

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mefford HC, Sharp AJ, Baker C, et al. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med* 2008;359. DOI: 10.1056/NEJMoa0805384.

SUPPLEMENTARY METHODS

Oligonucleotide array CGH

Rearrangements of 1q21.1 were analyzed utilizing custom oligonucleotide arrays (NimbleGen Systems, Madison, WI). The majority of cases (deletion cases 1-3, 6-15 and duplication cases 1-4) were analyzed on a custom array consisting of 166,000 isothermal probes (length 45-75 bp; mean probe density, 1 probe/130 bp) covering a number of chromosomal regions, including 27,958 probes covering a 4.5-Mb region of chromosome 1q21 (Build 35, chr11:142,000,000-146,500,000). Deletion cases 4, 5, and 16 and duplication cases 5 and 7 were analyzed using a second custom array consisting of 72,000 isothermal probes with 766 probes covering the same region of 1q21 (mean probe density 1 probe/5875 bp). Deletion cases 17-19 and duplication case 8 were analyzed using a third custom array consisting of 130,000 isothermal probes with 1155 probes in the same region based on Build 36 coordinates (chr1:143,500,000-148,000,000). Hybridizations were performed as described previously¹ and utilized a single normal male as a reference (GM15724, Coriell, Camden, NJ). Parent of origin was determined by analysis of microsatellite markers within the minimal deletion region. Insufficient DNA was available for additional oligo array CGH (after screening array CGH as in Table 3) for deletion case 21 and duplication case 6.

Quantitative PCR

Brilliant SYBR Green QPCR Master Mix (Stratagene) was used for quantitative PCR assays in 10 μ l reactions containing 2.5ng of template DNA from patient and control samples. Gene specific or control primers were used at a final concentration of 200nM with PCR conditions of 95°C denaturation for 10 mins followed by 40 cycles at 95°C for 30 secs, 60°C for 1 min and 72°C for 1 min followed by 1 min at 95°C, 30 secs at 55°C and a final step of 30 secs at 95°C to obtain a dissociation curve. Reactions were carried out in triplicate, and a passive internal reference dye (ROX) was used to correct any differences in reaction volume between wells. Results were analysed using MX3000P comparative quantitation software (Stratagene). Relative quantities of each gene were normalized by comparing to amplification levels of the control CFTR gene in each

sample using identical conditions. PCR amplification efficiencies for each primer pair were calculated from the slope of a standard curve obtained using a serial dilution of template DNA, where $\text{PCR Efficiency} = 10^{(-1/\text{slope}) - 1}$.

***TaqMan* quantitative PCR**

Patients from the USA (n=1040) were assayed for copy number of the 1q21.1 region using two *TaqMan* Gene Copy Number Assays. Primers and probes were designed from genomic sequence (Build 36) using Applied Biosystems proprietary software. Each assay was run as a duplex *TaqMan* real-time PCR reaction, utilizing a FAM dye-based assay targeted to 1q21.1 and a VIC dye-based assay for the reference gene, RNase P (PN 4316844 from Applied Biosystems, Foster City, CA). Each PCR assay was performed in quadruplicate and comprised 10 ng gDNA, 1xTaqMan probe/primer mix in 1xTaqMan Universal Master Mix in a 10 μ l reaction amplified using an Applied Biosystems 7900HT SDS instrument for 2 mins at 50°C, 10 mins at 95°C, followed by 40 cycles of 15 secs at 95°C and 60 secs 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 be two copies for diploid genome. Relative quantity is determined by the $\Delta\Delta\text{Ct}$ [(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. Gene copy number is 2 \times the relative quantity. The two regions assayed were (1) chr1:145,460,047-145,460,130 and (2) chr1:145,679,984-145,680,077 (Build 36 coordinates).

DNA methylation studies

Methylated DNA immunoprecipitation on patient C66 and her mother was performed as described previously.¹ Briefly, 10 μ g of genomic DNA from patient C66 and her mother were sonicated to a mean fragment size of \sim 500bp and immunoprecipitated using 5 μ g monoclonal mouse anti-5-methyl cytidine (Diagenode, Liege, Belgium).

Immunoprecipitated DNA was bound to Protein A agarose beads (Invitrogen, Basel, Switzerland), washed and purified by phenol:chloroform extraction. Immunoprecipitated DNA and input (sonicated) DNA were labeled by random-priming with Cy3/Cy5

nonamers,² purified and hybridized to the same custom oligonucleotide array (mean density, 1 probe/130 bp) as described above for array CGH (NimbleGen Systems, Madison, WI).

