *BDNF* polymorphisms in psychopathology, case reports describing large genomic alterations involving *BDNF* in subjects with psychiatric symptoms, and extensive phenotyping in animal models, we sought to better resolve the relationship between *BDNF* and psychopathology by identifying subjects with genomic copy number changes that include *BDNF*.

METHODS

Table 1 summarizes all subjects used in this study. From Signature Genomics (SG), we analyzed a total of 26 144 probands studied using oligonucleotide-based whole-genome array comparative genomic hybridization, using either a 105K-feature platform (SignatureChip OS version 1.0; custom-designed by SG, manufactured by Agilent Technologies) or a 135K-feature platform (SignatureChip OS version 2.0; custom-designed by SG, manufactured by Roche NimbleGen), according to previously described methods.[24,25] From this initial cohort, we divided subjects into those referred with an indication of a neurodevelopmental disorder (n = 14 616) and those referred with an indication for study that did not involve a known neurodevelopmental abnormality (n = 11 528). Unlike the microarrays used to analyze controls, these specific SG platforms are incapable of detecting intragenic *BNDF* variations and are limited to whole-gene *BDNF* deletions at a resolution of approximately 270 kb and 120 kb, respectively. The ethnic distribution in the samples from SG was estimated from a sampling cross-section previously described.[22] This sample (n = 144 subjects, self-reported) was composed of 75% white individuals, 7% African American individuals, and 18% individuals of other race/ethnicity. The sex distribution was 59% male and 41% female. The only alterations spanning *BDNF* observed in the SG group were patients with WAGR syndrome (n = 2), so there was no contribution to these analyses from this data set, although they are included in all statistical analyses. The ethnicity of each patient described herein with a copy gain or loss of *BDNF* was white.

The clinical cytogenetics laboratory at The Hospital for Sick Children in Toronto, Ontario, Canada, screened patients using either Agilent 4x44K array[26] or the 4x180K ISCA v2 microar-

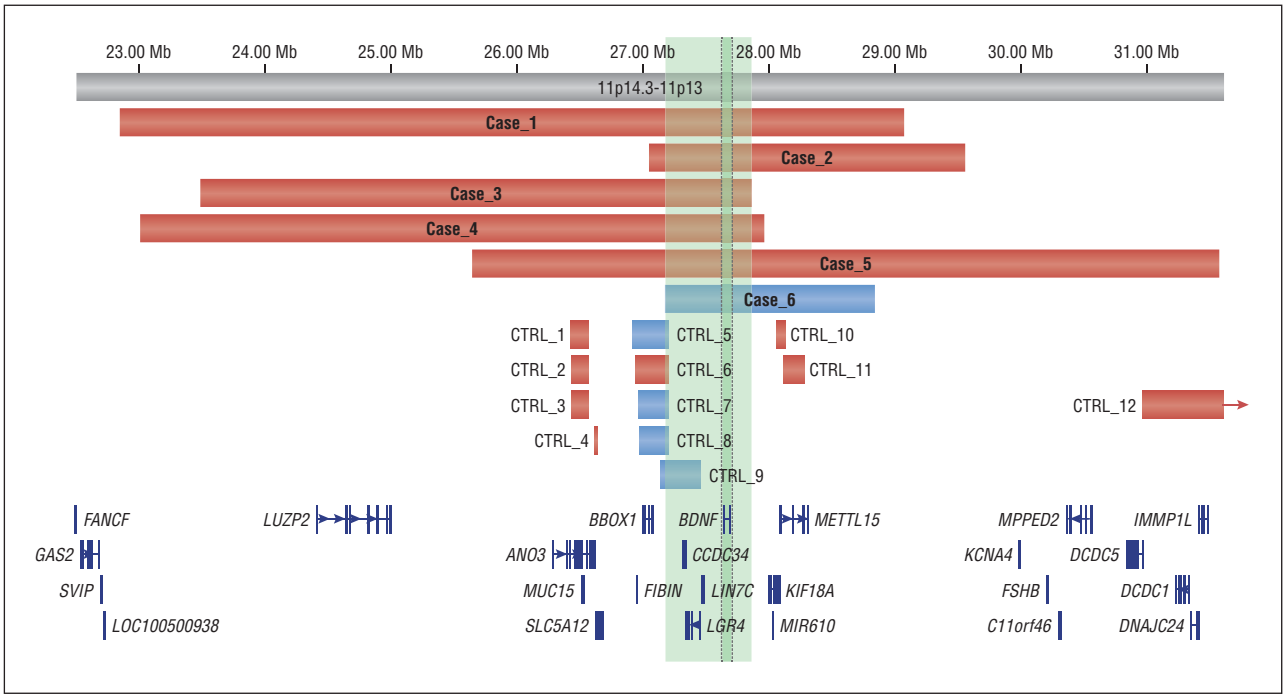

Figure 1. All cases and controls (CTRL) with copy gains (blue) or losses (red) near the *BDNF* locus. Megabase marks (Mb) represent human genome reference 18 build coordinates. Green shading represents an identified critical region while darker green shading corresponds specifically to the genomic location of *BDNF*.

ray manufactured by Agilent and designed by Oxford Gene Technologies. Previously published copy number variant data from 11 509 controls genotyped with high-resolution single-nucleotide polymorphism microarrays were compiled from several subject groups including 1234 Affymetrix 6.0 controls from Ottawa, Ontario,[27] 1123 Affymetrix 6.0 controls from PopGen,[27,28] 4783 Affymetrix 6.0 controls from the Wellcome Trust Case-Control Consortium,[29] 1056 Affymetrix 6.0 controls from the International HapMap 3 Consortium,[30] 1287 Illumina 1M data from Study of Addiction: Genetics and Environment controls,[31] and 2026 Hap550k controls.[23]

From the Developmental Genome Anatomy Project (DGAP) database (www.dgap.harvard.edu), we had access to information on 221 subjects, all of whom had a balanced chromosomal rearrangement. Identification of *BDNF* hemizygosity in 1 of these subjects (DGAP173) was assessed by an Agilent G3 1M array. Based on the available karyotype information of t(2;11)(q11.2;p13), this deletion appears to be independent of the chromosomal rearrangement but we cannot rule out a more complex rearrangement involving both regions. Array processing for all other clinical diagnostic centers was done using commercially available Agilent 244K arrays, except for the Mayo Clinic, which used 180K Agilent arrays.

