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A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures

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We report a recurrent microdeletion syndrome causing mental retardation, epilepsy and variable facial and digital dysmorphisms. We describe nine affected individuals, including six probands: two with *de novo* deletions, two who inherited the deletion from an affected parent and two with unknown inheritance. The proximal breakpoint of the largest deletion is contiguous with breakpoint 3 (BP3) of the Prader-Willi and Angelman syndrome region, extending 3.95 Mb distally to BP5. A smaller 1.5-Mb deletion has a proximal breakpoint within the larger deletion (BP4) and shares the same distal BP5. This recurrent 1.5-Mb deletion contains six genes, including a candidate gene for epilepsy (*CHRNA7*) that is probably responsible for the observed seizure phenotype. The BP4–BP5 region undergoes frequent inversion, suggesting a possible link between this inversion polymorphism and recurrent deletion. The frequency of these microdeletions in mental retardation cases is ~0.3% (6/2,082 tested), a prevalence comparable to that of Williams, Angelman and Prader-Willi syndromes.

Genomic disorders result from DNA rearrangements mediated by nonallelic homologous recombination (NAHR) between large, highly homologous flanking segmental duplications[1]. The clinical features of many of the known genomic disorders include mental retardation and developmental delay, and several recent studies of individuals with mental retardation have led to the identification of new recurrent genomic disorders[2]. By whole-genome array CGH screening of 757 individuals with mental retardation and/or congenital anomalies, we identified two unrelated individuals with mild to moderate mental retardation, dysmorphic features and abnormal electroencephalogram (EEG) findings who both have identical *de novo* 1.5-Mb deletions of 15q13.3. These two deletions share the same distal breakpoint (BP5) with a previously reported[3] 3.95-Mb deletion of 15q13 (**Fig. 1** and **Fig. 2a**) and a proximal breakpoint (BP4) within this larger deletion (**Fig. 1** and **Fig. 2b–d**). The shared 1.5-Mb region contains six known genes. Our array CGH screening also detected a single individual (543/06) with a proximal deletion breakpoint corresponding to breakpoint region 3 (BP3) of the Prader-Willi and Angelman syndrome region and a distal breakpoint at BP4 (**Fig. 1**). However, the deletion was also detected in the individual's unaffected father. Therefore, we interpret this BP3–BP4 deletion as probably representing a benign copy number variant, although we cannot exclude that it may instead be a pathogenic deletion with incomplete penetrance.

In order to rapidly screen a large collection of affected individuals for deletions in the shared 1.5-Mb interval between BP4 and BP5, we developed two TaqMan quantitative PCR (qPCR) assays targeted to this region and screened 1,040 individuals with mental retardation of unknown etiology. This cohort, obtained from the Greenwood Genetic Center, consists of an approximately equal number of individuals of European and African American descent. qPCR analyses identified four individuals as potentially harboring a deletion of the interval BP4–BP5. We subsequently validated deletions by BAC array CGH (data not shown) and a custom oligonucleotide array (**Fig. 1**). A review of pedigrees showed evidence of multiple affected individuals for each case (**Fig. 2** and **Supplementary Fig. 1** online), and review

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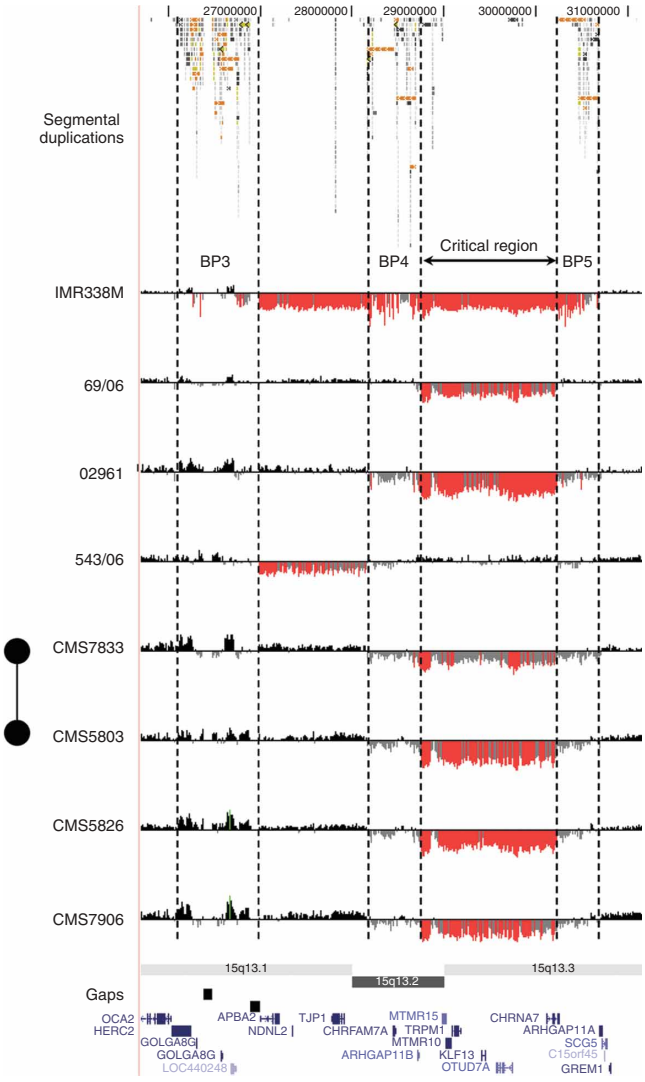