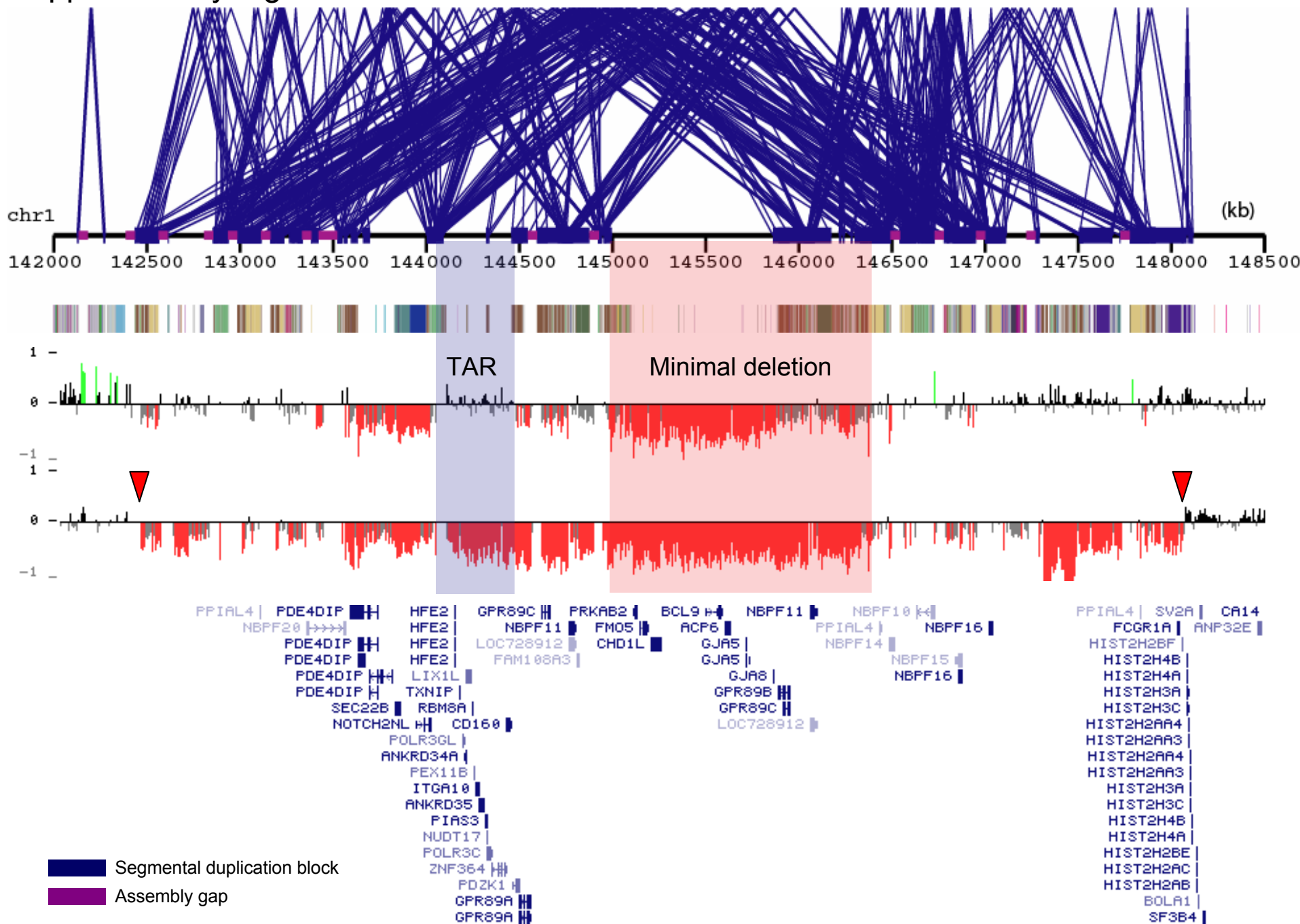
Fluorescence *in situ* hybridization

Metaphase spreads were obtained from lymphoblast immortalized cell lines. FISH experiments were performed using the fosmid ABC14_50192300_M1, directly labeled by nick-translation with Cy3-dUTP (Perkin-Elmer) as previously described³ with minor modifications. Briefly: 300 ng of labeled probe was hybridized at 37°C in 2 SSC, 50% (v/v) formamide, 10% (w/v) dextran sulphate, with 5µg COT1 DNA (Roche) and 3µg sonicated salmon sperm DNA in a volume of 10µL. Post-hybridization washing was at 60°C in 0.1 SSC (three times, high stringency). Nuclei were simultaneously DAPI stained. Digital images were obtained using a Leica DMRXA2 epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments). DAPI and Cy3 fluorescence signals, detected with specific filters, were recorded separately as gray scale images. Pseudocoloring and merging of images were performed using Adobe Photoshop software.

Sequence analysis

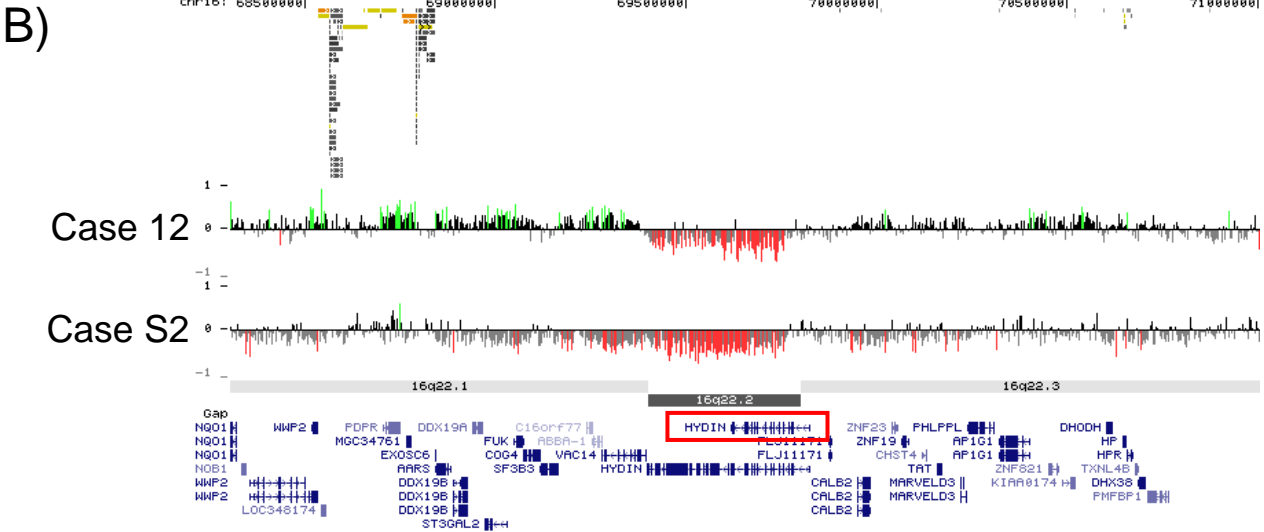
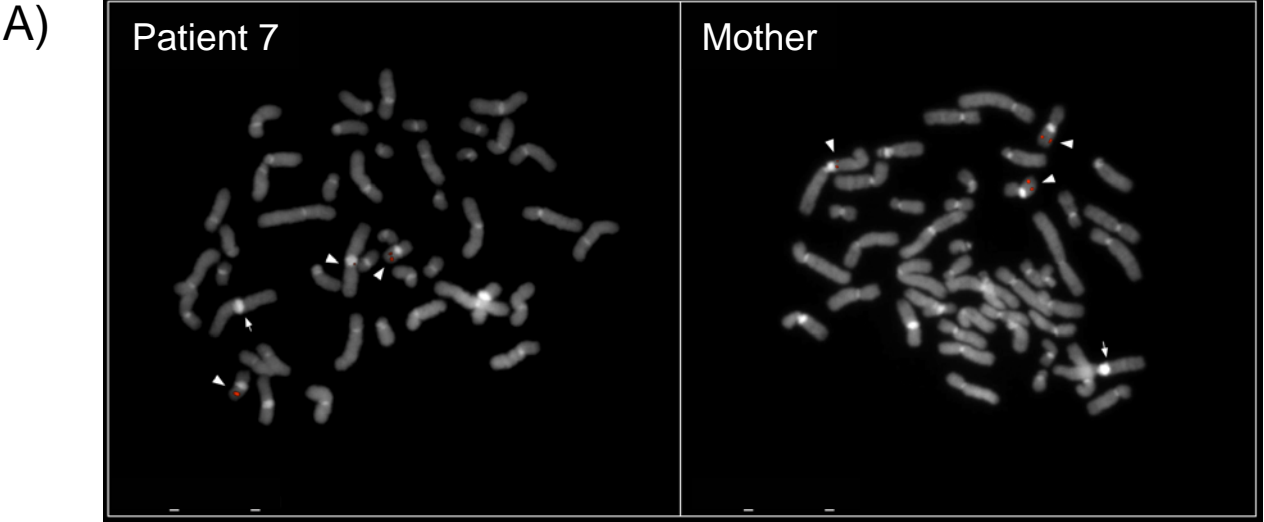
The coding exon and upstream region of *GJA8* and of *GJA5* were sequenced using standard BigDye terminator chemistry. Primer sequences are available upon request.