Control individuals were obtained from a variety of sources listed earlier as well as control data from the International Schizophrenia Consortium[32] and the Database of Genomic Variants[33] (filtered for overlap with other studies) and those described and publicly available from Cooper and colleagues,[22] filtering Wellcome Trust Case-Control Consortium controls to avoid redundancy with the previously mentioned control set. Table 1 describes these control subjects in more detail. All genomic coordinate positions are with reference to the human genome reference 18. Statistical analyses were performed using the Fisher exact test in the statistical package R.

Clinical diagnoses from all patients were performed by independent, qualified physicians who had seen the patient over a period of at least 2 years. We defined obesity as body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) more than 30 or if it was specifically indicated by the primary caregiver. We defined overweight as a BMI more than 25. Psychiatric diagnoses were done using *DSM-IV* criteria by caregiver interviews with affected subjects. In all *BDNF* deletion cases, referring physicians were contacted and provided clinical information for all subjects, allowing for psychiatric phenotyping.

These studies were approved by the institutional review boards of our institutions, and caregivers for each subject gave informed consent when needed.

RESULTS

We screened microarray-based comparative genomic hybridization data for more than 38 000 subjects from clinical diagnostic centers at Boston Children's Hospital; The Hospital for Sick Children, Toronto; Mayo Clinic, Rochester, Minnesota; Brigham and Women's Hospital, Boston, Massachusetts; Manchester Academic Health Science Centre, St Justine Hospital, Montreal, Quebec, Canada; and SG for any subjects with copy number changes of the *BDNF* region (see Table 1 for complete description of all subject groups). We identified 5 subjects with deletions encompassing the entire *BDNF* gene and 1 subject with a duplication spanning *BDNF* (**Figure 1** and **Table 2**). For all subjects, microarray-based comparative genomic hybridization was used to initially identify *BDNF* copy changes and **Figure 2** shows a visual example of microarray-based comparative genomic hybridization data in subject 2 from this study. The deletion group displayed varied phenotypes that included neurodevelopmental, behavioral, and mood disorders, in addition to being obese or overweight and insensitive to pain in some cases, as summarized in Table 2 and presented in greater detail later. The subject with a

Table 2. Characteristics of Current Subjects as Well as Those Previously Reported to Have Alterations in *BDNF*[a]

Patient No./ Sex/Age, y	Genotype	Nociception	Psychopathology	Overweight/Obese	Mental Dx
1/M/10	Deletion: 22,858,513-29,066,320	Pain insensitivity	ADHD, anxiety disorder, aggressive behaviors	BMI = 22.6	PDD-NOS
2/F/21	Deletion: 27,050,622-29,550,113	Self-injurious behaviors	Major depression, generalized anxiety disorder	BMI = 51.7	Mild MR
3/M/2.75	Deletion: 23,484,198-27,857,928	Unknown	Impaired behavior	Mother BMI = 39.5; proband BMI = 27.4	GDD in proband; ID in mother
4/M/16	Deletion: 23,002,186-27,956,720	Unknown	Adjustment disorder, major depression, generalized anxiety disorder	BMI = 50.5	PDD
5/M/7	Deletion: 25,649,116-31,566,599	Pain insensitivity	Anxiety, ADHD, temper tantrums, intolerance to frustration	BMI = 28.3	Moderate MR
6/F/3	Duplication: 27,179,904-28,837,666	Unknown	Not reported	No	Moderate MR and dystonia
Gül et al[34]/M/13	Deletion	Unknown	Not reported	Yes	MR
Brêmond-Gignac et al[12]/F/25	Deletion	Unknown	Mood disturbances, obsessive-compulsive behavior, temper tantrums, intolerance to frustration requiring antipsychotic medications	Yes	Mild/moderate MR
Brêmond-Gignac et al[12]/F/14	Deletion	Unknown	Chronic anxiety, poor acceptance of change, logorrhea, echolaly, poor social interactions, labile mood, and bouts of aggressiveness and motor agitation that required treatment with risperidone	Yes	PDD: moderate/severe MR
Gray et al[13]/F/9	Position effect due to an inversion	Pain insensitivity	Complex neurobehavioral phenotype, repetitive behaviors, extreme hyperactivity, no concept of danger	Yes	Low IQ

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); Dx, diagnosis; GDD, global developmental delay; ID, intellectual disability; MR, mental retardation; PDD-NOS, pervasive developmental delay, not objectively specified.

[a] All genomic coordinates according to human genome reference 18.

duplication also presented with developmental delay and dystonia, but no further information was available. Additional subjects identified with WAGR syndrome were excluded from this analysis (n = 2 subjects from SG) because of the very large number of genes in WAGR deletions, the severity of the associated neurodevelopmental phenotype,[35] and the inability to obtain any follow-up information on these subjects.