Figure 1 High-resolution oligonucleotide array mapping of 15q12–q13.3 rearrangements (chr15:25700000–31400000). Although there seems to be variation in the exact location of breakpoints, all map to large blocks of segmental duplication at BP3, BP4 and BP5 (indicated by dashed lines). For each individual, deviations of probe $\log_2$ ratios from 0 are depicted by gray and black lines. Those exceeding a threshold of 1.5 s.d. from the mean probe ratio are colored green and red to represent relative gains and losses, respectively. Segmental duplications of increasing similarity (90–98%, 98–99% and >99%) are represented by gray, yellow and orange bars, respectively. A number of other 15q rearrangements with breakpoints mapping to BP3, BP4 and BP5 are shown in **Supplementary Figure 5**.

of the sample collection showed that although the series was thought to have only unrelated individuals, two of the individuals we identified with deletions are mother (CMS7833) and son (CMS5803).

Review of the phenotypes observed in the nine individuals identified with deletions of 15q13 showed a number of phenotypic features (summarized in **Table 1** and **Fig. 2**, with full phenotypic details in the **Supplementary Note** online). The most consistent features among the individuals in our series were mild to moderate mental retardation (9/9 individuals) and epilepsy and/or abnormal EEG findings, which we noted in seven of nine individuals. All affected individuals also had mild facial dysmorphism, although these features were variable. Features shared among three or more individuals included hypertelorism, upslanting palpebral fissures, prominent philtrum with full everted lips, short and/or curved fifth finger and short fourth metacarpals. Related to the latter, it is noteworthy that we noted skeletal and/or joint defects of the hand in seven of nine individuals (**Table 1**). Therefore, testing for the 15q13.3 deletion should be considered in individuals with unexplained mental retardation, seizures and mild dysmorphic features.

Each breakpoint region corresponds to a complex set of segmental duplications, termed duplication blocks. BP4 is an 818-kb duplication block with three large regions of homology to BP5 (95 kb, 140 kb and 218 kb with 99.6% identity, according to the University of California Santa Cruz Genome Browser (see URLs section of Methods, below)), each of which lies in an inverted orientation relative to the corresponding region in BP5 (**Fig. 3**, **Supplementary Table 1** online and **Supplementary Fig. 2** online). Consistent with NAHR as the mechanism underlying rearrangements of 15q13.3, our data localized the breakpoints of all five BP4–BP5 deletions and a BP4–BP5 duplication to these large paralogous sequences (**Figs. 1**,**4** and **Supplementary Fig. 3** online). The BP3 region, which is the common distal breakpoint in Prader-Willi and Angelman syndrome deletions, corresponds to an 843-kb duplication block (hg17/Build 35, chr 15: 26053472–26896735). Duplicated sequence within BP3 shows relatively small stretches of homology to BP4 (17 kb with 93% identity) and BP5 (30 kb with 98% identity and 11 kb with 93% identity). BP3 also contains two assembly gaps, and our data from IMR338 and individual 543/06 suggest that the proximal breakpoint in these deletions occurs within the distal gap. Given the likelihood that NAHR underlies rearrangements of 15q13, we suggest that this region contains as-yet-uncharacterized segmental duplications, paralogous to those at BP4 and BP5, that catalyze recurrent rearrangements of 15q13. Thus, the increased prevalence of rearrangements observed between BP4–BP5 relative to those involving BP3–BP4–BP5 is consistent with the size and homology of duplicons in 15q13. We find it interesting that most of the breakpoint regions associated with genomic disorders on chromosome 15 correspond to duplication blocks that harbor copies of the *GOLGA* gene family[4,5]. Within the limits of oligonucleotide array CGH resolution, breakpoint regions seem to overlap with these specific duplicons (**Supplementary Fig. 4** online).