Supplementary Figure 1



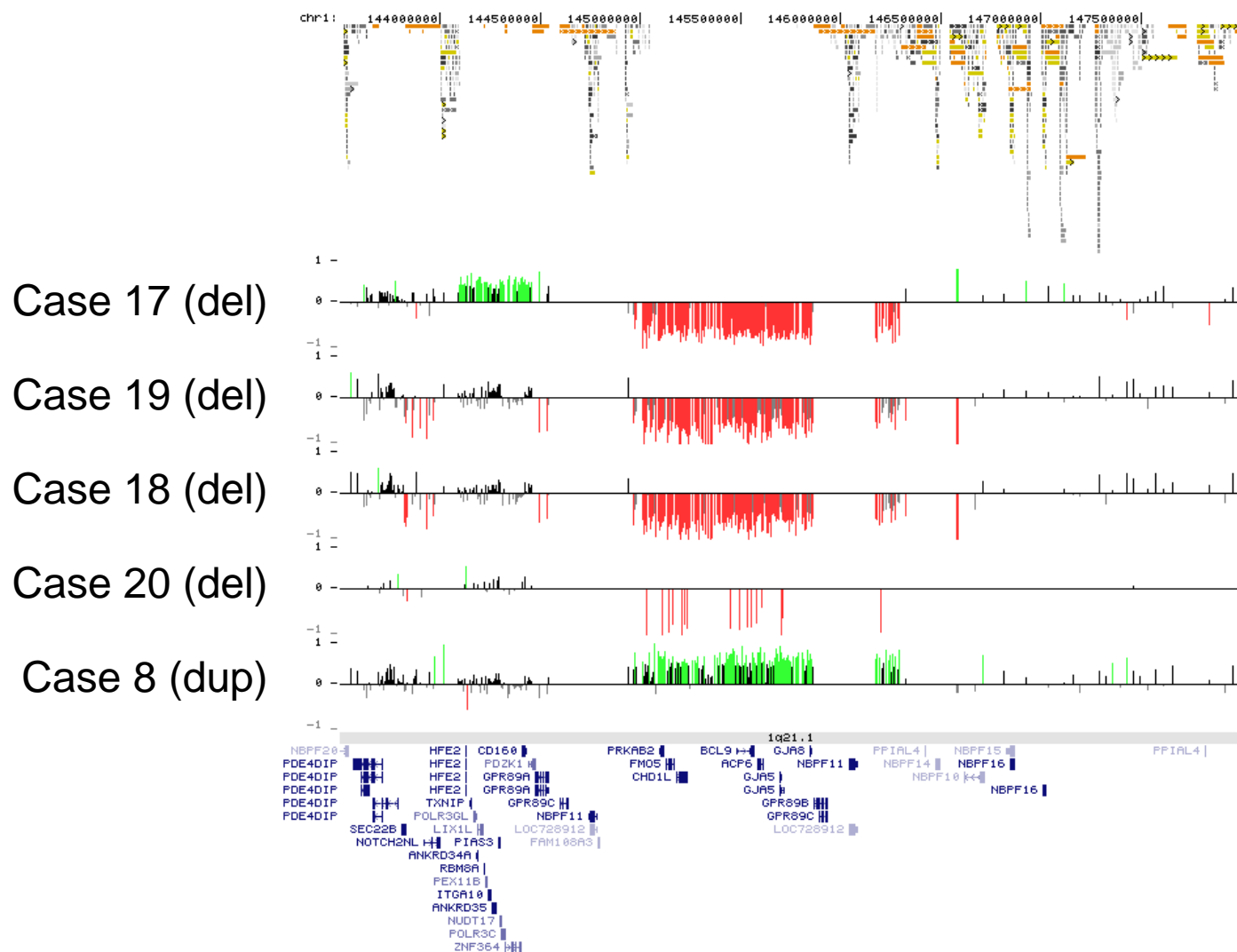
Supplementary Figure 1: Duplication architecture of 1q21 breakpoint regions and large atypical deletion. Build 36, chr1: 142,000,000-148,500,000: paralogy between large (≥ 10 kb), highly identical ($\geq 95\%$) intrachromosomal segmental duplications (*blue bars*) are shown as pairwise alignments (*blue lines*). Sequence assembly gaps are shown as purple bars. The underlying duplicon structure is shown, with blocks of identical color representing those that share the same evolutionary origin⁴. Oligo array CGH data (NimbleGen HD2 whole-genome oligo array) for deletion cases 10 and 12 are shown below. The breakpoints of the large deletion in case 12 are indicated by inverted red triangles. Pink shading indicates minimal rearrangement region and blue shading represents the TAR locus as in Figure 1. RefSeq genes are also shown. Note that case 10 appears to have a second deletion proximal to the TAR locus in addition to the minimal 1q21.1 deletion; this is a region of known copy number variation. An alternative explanation is these two apparently separate events actually represent one event, because the structure of 1q21.1 in this individual includes inversion(s), resulting in a structure different than that represented by the reference sequence.

Supplementary Figure 2



Supplementary Figure 2: A) FISH study in patient 7 and her mother, who each have a 1q21.1 deletion, using a chromosome-16 fosmid (ABC14_50192300_M1) that maps to the *HYDIN* locus. Both individuals have a positive signal on both homologs of chromosome 16 and one homolog of chromosome 1, suggesting that a *HYDIN* homolog maps to the deleted region of 1q21.1 in these individuals. B) Oligonucleotide array CGH data (NimbleGen HD2, 2.1-million oligo whole-genome chip) for two patients with 1q21.1 deletions; for each patient, there is also an apparent deletion on chromosome 16q22 encompassing most of the *HYDIN* gene. FISH validation experiments in three additional 1q21.1 deletion patients who exhibit similar 16q22 “deletions” failed to show deletion of 16q22 (data not shown) since the signal actually represents the deleted copy of *HYDIN2* on 1q21.1.