Subject 1 was identified with a *BDNF* deletion at chr11: 22,858,513-29,066,320 and a small deletion at chr19: 61,453,936-61,530,271 intersecting the testes-specific gene *ZSCAN5A*. The 10-year-old boy had been diagnosed with pervasive developmental disorder not otherwise specified, attention-deficit/hyperactivity disorder, anxiety, behavioral issues (eg, constantly hitting head against the wall), and mood dysregulation. At 4 years of age, his condition regressed markedly, and to date, he has been treated with escitalopram oxalate (Lexapro), aripiprazole (Abilify), citalopram hydrobromide (Celexa), guanfacine hydrochloride (Tenex), methylphenidate hydrochloride (Ritalin), atomoxetine hydrochloride (Strattera), and clonidine hydrochloride. His height and weight at age 9 years were 138.7 cm and 43.5 kg (95th-97th percentile), respectively, with a BMI of 22.6 (see the eFigure [http://www.archgenpsychiatry.com] for a weight chart for this subject taken at different points showing a progression toward obesity). He was extremely aggressive and parental report noted that the subject does not complain of pain when accidents occur. Array results were confirmed using clinically available multiplex ligation-dependent probe amplification probes targeting *BDNF* (SALSA MLPA P219; MRC-Holland).

Subject 2 (DGAP173) was a 21-year-old woman with a karyotype of 46,XX,t(2;11)(q11.2;p13) who also had a 2.5-megabase deletion (chr11:27,050,622-29,550,113) on chromosome 11 that included *BDNF* (Figure 2). Array comparative genomic hybridization results were confirmed using clinically available multiplex ligation-dependent probe amplification probes targeting *BDNF*. She had mild developmental delay (combined language and motor delay), major depression, generalized anxiety, sleep disturbance (sleep apnea), self-injurious behaviors, agitation, and tantrums. In 2009, at age 19 years, she weighed 167.6 kg and had a height of 180.1 cm, with a BMI of 51.7. Her head circumference was 61 cm, which is outside of the normal adult range of 55 to 58 cm. She had male-pattern hirsutism (thought to be associated with a tentative diagnosis of polycystic ovary syndrome, maternally inherited) and had had only a single period with no further menstruation even with trials of oral con-

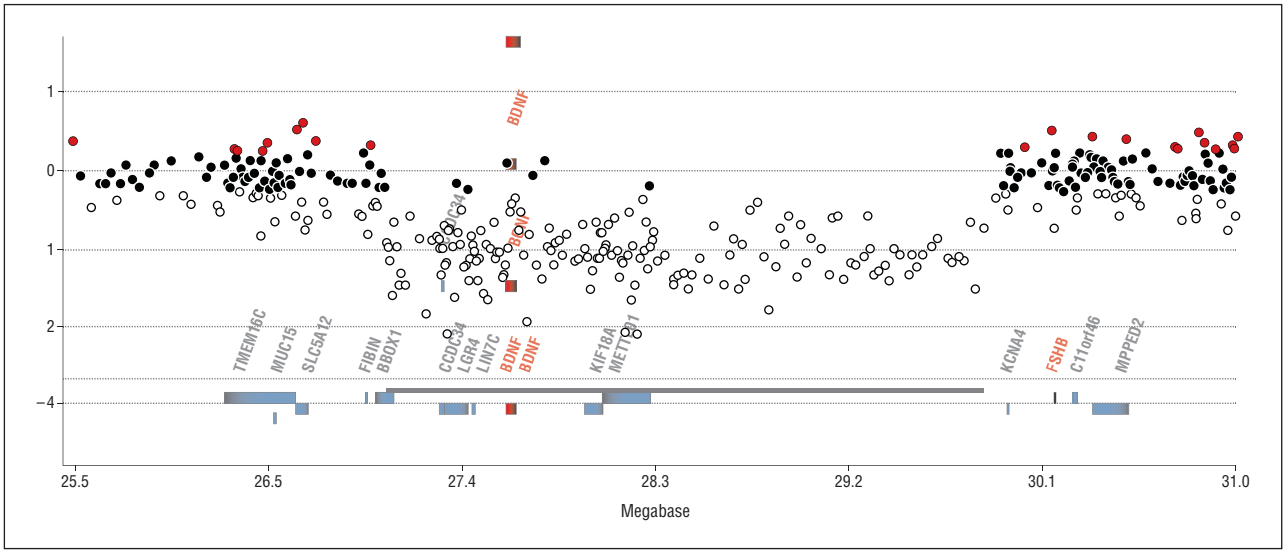

Figure 2. An approximately 2.5-megabase deletion in subject 2, including *BDNF*. Microarray-based comparative genomic hybridization results demonstrating a deletion on chromosome 11. Gray shading represents the predicted size of the deletion, while individual probes from the array are represented by black, white, or red dots. White probes represent decreased probe intensity from DNA from subject 2, reflecting copy loss. Human genome reference 18 build coordinates are shown on the x-axis.

traceptive pills. Impaired glucose tolerance without evidence of type 2 diabetes mellitus, poor lipid profile with elevated triglyceride and total cholesterol and low high-density lipoprotein cholesterol levels, elevated testosterone level, some deepening of the voice, and history of 1 nonfebrile seizure at 2 years of age were also noted. Her skin was remarkable for eczema, moles, and skin tags. She had dysmorphic features including bilateral epicanthal folds giving a saddle appearance to the nasal bridge, a small nose, and complex malocclusion with upper teeth more narrow and frontal than lower. Morphologically, she had somewhat short hands, slightly hyperkeratotic and sweaty palms, fifth-finger brachydactyly and clinodactyly, minor extension limitation of the right elbow, hypoplastic toenails, short feet, and copper-colored verrucous lesions in intertriginous regions (acanthosis nigricans vs epidermal nevi) present on the back, chest, and neck.