Observations in several genomic disorders suggest that microdeletions arise preferentially from chromosomes carrying an inversion of that same region[3,6–9]. Given the opposing orientation of the duplicons at BP4 relative to BP5 in the reference assembly (**Supplementary Fig. 2**), we hypothesized that 15q13.3 might also be a site of inversion polymorphism that could create a configuration predisposed to recurrent rearrangement. To investigate this possibility, we used FISH to assay the BP4–BP5 region. Testing of eight HapMap individuals of varied ethnicities showed the presence of a common inversion of this interval, which was present on 7 of the 16 chromosomes assayed (frequency of inversion relative to reference assembly: 0.44; 95% confidence interval (c.i.): 0.19–0.68) (**Fig. 5** and **Supplementary Table 2** online). In the two instances where we were able to study the parent-of-origin of BP4–BP5 deletions (the mother of individual 69/06 and the father of individual 02961), we found both to be heterozygous for the inversion. Although the high frequency of this inversion in the normal population and the lack of sufficient numbers of parental samples preclude any definitive conclusions, our observations are consistent with a model in which inversion polymorphism of the BP4–BP5 region results in these flanking duplicons being placed in

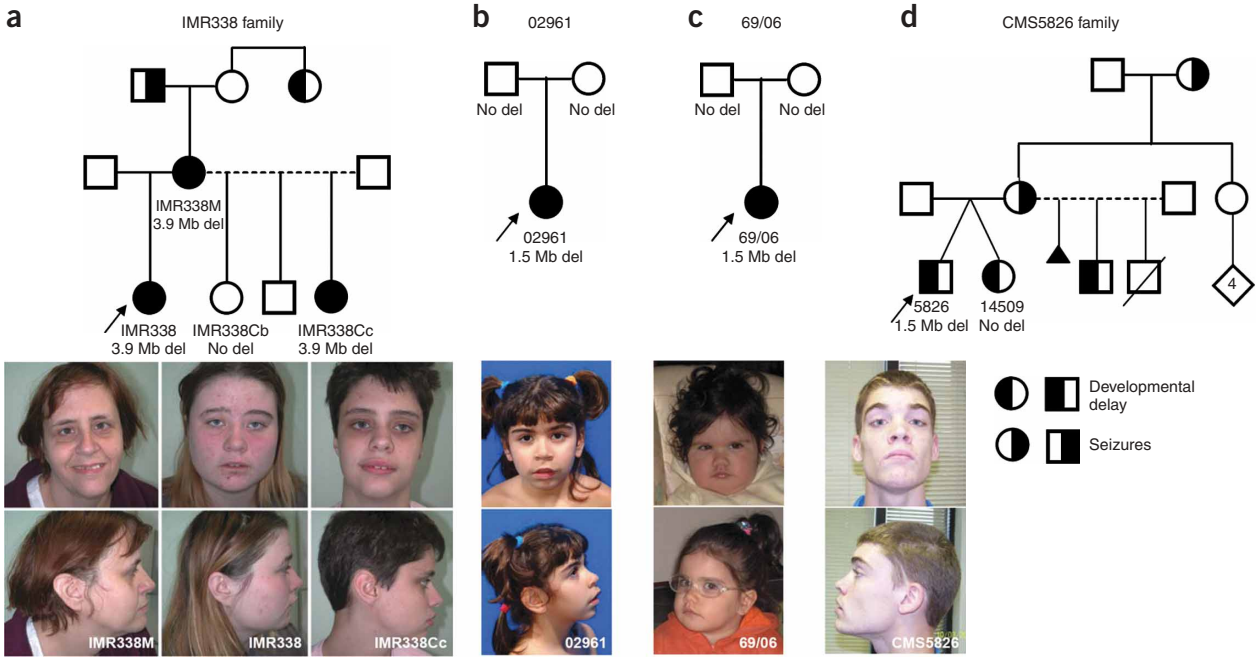

Figure 2 Pedigrees and photographs of individuals with 15q13 deletions. Half-filled symbols represent developmental delay and seizure phenotypes, as indicated in the figure. The presence or absence of 15q13 deletions is shown below each symbol in all individuals tested (the absence of text indicates that an individual was unavailable for testing). Photographs of affected family members are below each pedigree. We obtained consent to publish photographs from each individual included in this figure. (**a**) Family of proband IMR338. All affected individuals have 3.9-Mb deletions. Note the full everted lips and deep-set eyes evident in affected individuals. IMR338Cb is unaffected and does not have the deletion. (**b**) Individual 02961 (with a *de novo* deletion). Note the hypertelorism, synophrys, prominent philtrum, everted upper lip and hypotonic facies. (**c**) Individual 69/06 (with a *de novo* deletion). Note the prominent philtrum, everted upper lip, hypertelorism and hypotonic facies. (**d**) Family of proband CMS5826. Note the upslanting palpebral fissures and prominent philtrum in the affected individual.

direct orientation, creating a configuration predisposed to microdeletion or duplication by NAHR. These data strengthen the growing link between the occurrence of large inversion polymorphisms and genomic disorders (reviewed in ref. 10).