Supplementary Figure 3



Supplementary Figure 3: Array CGH results for deletion cases 17-20 and duplication case 8 (Build 36, chr1:143,500,000-148,000,000). Data for deletion cases 17-19 and duplication case 8 are from custom NimbleGen arrays (see Supplementary Methods). Data shown for case 20 is from the original screening array (Agilent 44k; see Table 3). Results for deletion case 21 and duplication case 6 are not shown as there was insufficient DNA for additional assays (aside from screening array CGH described in Table 1) and the screening array data is not available in this presentation format.

Supplementary Table 1A. Details on Enrollment and Results of Screening of the 5218 Study Patients, According to Series.*

| Series | Primary Center | Source of Referrals | Ascertainment and Inclusion Criteria | Exclusion Criteria | Screening Method† | Detected Cases of Deletion (N=25) |
|------------------|---|--|--|--|--|-----------------------------------|
| 1 (459 patients) | Wellcome Trust Center for Human Genetics, Oxford, United Kingdom | United Kingdom (50% of referrals from clinical geneticists, 35% from community-learning disability teams, and 15% from other physicians) | IMR±MCA (all had mild-to-severe MR with or without other features, normal karyotype and subtelomeric studies, and normal fragile X studies [when indicated]) | Normal intellect and abnormal karyotype, abnormal subtelomeric studies, or fragile X syndrome or other known causes of MR | Targeted BAC array | Patient 1 |
| 2 (104 patients) | Case Western Reserve University, Cleveland | Case Western Reserve University Hospital and University Hospitals of Cleveland | IMR (all had normal karyotype and normal fragile X studies, when indicated) | Normal intellect and abnormal karyotype, abnormal subtelomeric studies, or fragile X syndrome or other known causes of MR | Targeted BAC array | Patient 14 |
| 3 (84 patients) | Vanderbilt University, Nashville | Tufts Medical Center and Vanderbilt University | Autism (probands were recruited from families with two or more family members with autism, as determined with ADI-R and ADOS) | Fragile X syndrome, chromosomal abnormality, IQ <35, and significant dysmorphic features | Targeted BAC array | — |
| 4 (228 patients) | Istituto di Ricovero e Cura a Corattere Scientifico (IRCCS) Associazione Oasi Maria Santissima, Troina, Italy | Clinical geneticists in Sicily (95% of referrals) and mainland Italy (5% of referrals) | MR+DD±mild dysmorphic features (30% of patients) or MR+DD±major dysmorphic features or anomalies (70% of patients) (25% also had autistic features or another neuropsychiatric disorder; all patients had mild-to-severe MR [as defined by DSM-IV-TR criteria], normal karyotype, and normal subtelomeric screening by FISH or MLPA) | Normal intellect and abnormal karyotype, abnormal subtelomeric studies, or fragile X syndrome or other known cause of MR or DD | Agilent 44k oligonucleotide array (61 patients), Agilent 105k oligonucleotide array (60 patients), and Agilent 244k oligonucleotide array (107 patients) | Patients 4, 5 |

| | | | | | | |
|-------------------|---|---|---|---|---|------------------------|
| 5 (474 patients) | Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands | Clinical geneticists in south-east Netherlands (90% of referrals) and Ireland and United Kingdom (10% of referrals) | Isolated MR (30% of patients) or MR±MCA (70% of patients) (all patients had normal chromosomal G bands after trypsin and Giemsa staining and normal subtelomeric screening by MLPA) | Normal intellect, abnormal karyotype, abnormal subtelomeric studies, and fragile X syndrome or other known causes of MR | 32K BAC array | Patient S3 |
| 6 (1180 patients) | Centre for Human Genetics, Catholic University, Leuven, Belgium | Clinical geneticists in Belgium | IMR±dysmorphic features (95% of patients) or dysmorphic features + additional anomalies with normal intellect (5% of patients) | Recognizable syndrome and known karyotype abnormality or known cause of MR | 1-Mb BAC array | Patients 3, 6, 13 |
| 7 (367 patients) | Wessex Regional Genetics Laboratory, Salisbury, United Kingdom | Clinical geneticists in southern England | IMR or DD + MCA (85% of patients) or MCA without MR or in those too young for evaluation for MR (15% of patients) (all patients had normal karyotype and normal fragile X studies [when indicated]) | Known karyotype abnormality and fragile X syndrome or other known cause of MR | Custom 44k Agilent oligonucleotide array | Patients 8, 9, 11 |
| 8 (187 patients) | University of Manchester, Manchester, United Kingdom | Clinical geneticists in northern England | IMR + other features suggestive of chromosomal abnormality (all patients had mild to severe MR, normal karyotype, and normal subtelomeric studies) | Normal intellect and abnormal karyotype, other known cause of MR, or judgment by a panel of clinical geneticists of probable cause other than chromosomal | BlueGnome Cytochip BAC array or Affymetrix 250k Nsp SNP array | Patient 10 |
| 9 (180 patients) | University of Cambridge, Cambridge, United Kingdom | Clinical geneticists in East Anglia, United Kingdom | IMR + congenital anomalies or dysmorphic features (all patients had apparently normal karyotype) | Normal intellect and abnormal karyotype or other known cause of MR | Affymetrix 250k Nsp SNP array | Patients 2, 15, S1, S2 |
| 10 (302 patients) | Instituto G. Gaslini, Genoa, Italy, and Università Federico II, Naples, Italy | Neurologists (50% of referrals), clinical geneticists (35% of referrals) and other physicians (15% of referrals) in Italy | IMR±MCA with normal karyotype and normal subtelomeric studies | Normal intellect and abnormal karyotype, abnormal subtelomeric studies, or other known cause of MR | Agilent 44k oligonucleotide array | Patient 7 |

Supplementary Table 1A. (Continued.)