Subject 3 was identified with a maternally inherited deletion at chr11:23,484,198-27,857,928. He was referred for investigation at 2 years 9 months of age for severe receptive and expressive speech delay. He had impaired social, play, and behavioral skills as well as global developmental delay and a duplex left kidney. He was a large child with weight of 29.3 kg, height of 103.5 cm, and BMI of 27.4, all of which are greater than the 97th percentile. His head circumference was 52 cm, which is considered within the normal range at age 2.75 years but is at the 94th percentile. Family history is of note in that his mother had intellectual difficulties. Her height was 171 cm; weight, 114.3 kg; and BMI, 39.5. No further information was available for her. Fluorescence in situ hybridization analysis confirmed she had the same deletion. Maternal grandparents were of normal intellect and growth, and fluorescence in situ hybridization analyses were normal.

Subject 4 was a 16-year-old boy whose 36-week gestation was notable for the umbilical cord being wrapped around his neck. A 180K Agilent microarray screen revealed a chr11:23,002,186-27,956,720 (human genome reference 18) de novo deletion. He had hypercholesterolemia, a fatty liver, and hypertension and was prediabetic. At an assessment done at age 16 years, he was 151 kg and 1.73 m and had a BMI of 50.5. He had speech delay, pervasive developmental disorder, and an IQ/DQ of 58. With respect to psychopathology, he had been diagnosed with an adjustment disorder (mixed disturbance of emotion and conduct), depressive disorder, and anxiety disorder. Fluorescence in situ hybridization confirmed the array results using RP11-1150I2.

Subject 5 was a boy with a disruption in *BDNF* (chr11: 25,649,116-31,566,599). No other genetic anomalies were detected in this subject, initially ascertained through learning difficulties, severe speech and language delay, and obesity (BMI 28.3 at age 5 years 10 months; >97th percentile). He had a statement of special educational need, and at age 4.5 years, his overall general conceptual ability was limited (score on the British Ability Scales II was 47 [<0.4 percentile] in keeping with a severe learning disability); he was reported to be able to write his name at 6 years of age. He had poor fine motor skills and poor problem-solving skills. With respect to sensory systems, he had hyperacusis and a high pain threshold. He was described as having inappropriate toddlerlike tantrums triggered by not getting his own way or not being able to eat when he wishes. He had sleeping difficulties and was taking melatonin. A strengths-and-difficulties questionnaire completed by his teacher at age 5 years noted very high scores for overall stress, hyperactivity, and attentional difficulties and high scores for difficulties getting along with other children.

Subject 6 had a *BDNF* duplication and was indicated for screening because of developmental delay and dystonia (chr11:27,179,904-28,837,666). No further information was available on this subject.

There was a notable relationship between age and BMI in subjects with a *BDNF* deletion, strongly supporting a

role for a deletion in this region and obesity. Specifically, while all subjects were overweight at a young age, older subjects had even higher BMIs, suggesting a progression toward increasing obesity (BMI vs age, Pearson = 0.86; $P = .06$), with a particular increase after the later teen years (**Figure 3**). We were able to further support the hypothesis that people with a *BDNF* deletion have increased BMIs over time by acquiring data from a single subject (subject 1) who received multiple assessments over time. The supporting eFigure shows the increase in BMI over time compared with age standards.

While each of the *BDNF*-containing deletions reported herein disrupted multiple genes, the critical region of overlap included only *BBOX1, CCDC34, LGR4, BDNF*, and *LIN7C* (Figure 1). We therefore attempted to narrow the critical region responsible for the mood and behavior phenotypes by examining structural variations in data sets from individuals without a comparable phenotype. We found no structural variations affecting *BDNF* in copy number variant data from 28 705 control individuals with high-resolution chromosomal microarrays (Figure 1 and Table 1), despite the superior resolution of these platforms relative to those used to analyze most of the cases. There was also no disruption of the *BDNF* locus from clinical diagnostic cases not reported to have a neurological abnormality (n = 11 528) assayed through the SG Genoglyphix Chromosome Aberration Database. Collectively, though disruption of this locus was rare, we found a nominally significant burden of dosage alterations spanning *BDNF* in cases compared with all controls (Fisher exact test, $P = .04$) as well as the combination of controls and clinical diagnostic cases without a neurodevelopmental abnormality (n = 40 233; $P = .01$). Similar results were obtained if we restricted analyses to only those cases with deletion of the locus ($P = .08$ and .03, respectively). There was evidence for deletion of *BBOX1,* as well as for duplication of *BBOX1, CCDC34*, and *LGR4,* though there were no disruptions of *LIN7C*. *CCDC34* has previously been reported as disrupted in a case of translocation[36] without an associated neurodevelopmental phenotype. Taken together, these findings indicate that deletions encompassing *BDNF* are rare, but when they occur, they are highly penetrant in producing a distinct phenotypic spectrum that includes behavioral/psychiatric traits due to alterations in *BDNF, LIN7C*, *LGR4*, or some combination of these genes.

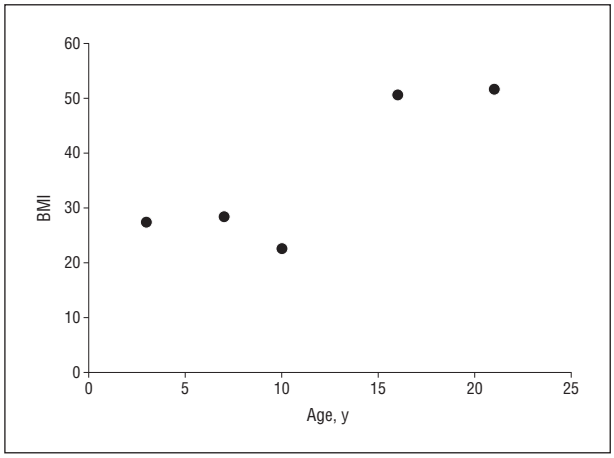

Figure 3. Positive correlation of body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) with age in subjects with a *BDNF* deletion.