To assess the frequency of copy number changes between BP3, BP4 and BP5 in the general population, we screened 960 control individuals at this locus ($n = 960$ unrelated European Americans genotyped with a high-density SNP Genotyping BeadChip[11] for variable efficacy of statin drug response on cardiovascular disease; R. Krauss, Children's Hospital Oakland Research Institute, and D. Nickerson, University of Washington, personal communication). We identified two individuals with duplications of 15q13: one individual with a duplication within the interval BP3–BP4 and a second with a duplication between BP4 and BP5 (**Fig. 3**). Within the limits of resolution of these data, the BP4–BP5 duplication seems to be reciprocal to the deletions shown in **Figure 1**, providing further evidence that NAHR probably underlies rearrangements of 15q13. However, the BP3–BP4 duplication is smaller than the deletion identified in individual 543/06, with breakpoints located within unique sequence (that is, not within the segmental duplications). We did not detect any large deletions of 15q13 in this control set. While some smaller regions of copy number polymorphism are present within 15q13 (mostly corresponding to the segmental duplication blocks at BP3, BP4 and BP5), deletions or duplications comparable to those that we describe have not been found in previous studies of an additional 2,002 control individuals[12–19]. Together, these results suggest that deletions of BP4–BP5 are pathogenic (6/2,082 probands with mental retardation versus 0/2,962 controls; $P = 0.005$, Fisher's exact test). In contrast, our limited data suggest that duplication events of BP3–BP4 and BP4–BP5 are probably either (i) benign copy number variants or (ii) frequently associated with milder phenotypic abnormalities, although additional studies are necessary to confirm this interpretation. Parental origin studies in the two individuals with *de novo* deletions showed paternal origin of the deletion in individual 02961 and maternal origin in individual 69/06. In addition, no imprinted genes have been identified thus far within the BP3–BP5 region (according to the Genomic Imprinting Website, February 2008; see URLs section of Methods below), suggesting that imprinting is unlikely to significantly affect the phenotype of these individuals (**Supplementary Fig. 5** online).

Notably, seven of nine deletion carriers presented with seizures or abnormal EEG findings (**Table 1**). One of the genes within the critical region is *CHRNA7* (cholinergic receptor, neuronal nicotinic, alpha polypeptide 7), encoding a synaptic ion channel protein that mediates neuronal signal transmission. Linkage studies have suggested *CHRNA7* as a susceptibility factor for both juvenile myoclonic epilepsy[20] and benign epilepsy of childhood with centrotemporal spikes[21]. Furthermore, it has been reported that mice with a knockout of *Chrna7* show a hypersynchronous neocortical EEG phenotype[22]. As a result, *CHRNA7* represents an excellent candidate gene, haploinsufficiency for which may underlie the epilepsy and seizure phenotype seen in the individuals we describe. As copy number variation of a region including *CHRNA7* has also been observed in the general population[13–16], the possible involvement of this gene as a genetic risk factor for epilepsy warrants further investigation.

Although, to our knowledge, we present the first description of recurrent BP4–BP5 microdeletions, there are single reports of

apparently similar deletions at BP3–BP4–BP5 (according to the Sanger Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (February 2008, see URLs section in Methods)). Several previous studies have also described a variety of different structural rearrangements involving 15q13, suggesting that this is a highly unstable genomic region. BAC array CGH studies of isodicentric(15q) chromosomes, marker(15) chromosomes, atypical deletions associated with Angelman syndrome and deletions of 15q14