| Series | Primary Center | Source of Referrals | Ascertainment and Inclusion Criteria | Exclusion Criteria | Screening Method [†] | Detected Cases of Deletion (N=25) |
|--------------------|--|---|---|---|------------------------------------|-----------------------------------|
| 11 (212 patients) | Geneva University Hospital and Inselspital, Bern, Switzerland | Clinical geneticists in Switzerland | IMR + MCA (90% of patients), isolated MR (5% of patients), or autistic spectrum disorder (5% of patients) (all had normal karyotype and normal fragile X studies [when indicated]) | Abnormal karyotype, fragile X syndrome, or other known cause of MR | Agilent 244k oligonucleotide array | Patient 16 |
| 12 (57 patients) | Irish Autism Genetics Study, Trinity College, Dublin | Clinical geneticists and other physicians in Ireland | Autism (diagnosis by ADI-R and ADOS criteria, IQ >35, or mental age score >18 mo) | Abnormal karyotype, fragile X syndrome, extreme prematurity, infantile rubella, and exposure in utero to medications known to cause autism or other known medical cause of autism | ROMA | — |
| 13 (1040 patients) | Greenwood Genetic Center, Greenwood, SC | South Carolina Department of Disabilities and Special Needs | IMR with normal karyotype and normal fragile X studies (when indicated) | Known cause of MR including fragile X syndrome, abnormal karyotype, and known metabolic disorder | TaqMan quantitative PCR | Patients 12, S4 |
| 14 (25 patients) | Pompeu Fabra University, Barcelona | Clinical geneticists in Sabadell, Spain | IMR±MCA (features suggestive of 22q11 deletion syndrome but normal karyotype, normal FISH results for deletion in 22q11, normal subtelomeric studies, and normal fragile X studies) | Abnormal karyotype, presence of del 22q11, CGG-repeat expansions in the fragile X mental retardation 1 gene (fragile X syndrome), and subtelomeric alteration | Targeted BAC array | Patient 17 |
| 15 (319 patients) | Centre for Medical Genetics, Ghent University Hospital, Ghent, Belgium | Clinical geneticists in Belgium | IMR±MCA (majority of patients with dysmorphic features) | Abnormal karyotype, fragile X studies, and other known causes of MR | Agilent 44k oligonucleotide array | Patients 18–21 |

* ADI-R denotes Autism Diagnostic Interview–Revised; ADOS Autism Diagnostic Observation Schedule; DD developmental delay; DSM-IV-TR *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition, text revision; FISH fluorescence in situ hybridization; IMR idiopathic mental retardation; MCA multiple congenital abnormalities; MLPA multiplex ligation-dependent probe amplification; MR mental retardation; PCR polymerase chain reaction; and SNP single-nucleotide polymorphism.

[†] Screening with the use of the targeted bacterial artificial chromosome (BAC) array as used in series 1, 2, and 3 is described by Sharp et al.¹⁸ and as used in series 14 is described by Cuscó et al.¹⁹ Screening with the use of the 32K BAC array as used in series 5 is described by de Vries et al.² Screening with the use of a custom 44k Agilent oligonucleotide array as used in series 7 is described by Barber et al.¹ Screening with the use of representational oligonucleotide microarray analysis (ROMA) as used in series 12 is described by Sebat et al.²⁰

| Supplementary Table 1B: Phenotypic features of 1q21.1 deletion (n=3) and duplication (n=3) patients from Dutch series* | | | | | | | | | |
|--|--------------------|--------|---|---|---|-------------|--|--------|--|
| Case | Inheritance | Origin | Cognitive | Growth | Facial features | Skeletal | Heart | Eyes | Neurologic |
| 1 (del) | De novo | Unk | MR | Postnatal growth parameters normal | Microcephaly, narrow eyes, periorbital fullness | Normal | Patent ductus arteriosus (surgically repaired) | Normal | No epilepsy; broad-based gait |
| 2 (del) | De novo | Unk | Expressive language delay, language dyspraxia, mild motor delay | Postnatal growth parameters low normal range | Prominent forehead, triangular face, broad nose and mouth | Polydactyly | Normal | Normal | No epilepsy; hypotonia; delayed myelination |
| 3 (del) | Unk | Unk | MR; self-harming behaviors | Postnatal growth parameters low normal range | Straight nose | Normal | Normal | Normal | No epilepsy |
| 4 (dup) | De novo | Unk | IQ within normal range | Mild intrauterine growth retardation; OFC normal at 2 years | Normal | Normal | Perimembranous VSD | Normal | No epilepsy Other: also has hypospadias |
| 5 (dup) | Inh, father normal | P | MR | Normal | Normal | Normal | Normal | Normal | Idiopathic generalized epilepsy |
| 6 (dup) | Unk | Unk | MR | Normal | Normal | Normal | Normal | Normal | Idiopathic generalized epilepsy; hypoplasia of the corpus callosum and cerebellar vermis |

* Additional patients analyzed at University Medical Center, Utrecht, The Netherlands included 788 patients with MR/MCA analyzed using the Cytochip BAC array (BlueGnome, Cambridge, UK) and 1-Mb BAC array⁵. Inclusion criteria were: DeVries score >3 (ref. 2)⁶, no known karyotype abnormality. Exclusion criteria were autism spectrum disorder, known karyotype abnormality, Fragile X or other known cause of MR.

Inh, inherited; P paternal; Unk, unknown; MR, mental retardation; Ht, height; Wt, weight; OFC, occipitofrontal circumference

| Supplementary Table 2: Evaluation of copy number variation in unaffected individuals | | | | | |
|---|-------------|---|--|---------------------------|------------------------------|
| STUDY | n | Evaluation method | Coverage of minimal deletion region | Deletions detected | Duplications detected |
| Itsara <i>et al</i> (in preparation)* | 2063 | Illumina Infinium arrays (HumanHap 300 or HumanHap 550) | 99 probes (HumanHap 300) 212 probes (HumanHap 550) | 0 | 0 |
| Cohort viii: Manchester, UK | 300 | SYBR Green quantitative PCR (see Methods) | 5 loci within region | 0 | 0 |
| Locke <i>et al</i> (2006), Redon <i>et al</i> , (2006) ^{7, 8} | 209 | Targeted BAC array CGH, 32K WGTP BAC array CGH and Affymetrix 5.0 | 13 clones (targeted BAC); 6 clones (WGTP); 348 probes (Affy 5.0) | 0 | 0 |
| Iafate <i>et al</i> (2004) ⁹ | 55 | 1-Mb BAC array CGH | 2 clones | 0 | 0 |
| Sebat <i>et al</i> (2004) ¹⁰ | 20 | ROMA | 37 probes (1 probe / 35kb) | 0 | 0 |
| Sharp <i>et al</i> (2005) ¹¹ | 47 | Targeted BAC array CGH | 13 clones | 0 | 0 |
| Simon-Sanchez <i>et al</i> (2007) ¹² | 276 | HumanHap 300 and Infinium Human-I | 99 probes (HumanHap 300) | 0 | 0 |
| Zogopolous <i>et al</i> (2007) ¹³ | 1190 | Affymetrix GeneChip 100K and 500K | 50 probes (100K) 221 probes (500K) | 0 | 1** |
| Pinto <i>et al</i> (2007) ¹⁴ | 506 | Affymetrix 500K SNP array | 221 probes | 0 | 0 |
| de Stahl <i>et al</i> (2008) ¹⁵ | 71 | 32K BAC array | 6 clones | 0 | 0 |
| TOTAL | 4737 | | | 0 | 1 |

* 22% of the individuals in this series are a subset of the Human Genome Diversity Panel (HGDP) reported by Jakobssen *et al* (ref. 12)¹⁶; 35% are adult neurologic controls from the NINDS collection screened for neurologic disorders including autism, bipolar disorder and schizophrenia.^{12, 17} The remaining 43% individuals are adult self-reported Caucasians from the United States who are enrolled in the PARC study, which aims to identify genetic contributors to the variable efficacy of statin drugs on cardiovascular disease risk (<http://www.pharmgkb.org/network/members/parc.jsp#team>).