COMMENT

To our knowledge, this study represents the largest and highest genomic resolution study to date investigating the role of *BDNF* in psychopathology. Previous reports identified single cases with large deletions encompassing *BDNF* or cases with *BDNF* deletions and WAGR syndrome, where 1 study identified 4 different subjects with WAGR syndrome with behavioral disturbances.[10] The current study included more than 38 000 probands collected internationally and found 5 subjects with *BDNF* deletions with heterogeneous, but always psychiatric, phenotypes. Despite being the most extensive study to date of the role of *BDNF* in psychopathology, this study should be considered supportive of the role of *BDNF* in psychopathology and not unequivocal, because the critical region included 2 other potentially causative genes affected in all *BDNF* deletion cases. Nonetheless, animal data and analysis of the function of these 2 genes in the critical region strongly suggest that *BDNF* hemizygosity leads to psychopathology.

Mouse studies of *LGR4* and *LIN7C* orthologs suggest a less central role for these genes in behavior. *Lgr4* knockout mice show embryonic lethality, thought to be due to its fundamental role in organogenesis, particularly of the kidney and the sex organs.[37,38] Subject 3 in our study had a duplex left kidney and subject 2 had polycystic ovary syndrome. Notably, expression of *Lgr4* is largely absent from the brain except in the olfactory bulb and periventricular area; expression is highest in the kidney, gallbladder, heart, bone, and spinal cord.[39] Thus, *LGR4* hemizygosity is unlikely to contribute to psychopathology in humans but could account for other observed abnormalities. *Lin7c* (aka *MALS-3*) has a role in maintaining cell polarity during development in the mouse,[40] though 2 paralogs, *Lin7a* and *Lin7b,* are suspected of being able to compensate for *Lin7c* deficiency.[41] Distribution of *Lin7c* expression in the mouse brain is low compared with *Lin7a* and *Lin7b* and is restricted to the dentate gyrus, cerebellum, and superior colliculus. In contrast, *Lin7a* and *Lin7b* are abundantly expressed in other brain regions, especially the cortex and dentate gyrus.[41] While this expression pattern does not suggest a primary role for *LIN7C* hemizygosity in psychopathology, such a contribution, alone or in interaction with *BDNF*, cannot be excluded.

The presence of psychiatric manifestations in subjects with *BDNF*-associated deletion is consistent with previously reported cases, as delineated in Table 2, along with their associated neurodevelopmental and behavioral phenotypes. Taken together, this collection of subjects supports the conclusion that gross disruption of *BDNF* in humans is associated with psychopathology, being obese or overweight, and, at least sometimes, pain insensitivity—phenotypes consistent with data from manipulation of *Bdnf* in rodents. No information was avail-

able for 3 deletion subjects with respect to pain insensitivity (1 of the subjects with a *BDNF* deletion was reported to engage in self-injurious behavior, a phenotype frequently associated with pain insensitivity in individuals with intellectual disability[42]), so we cannot draw a conclusion concerning the universality of pain insensitivity, but follow-up studies are warranted. Both the overweight/obese and nociceptive phenotypes in humans are also supported by a study of patients with WAGR syndrome, while those with deletions that extended to *BDNF* were more likely to be obese and insensitive to pain[11] than those without a *BDNF* deletion.

The consensus phenotype for individuals with a deletion in *BDNF* suggests that young children are hyperactive and anxious and have an intolerance to change. As subjects age, they likely develop more pronounced anxiety and mood disorders, exemplified by the 16-year-old and 21-year-old subjects with major depressive disorder and generalized anxiety disorder and by a 25-year-old woman with mood disturbances from a previous report.[12] Identification of a single locus that may be linked to major depression or anxiety highlights the heterogeneity of these psychiatric diseases—most subjects with major depression do not have deletions in *BDNF*, for example—and the need to possibly reassess how clinical categorization proceeds.[43]

Chromosomal aberrations at genomic loci that associate with mental retardation are common, but hemizygosity of a locus that can affect a spectrum of phenotypes including mood is less common, and the mechanisms that could contribute to such phenotypic diversity remain to be elucidated. Deeper investigation of the regulation of *BDNF* and of the molecular actions of the transcribed product will be required to better understand how hemizygosity at this locus contributes to psychopathology.