Table 1 Phenotypic features of nine individuals with deletions of 15q13

Individual ID; inheritance	Cognitive	Growth	Facial features	Hands	Neurologic	Other
69/06 *de novo*; maternal origin	**Moderate delays**, Brunet-Lezine global score 0.37	BW 3rd centile OFC (birth) 25th centile At 2 years: Wt 75th centile Ht 50th centile OFC 10th centile	**Mild hypertelorism, prominent philtrum, everted upper lip**, small L eye, strabismus, astigmatism		Moderate to severe **hypotonia**, stereotypic hand movements, **abnormal EEG**	EEG: unusual delta-theta activity with sporadic slow waves Normal brain MRI scan Right-side heart defect
02961 *de novo*; paternal origin	**Mild MR**, speech delay	Wt 25th centile Ht 3–10th centile OFC 10–25th centile	Brachycephaly, **hypertelorism**, astigmatism, synophris, wide nasal bridge, anteverted nares, **short thick philtrum**, **full everted upper lip**, low-set ears	Tapering fingers, 5th-finger clinodactyly	ADHD, **hypotonia**, **myoclonic epilepsy**	EEG: 3 c/s Sharps-Waves complexes, high voltage spikes in frontal region Normal brain MRI scan Early pubarche, hypertrichosis
IMR338; maternal inheritance	**Developmental delay**, speech difficulties	Ht 97th centile OFC >97th centile	Round flat face, **upslanting PF**, epicanthic folds, optic pit vs. coloboma, **everted upper lip**, nasal speech	5th-finger clinodactyly, lax thumb joint, single palmar crease	**Absence seizures**, **one tonic clonic seizure**	EEG: abnormal spike and wave activity, focus in left frontal area MRI: patchy change in white matter adjacent to left ventricle Hyperphagia, obesity, type II DM
IMR338M; unknown inheritance	**Developmental delay**, mild learning difficulties		Squint	Lax thumb joint	**Grand mal seizures**	Normal head CT scan
IMR338Cc; maternal inheritance	**Developmental delay**, moderate to severe learning difficulties	OFC 90th centile	Deep-set eyes, squint, **everted upper lip**	Lax thumb joint, long fingers	**Intractable epilepsy**	
CMS7906; unknown inheritance	**Mild to moderate MR** IQ 52	Ht 30th centile OFC 35th centile	Full round face, **upslanting PF**, widely spaced teeth	Short 4th MCPs, limited elbow extension	**Single seizure, 12 years old**	
CMS5826; unknown inheritance	**Mild to moderate MR** IQ 62 at 4 years IQ 44 at 15 years Speech delay	BW 50th centile OFC 30th centile	Long face, **upslanting PF**, **prominent philtrum**, depressed nasal bridge, anteverted nares, thick ear helices	Short 5th finger	Mild autism	Normal brain MRI Aggressive behavior, rage
CMS5803; maternal inheritance	**Mild MR** IQ 56	Ht 5–10th centile OFC <3rd centile	**Short philtrum**, full lips, everted lower lip	Short 4th MCPs, stiff fingers	**Hypotonia**	
CMS7833; unknown inheritance	**Moderate MR** IQ 34–46	Ht 5th centile OFC 3rd centile	Round face, depressed nasal bridge, smooth philtrum, everted lower lip	Short 4th MCPs, stiff fingers	**Seizures** under good control with medication	

BW, birth weight; Wt, weight; Ht, height; OFC, occipitofrontal circumference; PF, palpebral fissures; MCP, metacarpals; ADHD, attention deficit hyperactivity disorder; DM, diabetes mellitus; MR, mental retardation. Boldface indicates features shared by three or more individuals.

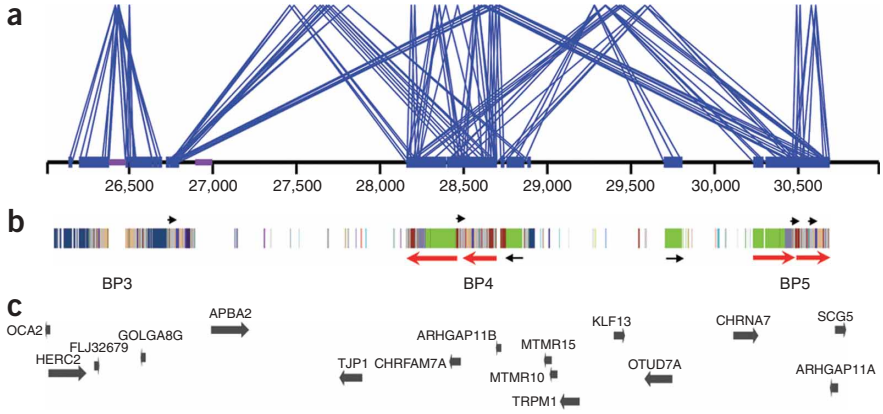

Figure 3 Duplication architecture of 15q13 breakpoint regions. (**a**) Paralogy between large (≥10 kb), highly identical (≥95%) segmental duplications (blue bars) is shown between BP3, BP4 and BP5 as pairwise alignments (blue lines). Sequence assembly gaps are shown as purple bars. (**b**) Underlying duplicon structure (where blocks of identical color represent those that share the same evolutionary origin) and orientation (red and black arrows) of pairwise alignments between the blocks[9]. (**c**) RefSeq genes. The BP3–BP4 and BP3–BP5 regions share fewer large, high-identity duplications when compared to BP4–BP5 (BP4–BP5, total number of base pairs aligned is 571.8 kb, mean identity = 98.6%) (**Supplementary Table 1**). Most notable are three segmental duplications, each with >99.4% identity, ranging in length from 96 kb to 218 kb, in which the breakpoints of all recurrent 1.5-Mb deletions we describe occur (red arrows). All three of these large duplications lie in an opposing orientation between BP4 and BP5 in the reference assembly. As a result, inversions of this region could result in these duplications being placed in a direct orientation, creating a configuration predisposed to microdeletion by NAHR.

have suggested that the breakpoints of these rearrangements often map to the segmental duplication blocks at BP3, BP4 and BP5 (refs. 23–26). Therefore, we investigated a number of other rearrangements of chromosome 15 using high-resolution oligonucleotide array CGH. Consistent with previous studies[25], data obtained in two unrelated inv dup(15) carriers were similar. Results showed that both inv dup(15) chromosomes are complex, being composed of two copies of the region 15cen-BP4 and a single copy of region BP4–BP5. We subsequently confirmed this by FISH (data not shown). Results in an individual with an Angelman syndrome class II deletion and in a second individual with a mar(15) showed that, in both cases, the distal breakpoints mapped to the large segmental duplication block at BP3 (**Supplementary Fig. 6** online).