** 4 duplications were reported in this study; however only 1 was validated (L. Feuk, personal communication).

Supplementary Table 3. Phenotypic Features of Probands with a Duplication in Chromosome 1q21.1.*

| Patient† | Inheritance | Parental Origin | Cognitive Features | Growth Features | Facial Features | Skeletal Features | Features of the Heart | Features of the Eyes | Neurologic Features |
|----------|---|-----------------|---|--|--|-------------------|---|----------------------|-------------------------------------|
| 1 | Inherited (normal parent and affected brother carry 1q21.1 duplication) | P | Autism; no other data available | Unknown | No notable dysmorphic features | Unknown | Unknown | Unknown | No known seizures |
| 2 | Unknown | Unknown | Moderate MR | Height, <3rd percentile for age; OFC, 85th percentile for age | Frontal bossing, down-slanting palpebral fissures, epicanthic folds, hypertelorism, high palate, bifid uvula, low ears | Normal | Normal | Rotational nystagmus | Normal |
| 3 | De novo | Unknown | MR | Macrocephaly | Frontal bossing, down-slanting palpebral fissures, low set ears | Normal | Normal | Normal | Normal |
| 4 | Unknown | Unknown | DD, mild learning disability, challenging behavior | Height, <3rd percentile for age; weight, 50th percentile for age; OFC, 90th percentile for age | Small low-set ears, up-turned lobes; small mouth and chin; high palate; broad nasal bridge; telecanthus | Joint laxity | Small ventricular septal defect, closed spontaneously (no intervention) | Normal | Small sacral dimple, mild hypotonia |
| 5 | Inherited (normal father) | P | Autism, learning disabilities | Height and weight, 5th–10th percentile for age | Normal | Hemivertebra L4 | Univentricular heart | Papillary atrophy‡ | Seizure disorder |
| 6 | Unknown | Unknown | Moderate MR, autistic features, severe speech delay | Height, 75th percentile for age; gross obesity; OFC, 97th percentile for age | Everted lower lip | Normal | Normal | Normal | Normal findings on head CT |
| 7 | De novo | Unknown | Autism, borderline mild MR | Height, 90th percentile for age; OFC, >90th percentile for age | Normal | Normal | Normal | Normal | Normal |
| 8 | Unknown | Unknown | Psychomotor retardation | Weight, 90th percentile for age; OFC, 97th percentile for age | Hypertelorism | Normal | Normal | Normal | Normal findings on brain MRI |

* CT denotes computed tomography, DD developmental delay, M maternal, MR mental retardation, MRI magnetic resonance imaging, OFC occipitofrontal circumference, and P paternal.

† Patient 1 is from series 3, Patients 2 and 3, series 5; Patient 4, series 8; Patients 5 and 6, series 6; Patient 7, series 12; and Patient 8, series 15.

‡ Patient 5 also had a choroid plexus carcinoma for which he had surgery, radiotherapy, and chemotherapy. Papillary atrophy is probably secondary to repeated episodes of increased intracranial hypertension related to the tumor.

| Supplementary Table 4: Phenotypic features for patients with 1q21.1 rearrangements and additional chromosomal abnormalities | | | | | | | | | | |
|---|--|--------|--|---|--|--|---|--|-----------------------|--|
| Case | Inheritance | Origin | Additional imbalances | Cognitive | Growth | Facial features | Skeletal | Heart | Eyes | Neurologic |
| S1 (del) | De novo | Unk | 5q14 deletion (5.3 Mb; de novo) | N.I. | Wt P0.4-2 OFC <P0.4 | Hypotelorism, short stub nose | Bilateral post-axial skin tags | Small persistent patent foramen ovale (asymptomatic) | N.I. | Hypotonia |
| S2 (del) | Inherited | P | 5p15.3 duplication (6.1 Mb; inherited from father) der(8)t(8;21)(q24.3;q22.3) | Early delays (sat >12 mo, walked at 20 mo); now progressing | At 2 yrs: Wt P9 Ht P20 OFC <P0.4 | Asymmetry of cranial vault, downslanting palpebral fissures, tubular nose | Bilateral 5 th finger clinodactyly | N.I. | Strabismus | Hypotonia |
| S3 (del) | Inherited | P | t(6,9) 13q21-q31 del (6.6 Mb) and 13q31 del (1 Mb) | MR, motor delays | Pre- and post-natal growth retardation; microcephaly | Palatoschisis, ptosis, long eyelashes, high/broad nasal bridge, dysplastic and low set ears micrognathia | Bilateral 5 th finger clinodactyly | Atrial septal defect, no intervention required | Strabismus | Ataxia, hypotonia |
| S4 (del) | Unk; half-siblings and maternal uncles with MR | Unk | <i>DLG3</i> mutation | Moderate MR (IQ 41) | Microcephaly | Sloping forehead, L ptosis, wide-spaced teeth | Normal | Murmur, normal echocardiogram | Normal | No seizures |
| S5* (del) | Unk | Unk | 2p16.3 duplication chr2:48,648,754-49,319,683 (671 kb) | Likely normal; achieved GED; honorably discharged from military service | Mildly obese | N.I. | N.I. | N.I. | N.I. | Schizophrenia, onset age 25; severe paranoia, delusions, catatonia |
| S6 (dup) | De novo | Unk | 1q21-q23 duplication (9.4 Mb) | MR, severe with little progress | Normal but poor feeding | Myopathic facies, prominent lower lip | Normal | Normal | Myopia and strabismus | Deafness, delayed milestones |