Submitted for Publication: January 25, 2012; final revision received March 26, 2012; accepted April 26, 2012.
Published Online: October 8, 2012. doi:10.1001/archgenpsychiatry.2012.660
Author Affiliations: Department of Psychiatry, McGill University (Drs Ernst and Diallo and Mr Crapper), and St Justine Hospital (Dr Lemyre), Montreal, Quebec, Program in Genetics and Genome Biology, The Hospital for Sick Children (Drs Marshall and Scherer), and Departments of Laboratory Medicine and Pathobiology (Dr Stavropoulos) and Molecular Genetics (Dr Scherer) and McLaughlin Centre (Dr Scherer), University of Toronto, Toronto, and Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa (Mr Geraghty), Ontario, Canada; Center for Human Genetic Research, Massachusetts General Hospital (Drs Ernst, Shen, Sklar, Purcell, Gusella, and Talkowski and Messrs Blumenthal, Chiang, Pillalamarri, and Ruderfer), Departments of Neurology (Drs Ernst, Gusella, and Talkowski), Pathology (Dr Morton), and Genetics (Dr Gusella), Harvard Medical School, Departments of Laboratory Medicine and Pathology (Drs Shen, Harris, and Wu) and Pediatrics (Dr Torres), and Division of Clinical Genetics (Dr Harris), Boston Children's Hospital, Departments of Pathology (Drs Quade and Morton) and Obstetrics, Gynecology, and Reproductive Biology (Ms Pereira and Drs Quade and Morton), Brigham and Women's Hospital, Boston, and Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge (Drs Ernst, Morton, Gusella, and Talkowski); Shanghai Children's Medical Center, Shanghai Jiaotong University School of Medicine (Dr Shen), and Children's Hospital and Institutes of Biomedical Science, Shanghai Medical College, Fudan University (Dr Wu), Shanghai, China; Manchester Academic Health Science Centre, Manchester, England (Dr Metcalfe); Signature Genomic Laboratories, PerkinElmer Inc, Spokane, Washington (Drs Rosenfeld and Shaffer); Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota (Dr Hodge); Mount Sinai School of Medicine, New York, New York (Dr Sklar); and Idaho State Genetic Services Program and St Luke's Children's Hospital, Boise (Dr Wildin), and Department of Philosophy and Religion, College of Idaho, Caldwell (Ms Spencer).
Correspondence: Carl Ernst, PhD, Department of Psychiatry, McGill University and Douglas Hospital, 6875 LaSalle Blvd, Frank Common Bldg, Room 2101.2, Verdun, QC H4H 1R3, Canada (carl.ernst@mcgill.ca) and Michael E. Talkowski, PhD, Department of Neurology, Harvard Medical School, Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge St, Boston, MA 02114 (talkowski@chgr.mgh.harvard.edu).
Financial Disclosure: Drs Rosenfeld and Shaffer are employees of Signature Genomic Laboratories, PerkinElmer Inc.
Funding/Support: This work was funded by a Canada Research Chair in Psychiatric Genetics from the Canadian Institute of Health Research (Dr Ernst), grants GM061354 (Drs Morton and Gusella), HD065286 (Dr Gusella), and MH095687 (Dr Talkowski) from the National Institutes of Health, the Simons Foundation Autism Research Initiative (Drs Gusella and Talkowski), grant 2010CB529601 from the National Basic Research Program of China (973 Program) (Dr Wu), and grants 09JC1402400 and 09ZR1404500 from the Science and Technology Council of Shanghai (Dr Wu).
Online-Only Material: The eFigure is available at http://www.archgenpsychiatry.com.
Additional Contributions: We are grateful to the members of the International Schizophrenia Consortium and Wellcome Trust Case-Control Consortium, Evan Eichler, PhD, and Bradley Coe, PhD, for their invaluable contribution of control resources. We are deeply thankful to all patients, families, and referring physicians who participated in this study.

REFERENCES

1. Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. *Cell*. 1993;75(1):113-122.
2. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2011;10(3):209-219.
3. Anand P. Neurotrophic factors and their receptors in human sensory neuropathies. *Prog Brain Res*. 2004;146:477-492.
4. Lu B, Gottschalk W. Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. *Prog Brain Res*. 2000;128:231-241.