Our screen of 2,082 individuals with idiopathic mental retardation identified six unrelated individuals with 15q13.3 deletions, suggesting that this microdeletion accounts for ~0.3% of mental retardation of unknown etiology. The 1,040 individuals screened by qPCR are a subset of a larger population of individuals who receive services through the South Carolina Department of Disabilities and Special Needs[27]. We compared the frequency of the 15q13.3 deletion to that of other known microdeletions resulting in mental retardation in the

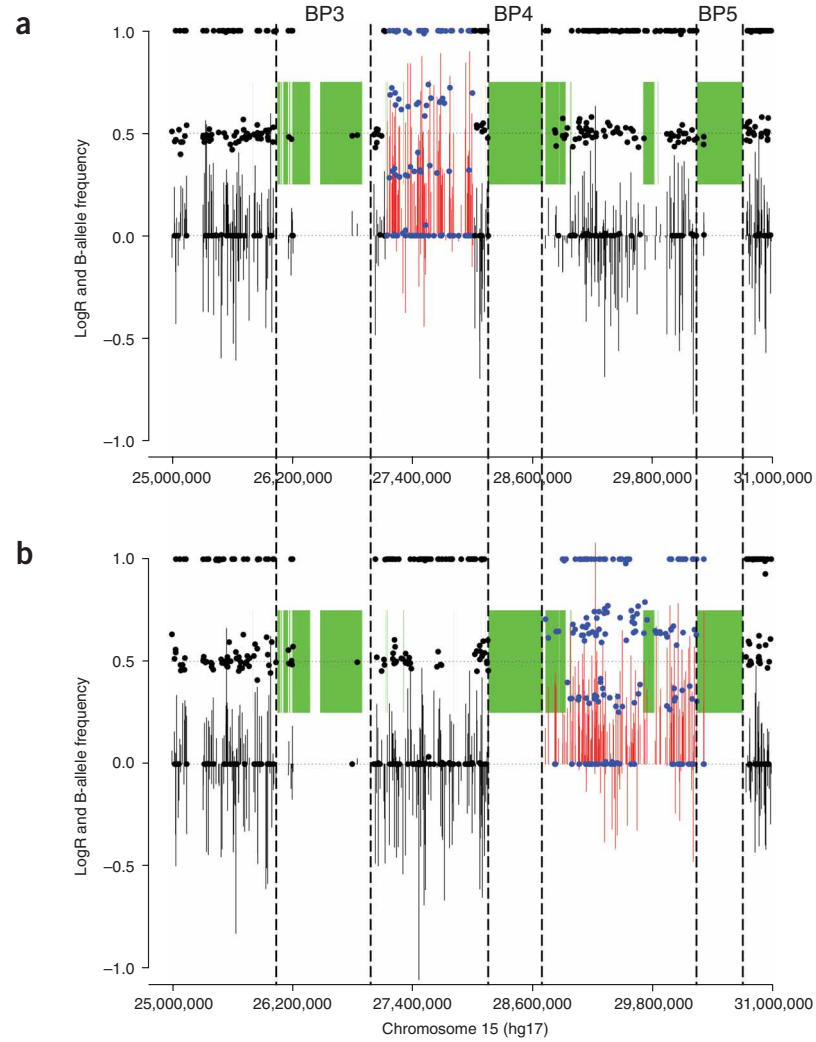

Figure 4 Duplications identified in two of 960 normal control samples using the Illumina HumanHap300 Genotyping BeadChip. (**a**) 15q13.1–q13.2 (BP3-BP4). (**b**) 15q13.3 (BP4–BP5). Data show probe position (*x* axis) against smoothed logR intensity ratio (red and black bars) and B allele frequency (blue and black circles) (*y* axis) for probes on chromosome 15 (ref. 3). Data points within the duplicated regions are shown in color, and those outside are shown in black. Green shaded boxes indicated segmental duplications. Within the limits of resolution of this data, the BP4–BP5 duplication seems to be the reciprocal event to the recurrent deletion shown in **Figure 1**. However, the BP3–BP4 duplication is clearly smaller than the deletion identified in individual 543/06, with breakpoints located in unique sequence (not in segmental duplications). No deletions of BP3–BP4 or BP4–BP5 were observed in this control population.