Del, deletion; dup, duplication; Unk, unknown; M, maternal; P, paternal; MR, mental retardation; Ht, height; Wt, weight; OFC, occipitofrontal circumference; N.I., no information; *previously reported by Walsh and colleagues¹⁸

Supplementary Table 5: All pairwise alignments between breakpoint regions
(Pairwise alignments with longest stretch of highest identity for each pair in bold)

| A) Paired duplications between BP1 and BP2 | | | | | | |
|---|--------------------|--------------------|--------------------|------------------|-----------------|--------------------|
| BP1 start | BP1 stop | BP2 start | BP2 stop | Size (kb) | Identity | orientation |
| 142,857,141 | 142,927,783 | 144,010,043 | 144,080,196 | 70.6 | 95.52% | + |
| 143,522,082 | 143,540,019 | 144,010,043 | 144,027,990 | 17.9 | 98.51% | + |
| 143,534,672 | 143,552,732 | 144,074,521 | 144,092,620 | 18.1 | 96.07% | + |
| B) Paired duplications between BP1 and BP3 | | | | | | |
| BP1 start | BP1 stop | BP3 start | BP3 stop | Size (kb) | Identity | orientation |
| 142,857,141 | 142,875,105 | 144,763,589 | 144,745,644 | 18.0 | 97.52% | - |
| 143,308,712 | 143,333,770 | 144,786,645 | 144,761,595 | 25.1 | 97.49% | - |
| 143,522,082 | 143,545,357 | 144,763,589 | 144,740,310 | 23.3 | 97.98% | - |
| 143,559,134 | 143,575,976 | 144,740,310 | 144,723,753 | 16.8 | 96.28% | - |
| 143,594,887 | 143,627,819 | 144,963,196 | 144,995,604 | 32.9 | 95.72% | + |
| C) Paired duplications between BP1 and BP4 | | | | | | |
| BP1 start | BP1 stop | BP4 start | BP4 stop | Size (kb) | Identity | orientation |
| 142,436,049 | 142,562,525 | 146,298,638 | 146,425,103 | 126.5 | 99.59% | + |
| 142,535,781 | 142,562,525 | 146,542,663 | 146,572,240 | 26.7 | 99.09% | + |
| 142,542,168 | 142,562,525 | 146,927,802 | 146,907,552 | 20.4 | 98.94% | - |
| 142,542,168 | 142,562,525 | 147,089,711 | 147,069,563 | 20.4 | 99.03% | - |
| 142,857,141 | 142,875,105 | 146,060,642 | 146,042,692 | 18.0 | 97.52% | - |
| 142,858,092 | 142,875,057 | 147,006,246 | 147,023,138 | 17.0 | 98.44% | + |
| 142,858,092 | 142,875,058 | 146,844,161 | 146,861,095 | 17.0 | 98.47% | + |
| 142,859,972 | 142,881,664 | 146,492,662 | 146,471,017 | 21.7 | 95.60% | - |
| 142,985,839 | 143,002,546 | 147,523,840 | 147,505,292 | 16.7 | 96.49% | - |
| 142,985,839 | 143,006,944 | 146,369,533 | 146,348,380 | 21.1 | 97.39% | - |
| 142,985,839 | 143,113,101 | 147,909,455 | 147,782,119 | 127.3 | 99.68% | - |
| 143,015,785 | 143,031,533 | 146,348,462 | 146,332,665 | 15.7 | 97.94% | - |
| 143,032,741 | 143,113,101 | 146,379,171 | 146,459,532 | 80.4 | 99.61% | + |
| 143,051,940 | 143,113,038 | 146,542,663 | 146,606,116 | 61.1 | 98.79% | + |
| 143,058,333 | 143,113,038 | 146,927,802 | 146,873,659 | 54.7 | 98.71% | - |
| 143,058,333 | 143,113,038 | 147,089,711 | 147,035,687 | 54.7 | 98.75% | - |
| 143,163,102 | 143,182,782 | 147,526,837 | 147,505,292 | 19.7 | 96.86% | - |
| 143,163,102 | 143,187,183 | 146,372,523 | 146,348,380 | 24.1 | 97.17% | - |
| 143,163,102 | 143,238,033 | 147,912,434 | 147,837,397 | 74.9 | 99.43% | - |
| 143,196,013 | 143,211,780 | 146,348,462 | 146,332,665 | 15.8 | 97.92% | - |
| 143,212,991 | 143,238,033 | 146,379,171 | 146,404,289 | 25.0 | 99.08% | + |
| 143,263,101 | 143,331,520 | 146,777,983 | 146,849,231 | 68.4 | 98.49% | + |
| 143,281,711 | 143,292,847 | 146,286,961 | 146,278,091 | 11.1 | 95.37% | - |
| 143,308,712 | 143,333,770 | 146,083,748 | 146,058,648 | 25.1 | 97.51% | - |
| 143,310,744 | 143,333,770 | 146,727,982 | 146,706,108 | 23.0 | 98.74% | - |
| 143,321,088 | 143,331,520 | 147,000,772 | 147,011,318 | 10.4 | 95.64% | + |
| 143,522,082 | 143,540,019 | 146,708,099 | 146,690,133 | 17.9 | 98.21% | - |
| 143,522,082 | 143,545,357 | 146,060,642 | 146,037,358 | 23.3 | 97.96% | - |
| 143,523,027 | 143,542,193 | 146,844,161 | 146,863,310 | 19.2 | 98.38% | + |
| 143,523,027 | 143,542,193 | 147,006,246 | 147,025,354 | 19.2 | 98.40% | + |
| 143,524,924 | 143,540,019 | 146,492,662 | 146,477,559 | 15.1 | 98.53% | - |
| 143,559,134 | 143,575,976 | 146,037,358 | 146,020,797 | 16.8 | 96.27% | - |

D) Paired duplications between BP2 and BP3

| BP2 start | BP2 stop | BP3 start | BP3 stop | Size (kb) | Identity | orientation |
|--------------------|--------------------|--------------------|--------------------|-------------|---------------|-------------|
| 144,004,163 | 144,027,990 | 144,769,399 | 144,745,644 | 23.8 | 97.84% | - |
| 144,073,367 | 144,092,620 | 144,926,035 | 144,945,324 | 19.3 | 95.08% | + |