5. Krishnan V, Nestler EJ. Linking molecules to mood: new insight into the biology of depression. *Am J Psychiatry*. 2010;167(11):1305-1320.
6. Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, Bardoni R. BDNF as a pain modulator. *Prog Neurobiol*. 2008;85(3):297-317.
7. Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol*. 2001;15(10):1748-1757.
8. Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet*. 2002;71(3):651-655.
9. Chen ZY, Bath K, McEwen B, Hempstead B, Lee F. Impact of genetic variant BDNF (Val66Met) on brain structure and function. *Novartis Found Symp*. 2008;289:180-188, discussion 188-195.
10. Shinawi M, Sahoo T, Maranda B, Skinner SA, Skinner C, Chinault C, Zascavage R, Peters SU, Patel A, Stevenson RE, Beaudet AL. 11p14.1 Microdeletions associated with ADHD, autism, developmental delay, and obesity. *Am J Med Genet A*. 2011;155A(6):1272-1280.
11. Han JC, Liu QR, Jones M, Levinn RL, Menzie CM, Jefferson-George KS, Adler-Wailes DC, Sanford EL, Lacbawan FL, Uhl GR, Rennert OM, Yanovski JA. Brain-derived neurotrophic factor and obesity in the WAGR syndrome. *N Engl J Med*. 2008;359(9):918-927.
12. Brémond-Gignac D, Crolla JA, Copin H, Guichet A, Bonneau D, Taine L, Lacombe D, Baumann C, Benzacken B, Verloes A. Combination of WAGR and Potocki-Shaffer contiguous deletion syndromes in a patient with an 11p11.2-p14 deletion. *Eur J Hum Genet*. 2005;13(4):409-413.
13. Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'rahilly S, Farooqi IS. Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes*. 2006;55(12):3366-3371.
14. Licinio J, Dong C, Wong ML. Novel sequence variations in the brain-derived neurotrophic factor gene and association with major depression and antidepressant treatment response. *Arch Gen Psychiatry*. 2009;66(5):488-497.
15. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*. 2006;311(5762):864-868.
16. Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*. 2006;314(5796):140-143.
17. MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M. Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. *Behav Neurosci*. 2001;115(5):1145-1153.
18. Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, Tessarollo L. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A*. 1999;96(26):15239-15244.
19. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 2007;131(2):391-404.
20. Ernfors P, Lee KF, Jaenisch R. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature*. 1994;368(6467):147-150.
21. Jones KR, Fariñas I, Backus C, Reichardt LF. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell*. 1994;76(6):989-999.
22. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. A copy number variation morbidity map of developmental delay. *Nat Genet*. 2011;43(9):838-846.
23. Shaikh TH, Gai X, Perin JC, Glessner JT, Xie H, Murphy K, O'Hara R, Casalunovo T, Conlin LK, D'Arcy M, Frackelton EC, Geiger EA, Haldeman-Englert C, Imielinski M, Kim CE, Medne L, Annaiah K, Bradfield JP, Dabaghyan E, Eckert A, Onyiah CC, Ostapenko S, Otieno FG, Santa E, Shaner JL, Skraban R, Smith RM, Elia J, Goldmuntz E, Spinner NB, Zackai EH, Chiavacci RM, Grundmeier R, Rappaport EF, Grant SF, White PS, Hakonarson H. High-resolution mapping and analysis of copy number variations in the human genome: a data resource for clinical and research applications. *Genome Res*. 2009;19(9):1682-1690.
24. Ballif BC, Theisen A, McDonald-McGinn DM, Zackai EH, Hersh JH, Bejjani BA, Shaffer LG. Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. *Clin Genet*. 2008;74(5):469-475.
25. Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, Thompson R, Traylor R, Bejjani BA, Shaffer LG, Rosenfeld JA, Lamb AN, Sahoo T. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet*. 2010;18(11):1196-1201.
26. Baldwin EL, Lee JY, Blake DM, Bunke BP, Alexander CR, Kogan AL, Ledbetter DH, Martin CL. Enhanced detection of clinically relevant genomic imbalances using a targeted plus whole genome oligonucleotide microarray. *Genet Med*. 2008;10(6):415-429.
27. Lionel AC, Crosbie J, Barbosa N, Goodale T, Thiruvahindrapuram B, Rickaby J, Gazzellone M, Carson AR, Howe JL, Wang Z, Wei J, Stewart AF, Roberts R, McPherson R, Fiebig A, Franke A, Schreiber S, Zwaigenbaum L, Fernandez BA, Roberts W, Arnold PD, Szatmari P, Marshall CR, Schachar R, Scherer SW. Rare copy number variation discovery and cross-disorder comparisons identify risk genes for ADHD. *Sci Transl Med*. 2011;3(95):ra75.
28. Krawczak M, Nikolaus S, von Eberstein H, Croucher PJ, El Mokhtari NE, Schreiber S. PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. *Community Genet*. 2006;9(1):55-61.
29. Craddock N, Hurles ME, Cardin N, Pearson RD, Plagnol V, Robson S, Vukcevic D, Barnes C, Conrad DF, Giannoulatou E, Holmes C, Marchini JL, Stirrups K, Tobin MD, Wain LV, Yau C, Aerts J, Ahmad T, Andrews TD, Arbury H, Attwood A, Auton A, Ball SG, Balmforth AJ, Barrett JC, Barroso I, Barton A, Bennett AJ, Bhaskar S, Blaszczyk K, Bowes J, Brand OJ, Braund PS, Bredin F, Breen G, Brown MJ, Bruce IN, Bull J, Burren OS, Burton J, Byrnes J, Caesar S, Clee CM, Coffey AJ, Connell JM, Cooper JD, Dominiczak AF, Downes K, Drummond HE, Dudakia D, Dunham A, Ebbs B, Eccles D, Edkins S, Edwards C, Elliot A, Emery P, Evans DM, Evans G, Eyre S, Farmer A, Ferrier IN, Feuk L, Fitzgerald T, Flynn E, Forbes A, Forty L, Franklyn JA, Freathy RM, Gibbs P, Gilbert P, Gokumen O, Gordon-Smith K, Gray E, Green E, Groves CJ, Grozeva D, Gwilliam R, Hall A, Hammond N, Hardy M, Harrison P, Hassanali N, Hebaishi H, Hines S, Hinks A, Hitman GA, Hocking L, Howard E, Howard P, Howson JM, Hughes D, Hunt S, Isaacs JD, Jain M, Jewell DP, Johnson T, Jolley JD, Jones IR, Jones LA, Kirov G, Langford CF, Lango-Allen H, Lathrop GM, Lee J, Lee KL, Lees C, Lewis K, Lindgren CM, Maisuria-Armer M, Maller J, Mansfield J, Martin P, Massey DC, McArdle WL, McGuffin P, McLay KE, Mentzer A, Mimmack ML, Morgan AE, Morris AP, Mowat C, Myers S, Newman W, Nimmo ER, O'Donovan MC, Onipinla A, Onyiah I, Ovington NR, Owen MJ, Palin K, Parnell K, Pernet D, Perry JR, Phillips A, Pinto D, Prescott NJ, Prokopenko I, Quail MA, Rafelt S, Rayner NW, Redon R, Reid DM, Renwick, Ring SM, Robertson N, Russell E, St Clair D, Sambrook JG, Sanderson JD, Schuilenburg H, Scott CE, Scott R, Seal S, Shaw-Hawkins S, Shields BM, Simmonds MJ, Smyth DJ, Somaskantharajah E, Spanova K, Steer S, Stephens J, Stevens HE, Stone MA, Su Z, Symmons DP, Thompson JR, Thomson W, Travers ME, Turnbull C, Valsesia A, Walker M, Walker NM, Wallace C, Warren-Perry M, Watkins NA, Webster J, Weedon MN, Wilson AG, Woodburn M, Wordsworth BP, Young AH, Zeggini E, Carter NP, Frayling TM, Lee C, McVean G, Munroe PB, Palotie A, Sawcer SJ, Scherer SW, Strachan DP, Tyler-Smith C, Brown MA, Burton PR, Caulfield MJ, Compston A, Farrall M, Gough SC, Hall AS, Hattersley AT, Hill AV, Mathew CG, Pembrey M, Satsangi J, Stratton MR, Worthington J, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand W, Parkes M, Rahman N, Todd JA, Samani NJ, Donnelly P; Wellcome Trust Case Control Consortium. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature*. 2010;464(7289):713-720.
30. Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Peltonen L, Dermitzakis E, Bonnen PE, Altshuler DM, Gibbs RA, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Yu F, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Gibbs RA, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Dermitzakis E, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Bonnen PE, Gibbs RA, Gonzaga-Jauregui C, Keinan A, Price AL, Yu F, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Schaffner SF, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Pollack S, Price AL, Schaffner SF, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE; International HapMap 3 Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature*. 2010;467(7311):52-58.
31. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, Conroy J, Magalhaes TR, Correia C, Abrahams BS, Almeida J, Bacchelli E, Bader GD, Bailey AJ, Baird G, Battaglia A, Berney T, Bolshakova N, Bölte S, Bolton PF, Bourgeron T, Brennan S, Brian J, Bryson SE, Carson AR, Casallo G, Casey J, Chung BH, Cochrane L, Corsello C, Crawford EL, Crossett A, Cytrynbaum C, Dawson G, de Jonge M, Delorme R, Drmic I, Duketis E, Duque F, Estes A, Farrar P, Fernandez BA, Folstein SE, Fombonne E, Freitag CM, Gilbert J, Gillberg C, Glessner JT, Gold-