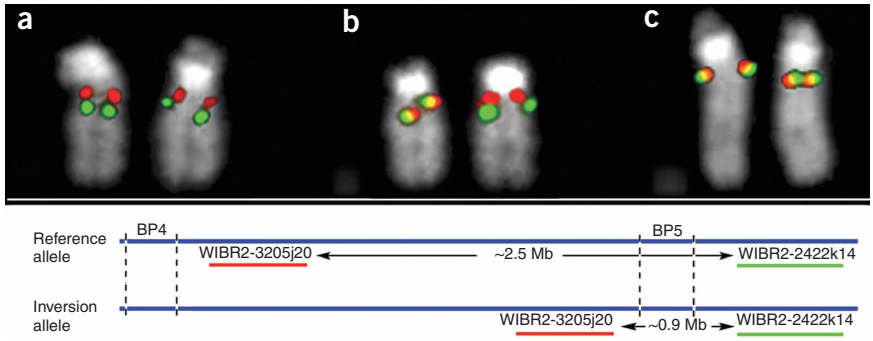

Figure 5 Identification of a common inversion polymorphism in 15q13.3. To detect inversions of the BP4–BP5 microdeletion region, we performed FISH mapping using fosmid probes located proximal and distal to BP5. The separation of these probes in the reference assembly is ~2.5 Mb, enough to visualize these as two separate signals on metaphase chromosomes. Inversion of the region BP4–BP5 moves the two probes within close proximity to each other, visualized as overlapping yellow signals. Using this assay on eight HapMap individuals of different ethnicities, we observed the inversion on 7/16 chromosomes (~44% of the population; **Supplementary Table 2**). We also tested the mother of individual 69/06 (in whose germline the BP4–BP5 deletion arose), who was found to be heterozygous. (**a**) Individuals homozygous for the reference allele. (**b**) Individuals heterozygous for the inversion allele. (**c**) Individuals homozygous for the inversion allele.

larger cohort from which our population was obtained (10,997 individuals of both European and African ancestry from South Carolina). Within the larger cohort, Prader-Willi syndrome was diagnosed in 0.22%, Angelman syndrome in 0.34% and Williams syndrome in 0.31%[27]. In comparison, we identified 15q13.3 deletions in 0.29% of the subgroup (three unrelated probands out of 1,040 individuals tested) and 0.29% of the European populations tested by array CGH (three unrelated probands out of 1,042 individuals tested), suggesting the frequency of this 15q13.3 microdeletion syndrome may be comparable to that of the disorders listed above (estimated frequency in individuals with mental retardation: 0.29%; 95% c.i.: 0.06–0.52%). Two pedigrees were of African American descent, and the remainder were of European origin, indicating that this syndrome is found in individuals of different ethnic backgrounds. Given that this microdeletion is well below the resolution of conventional cytogenetics—we detected it only when using techniques such as array CGH—we anticipate that the increasing resolution of these studies will lead to the identification of additional microdeletion syndromes. Assuming a prevalence of moderate mental retardation in the general population of ~0.8% (ref. 28), we estimate an approximate population incidence for this 15q13.3 microdeletion of 1/40,000. Given this relatively high frequency and the multiethnic distribution, we recommend testing for this disorder in individuals with features similar to those presented here.

METHODS

DNA samples. DNA samples were obtained from the following cohorts after obtaining informed consent and approval by the appropriate institutional review boards: (i) children and young adults from a variety of UK clinical genetics centers, community learning disability teams and other sources (including hospital neuropediatricians) in whom common causes of mental retardation, including karyotype and subtelomeric abnormalities and Fragile X, had been previously excluded ($n = 394$)[3]; (ii) karyotypically normal individuals presenting with mental retardation and/or dysmorphism collected by the University of Pavia ($n = 510$); (iii) individuals admitted to the IRCCS Associazione Oasi Maria Santissima (an Institute for Research and Care in Mental Retardation and Brain Aging) screened for mental retardation according to the DSM-IV-TR criteria, in whom the common causes of mental retardation were excluded ($n = 138$, all Caucasian, 75 female, 63 male); and (iv) individuals who received services through the South Carolina Department of Disabilities and Special Needs and for whom common causes of mental retardation had been excluded by fragile X testing, chromosome analysis, amino acid and organic analyses and urinary metabolic screening ($n = 1{,}040$, 540 female, 500 male, 562 African American, 448 European American, 30 unstated or other ethnicity; all females and all but 10 of the males had IQ scores <70)[29]. Two control groups were used to assess the extent of normal copy number variation. The first control population consisted of 316 unrelated individuals who had been tested using the same BAC duplication microarray[13,15]. A second control population, comprising 960 unrelated European American adults (age 40–70 years), was genotyped using the HumanHap300 Genotyping BeadChips (Illumina), which comprise ~317,000 HapMap SNPs spread throughout the genome. Each individual was enrolled in the Pharmacogenomics and Risk of Cardiovascular Disease (PARC) study, which aims to identify genetic contributors to the variable efficacy of statin drugs on cardiovascular disease risk (see URLs section below). Hybridizations, data analysis and copy number analysis, with particular reference to chromosome 15q13 (155 probes between BP3–BP4, 167 probes between BP4–BP5), were performed according to published protocols[11].