E) Paired duplications between BP2 and BP4

| BP2 start | BP2 stop | BP4 start | BP4 stop | Size (kb) | Identity | orientation |
|--------------------|--------------------|--------------------|--------------------|-------------|---------------|-------------|
| 144,004,163 | 144,027,990 | 146,066,503 | 146,042,692 | 23.8 | 97.81% | - |
| 144,004,163 | 144,079,919 | 146,713,980 | 146,637,664 | 75.8 | 98.19% | - |
| 144,011,009 | 144,027,942 | 147,006,246 | 147,023,138 | 16.9 | 98.41% | + |
| 144,011,009 | 144,027,943 | 146,844,161 | 146,861,095 | 16.9 | 98.42% | + |
| 144,012,902 | 144,034,531 | 146,492,662 | 146,471,013 | 21.6 | 95.65% | - |
| 144,065,165 | 144,076,718 | 146,482,897 | 146,471,341 | 11.6 | 97.86% | - |

F) Paired duplications between BP3 and BP4

| BP3 start | BP3 stop | BP4 start | BP4 stop | Size (kb) | Identity | orientation |
|--------------------|--------------------|--------------------|--------------------|--------------|---------------|-------------|
| 144,322,642 | 144,337,061 | 146,017,954 | 146,003,508 | 14.4 | 99.23% | - |
| 144,459,423 | 144,544,474 | 145,948,719 | 145,861,130 | 85.1 | 99.12% | - |
| 144,594,476 | 144,876,007 | 145,891,442 | 146,173,101 | 281.5 | 99.94% | + |
| 144,743,482 | 144,762,642 | 146,863,310 | 146,844,161 | 19.2 | 97.94% | - |
| 144,743,482 | 144,762,642 | 147,025,354 | 147,006,246 | 19.2 | 97.99% | - |
| 144,745,644 | 144,760,751 | 146,477,559 | 146,492,662 | 15.1 | 97.90% | + |
| 144,745,644 | 144,784,610 | 146,690,133 | 146,727,982 | 39.0 | 97.85% | + |
| 144,763,840 | 144,774,233 | 147,011,318 | 147,000,772 | 10.4 | 95.80% | - |
| 144,763,840 | 144,786,645 | 146,849,231 | 146,826,314 | 22.8 | 96.54% | - |
| 144,927,245 | 144,937,953 | 146,048,059 | 146,037,358 | 10.7 | 98.49% | - |
| 144,953,161 | 144,963,193 | 146,037,358 | 146,027,332 | 10.0 | 98.12% | - |

| Supplementary Table 6: Sequencing results | | | | | | | |
|---|-----|-----------|---|--------------------------------|-----------------------|---------------------|-------------------------------------|
| | | | GJA5 | | | | GJA8 |
| | | | rs1692137 upstream of tss (-32bp) | rs791286 Exon 1 (5' UTR) | rs35594137 Intron1 | rs1692141 3' UTR | rs3766503 Exon 2 (synonymous) |
| Patient | CHD | Cataracts | | | | | |
| Case 2* | + | + | G | C | A | A | G |
| Case 3 | -- | -- | G | C | A | A | G |
| Case 6 | -- | -- | G | C | G | G | G |
| Case 7 | + | -- | G | C | G | G | G |
| Case 8 | -- | -- | G | C | G | G | G |
| Case 9 | -- | + | G | C | G | N.D. | G |
| Case 10 | -- | -- | G | C | G | G | G |
| Case 11 | -- | -- | G | C | G | G | A |
| Case 12 | -- | -- | G | C | G | G | G |
| Case 15 | -- | -- | G | C | G | G | G |
| Case 16 | + | + | G | C | A | A | G |

* Case 2 also has an A>C change 12 bp downstream of the stop codon in GJA8

All other changes were at sites of known SNPs, as indicated in table.

CHD, congenital heart disease

References

1. Weber M, Davies JJ, Wittig D, et al. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 2005;37(8):853-62.
2. Selzer RR, Richmond TA, Pofahl NJ, et al. Analysis of chromosome breakpoints in neuroblastoma at sub-kilobase resolution using fine-tiling oligonucleotide array CGH. *Genes Chromosomes Cancer* 2005;44(3):305-19.
3. Lichter P, Tang CJ, Call K, et al. High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. *Science* 1990;247(4938):64-9.
4. Jiang Z, Tang H, Ventura M, et al. Ancestral reconstruction of segmental duplications reveals punctuated cores of human genome evolution. *Nat Genet* 2007;39(11):1361-8.
5. Vissers LE, de Vries BB, Osoegawa K, et al. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003;73(6):1261-70.
6. de Vries BB, White SM, Knight SJ, et al. Clinical studies on submicroscopic subtelomeric rearrangements: a checklist. *J Med Genet* 2001;38(3):145-50.
7. Locke DP, Sharp AJ, McCarroll SA, et al. Linkage disequilibrium and heritability of copy-number polymorphisms within duplicated regions of the human genome. *Am J Hum Genet* 2006;79(2):275-90.
8. Redon R, Ishikawa S, Fitch KR, et al. Global variation in copy number in the human genome. *Nature* 2006;444(7118):444-54.
9. Iafrate AJ, Feuk L, Rivera MN, et al. Detection of large-scale variation in the human genome. *Nat Genet* 2004;36(9):949-51.
10. Sebat J, Lakshmi B, Troge J, et al. Large-scale copy number polymorphism in the human genome. *Science* 2004;305(5683):525-8.
11. Sharp AJ, Locke DP, McGrath SD, et al. Segmental duplications and copy-number variation in the human genome. *Am J Hum Genet* 2005;77(1):78-88.
12. Simon-Sanchez J, Scholz S, Fung HC, et al. Genome-wide SNP assay reveals structural genomic variation, extended homozygosity and cell-line induced alterations in normal individuals. *Hum Mol Genet* 2007;16(1):1-14.
13. Zogopoulos G, Ha KC, Naqib F, et al. Germ-line DNA copy number variation frequencies in a large North American population. *Hum Genet* 2007;122(3-4):345-53.
14. Pinto D, Marshall C, Feuk L, Scherer SW. Copy-number variation in control population cohorts. *Hum Mol Genet* 2007;16 Spec No. 2:R168-73.
15. de Stahl TD, Sandgren J, Piotrowski A, et al. Profiling of copy number variations (CNVs) in healthy individuals from three ethnic groups using a human genome 32 K BAC-clone-based array. *Hum Mutat* 2008;29(3):398-408.
16. Jakobsson M, Scholz SW, Scheet P, et al. Genotype, haplotype and copy-number variation in worldwide human populations. *Nature* 2008;451(7181):998-1003.
17. Schymick JC, Scholz SW, Fung HC, et al. Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: first stage analysis and public release of data. *Lancet Neurol* 2007;6(4):322-8.
18. Walsh T, McClellan JM, McCarthy SE, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008;320(5875):539-43.