berg J, Green A, Green J, Guter SJ, Hakonarson H, Heron EA, Hill M, Holt R, Howe JL, Hughes G, Hus V, Igliozzi R, Kim C, Klauck SM, Kolevzon A, Korvatska O, Kustanovich V, Lajonchere CM, Lamb JA, Laskawiec M, Leboyer M, Le Couteur A, Leventhal BL, Lionel AC, Liu XQ, Lord C, Lotspeich L, Lund SC, Maestrini E, Mahoney W, Mantoulan C, Marshall CR, McConachie H, McDougle CJ, McGrath J, McMahon WM, Merikangas A, Migita O, Minshew NJ, Mirza GK, Munson J, Nelson SF, Noakes C, Noor A, Nygren G, Oliveira G, Papanikolaou K, Parr JR, Parrini B, Paton T, Pickles A, Pilorge M, Piven J, Ponting CP, Posey DJ, Poustka A, Poustka F, Prasad A, Ragoussis J, Renshaw K, Rickaby J, Roberts W, Roeder K, Roge B, Rutter ML, Bierut LJ, Rice JP, Salt J, Sansom K, Sato D, Segurado R, Sequeira AF, Senman L, Shah N, Sheffield VC, Soorya L, Sousa I, Stein O, Sykes N, Stoppioni V, Strawbridge C, Tancredi R, Tansey K, Thiruvahindrapduram B, Thompson AP, Thomson S, Tryfon A, Tsiantis J, Van Engeland H, Vincent JB, Volkmar F, Wallace S, Wang K, Wang Z, Wassink TH, Webber C, Weksberg R, Wing K, Wittemeyer K, Wood S, Wu J, Yaspan BL, Zurawiecki D, Zwaigenbaum L, Buxbaum JD, Cantor RM, Cook EH, Coon H, Cuccaro ML, Devlin B, Ennis S, Gallagher L, Geschwind DH, Gill M, Haines JL, Hallmayer J, Miller J, Monaco AP, Nurnberger JI Jr, Paterson AD, Pericak-Vance MA, Schellenberg GD, Szatmari P, Vicente AM, Vieland VJ, Wijsman EM, Scherer SW, Sutcliffe JS, Betancur C. Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*. 2010;466(7304):368-372.

32. International Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature*. 2008;455(7210):237-241.
33. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. *Nat Genet*. 2004; 36(9):949-951.
34. Gül D, Oğur G, Tunca Y, Ozcan O. Third case of WAGR syndrome with severe obesity and constitutional deletion of chromosome (11)(p12p14). *Am J Med Genet*. 2002;107(1):70-71.
35. Miller RW, Fraumeni JF Jr, Manning MD. Association of Wilms's tumor with aniridia, hemihypertrophy and other congenital malformations. *N Engl J Med*. 1964; 270:922-927.
36. Kutsche K, Glauner E, Knauf S, Pomarino A, Schmidt M, Schröder B, Nothwang H, Schüler H, Goecke T, Kersten A, Althaus C, Gal A. Cloning and characterization of the breakpoint regions of a chromosome 11;18 translocation in a patient with hamartoma of the retinal pigment epithelium. *Cytogenet Cell Genet*. 2000; 91(1-4):141-147.
37. Mohri Y, Oyama K, Akamatsu A, Kato S, Nishimori K. Lgr4-deficient mice showed premature differentiation of ureteric bud with reduced expression of Wnt effector Lef1 and Gata3. *Dev Dyn*. 2011;240(6):1626-1634.
38. Mendive F, Laurent P, Van Schoore G, Skarnes W, Pochet R, Vassart G. Defective postnatal development of the male reproductive tract in LGR4 knockout mice. *Dev Biol*. 2006;290(2):421-434.
39. Mazerbourg S, Bouley DM, Sudo S, Klein CA, Zhang JV, Kawamura K, Goodrich LV, Rayburn H, Tessier-Lavigne M, Hsueh AJ. Leucine-rich repeat-containing, G protein-coupled receptor 4 null mice exhibit intrauterine growth retardation associated with embryonic and perinatal lethality. *Mol Endocrinol*. 2004;18(9): 2241-2254.
40. Srinivasan K, Roosa J, Olsen O, Lee SH, Bredt DS, McConnell SK. MALS-3 regulates polarity and early neurogenesis in the developing cerebral cortex. *Development*. 2008;135(10):1781-1790.
41. Misawa H, Kawasaki Y, Mellor J, Sweeney N, Jo K, Nicoll RA, Bredt DS. Contrasting localizations of MALS/LIN-7 PDZ proteins in brain and molecular compensation in knockout mice. *J Biol Chem*. 2001;276(12):9264-9272.
42. Barron JL, Sandman CA. Self-injurious behavior and stereotypy in an institutionalized mentally retarded population. *Appl Res Ment Retard*. 1984;5(4):499-511.
43. Holtzheimer PE, Mayberg HS. Stuck in a rut: rethinking depression and its treatment. *Trends Neurosci*. 2011;34(1):1-9.