Array CGH. DNA from affected individuals from the UK ($n = 394$) were hybridized to a custom BAC array consisting of 2,007 clones targeted to regions of the genome flanked by segmental duplications, as described previously[15]. This array includes all regions associated with known genomic disorders and an additional 105 regions with similar genomic architecture. Because of the targeted nature of this array, it has reduced power to detect rearrangements not mediated by segmental duplications[15]. Regions were scored as copy number variant if the $\log_2$ ratio of two or more consecutive clones each exceeded twice the s.d. of the autosomal clones in dye-swap replicate experiments[7]. Affected individuals from Italy ($n = 648$) were molecularly karyotyped using Agilent 244A Human Genome CGH Microarrays. Parents of probands showing cryptic deletions were also analyzed to exclude inherited imbalances. Rearrangements of 15q were analyzed using a custom oligonucleotide array (NimbleGen Systems) consisting of 166,000 isothermal probes (length, 45–75 bp; mean probe density, 1 probe/130 bp) covering a number of chromosomal regions, including 42,698 probes covering a 6-Mb region of chromosome 15q13 (chr15:25500000–31500000). Hybridizations were performed as described previously[3] and used a single normal male as a reference (GM15724, Coriell). Microsatellite analysis to determine parental origin of the deletions used markers STS6_chr15, D15S1031 and D15S165 located within the interval BP4–BP5.

TaqMan quantitative PCR. Affected individuals from the USA ($n = 1{,}040$) were assayed for copy number of the region BP4–BP5 using two TaqMan Gene Copy Number Assays. Primers and probes were designed from genomic sequence (hg18/Build 36) using Applied Biosystems proprietary software. Each assay was run as a duplex TaqMan real-time PCR reaction, using a FAM dye–based assay targeted to 15q13.3 and a VIC dye–based assay for the reference gene, RNase P (PN 4316844 from Applied Biosystems). Each PCR assay was performed in quadruplicate and comprised 10 ng gDNA and 1× TaqMan probe/primer mix in 1× TaqMan Universal Master Mix in a 10-µl reaction amplified using an Applied Biosystems 7900HT SDS instrument. Cycling conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 92 °C and 60 s at 60 °C. Real-time data were collected by the SDS 2.3 software. The method involves relative quantification of the test sequence

versus a reference gene known to have two copies per diploid genome. Relative quantity is determined by the ΔΔCt ((FAM Ct – VIC Ct)$_{sample}$ – (FAM Ct – VIC Ct)$_{calibrator}$) method, where a reference sample or calibrator known to have two copies of the test sequence is used as the basis for comparative results. The gene copy number is two times the relative quantity[30]. The two regions assayed were (i) chr15:29000001–29000077 and (ii) chr15:29239994–29240092.

FISH inversion assay. Metaphase spreads were obtained from lymphoblast cell lines from eight HapMap individuals (Coriell Cell Repository) and from cultured peripheral blood lymphocytes from the mother of individual 69/06. FISH was performed using fosmid WIBR2-3205j20 (chr15:29009842–29052657) directly labeled by nick translation with Cy3-dUTP (Perkin-Elmer) and WIBR2-2422k14 (chr15:31531310–31570294) labeled with fluorescein-dUTP (Enzo). Each hybridization used 300 ng of labeled probe, 5 μg COT1 DNA (Roche) and 3 μg sonicated salmon sperm DNA at 37 °C in 10 μl 2× SSC/50% formamide/10% dextran sulfate, followed by three post-hybridization washes at 60 °C in 0.1× SSC. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) and digital images obtained using a Leica DMRXA2 epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments).

Accession number. Microarray data have been deposited in the Gene Expression Omnibus database under accession number GSE10189.

URLs. University of California Santa Cruz genome browser, http://genome.ucsc.edu/; Genomic Imprinting Website, http://www.geneimprint.com/site/genes-by-species; Sanger DECIPER, http://decipher.sanger.ac.uk/; PARC study, http://www.pharmgkb.org/network/members/parc.jsp#team.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

A.J.S., H.C.M. and E.E.E. contributed to the writing of this paper. The study was coordinated by A.J.S., H.C.M., S.J.L.K., C.R., O.Z., C.C., C.E.S. and E.E.E.; experimental work was done by A.J.S., H.C.M., K.L., C. Baker, F.N., M.D.G., R.C., A.B., G.G., R.R., M.F., L.C., P.F. and M.V.; clinical work was done by R.E.S., R.J.S., B.D.B., C.T., R.G., V.M., S.M., C.S. and C.R.; computational analysis was performed by Y.W., C.X., C. Barbacioru, Z.J., I.C. and G.M.C.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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