Supplementary Note. Phenotype details in ten patients with deletions of 15q13

IMR338, IMR338M, and IMR338Cc – familial 3.95 Mb deletion BP3-BP5

The proband, IMR338, was initially reported by Sharp *et al.* (2006). Her mother (IMR338M) and one maternal half sister (IMR338Cc) are also affected and carry the same deletion. Her other half-sister (IMR338Cb, who does not carry the deletion) and brother (not tested) are not affected. Common features of affected individuals include: developmental delay, seizures, squint, gaunt appearance, speech difficulty, increased appetite, and a lax thumb joint. The proband, IMR338, is moderately delayed and has had some absence and one tonic clonic seizure. She has a round flat face, upslanting palpebral fissures, epicanthic folds, posteriorly rotated ears, mild 5th-finger clinodactyly, a single palmar crease on the left, nasal speech, and a right optic pit (possible coloboma) on eye exam. She has hyperphagia, is overweight, and suffers from type II diabetes. Height is 175 cm (97th centile) and head circumference is 58.7 cm (>97% centile). Her EEG is abnormal with a good deal of spike and wave activity and a suggestion of a focus in the left frontal area. Brain MRI showed a small area of patchy change in the white matter posteriorly adjacent to the lateral ventricle.

Her half-sister, IMR338Cc, has moderate to severe learning difficulties and requires special education. She also suffers from intractable epilepsy. She had a hiatus hernia repaired in infancy. Exam shows a normal head circumference (55.7 cm), deep set eyes, long fingers, and lax joints. She does not have nasal speech and is not obese or diabetic.

The mother, IMR338M, is mildly dysmorphic with mild delays. She is overweight and developed grand mal seizures at the age of 42. A CT scan was normal. She has had operations for fecal incontinence and has had 7 miscarriages. Photographs of the proband's maternal grandmother and great aunt show a very similar appearance to the proband (not shown). Both were also reported to have learning difficulties and diabetes. The maternal grandfather suffered from epilepsy and was illiterate.

69/06 – *de novo* 1.5 Mb deletion BP4-BP5

This patient is the first child of healthy, unrelated parents, born at 37 weeks after spontaneous delivery. Pregnancy was complicated by a partial placental detachment at the 8th month and gestational diabetes treated with diet. Birth weight was 2510g (3rd percentile) and head circumference (OFC) was 33 cm (25th percentile). Renal ultrasound performed because of urinary tract infection at 2 months was normal. She required gavage feeding in the neonatal period because of hypotonia and weight loss. Exam at 14 months showed mild hypertelorism, high-arched palate, prominent philtrum, and generalized moderate hypotonia. She was able to sit down with support. When prone, she rested her forearm with a frog-like attitude, but was unable to perform other postural movements. She showed stereotyped hand movements more evident with the right hand. She was attracted to light sources and she observed a toy for some seconds. Vocalization was poor. Evaluation using the Brunet-Lezine test gave a global score of 0.37 with the following partial score: Posture 0.35, Coordination 0.28, Language 0.35, Sociality 0.45. Echocardiogram showed a defect of the right side of the heart. Ocular examination showed an asymmetry of left eye, with the left eye smaller and deeper than the right, microexotrophia right/left, good eye convergence, and astigmatism corrected by glasses. Auditory evoked potentials were normal. Brain MRI was normal. EEG showed unusual delta-theta activity (arceaux-like) on frontal and vertex regions with sporadic slow waves on the same regions.

02961 – *de novo* 1.5 Mb deletion BP4-BP5

The proband is a nine-year-old single child of non-consanguineous parents. She has mild mental retardation, epilepsy, ADHD, speech delay, and dysmorphic features such as brachycephaly, hypertelorism, synophris, wide nasal bridge, anteverted nares, short thick philtrum, low-set ears, tapering fingers, and 5^{th} finger clinodactyly. There is a family history of mental retardation and language impairment in two paternal cousins. She was born at term, after a pregnancy characterized by IUGR. Birth weight was 2.55 kg (10th-25th centile). Developmental milestones were reached within normal range, but a speech delay was present. The psychomotor testing (WISC-R, CPM of Raven, Leiter-R) revealed a mild mental retardation and Attention Deficit Hyperactivity Disorder (ADHD). At the age of nine years her weight was 26.5 kg (25th centile), height was 122 cm (3rd-10th centile) and OFC was 50 cm (10th-25th centile). Her phenotype is also characterized by early pubarche, muscular hypotonia, hypertrichosis, hypertelorism, wide nasal bridge, synophris, short thick philtrum, anteverted nostrils, brachycephaly, low-set and

large ears with wide concha, malocclusion, tapering fingers, clinodactyly, and hallux valgus. Neurologic examination showed brisk reflexes. From the age of 7 years she has had seizures which are treated with sodium valproate. The EEG shows 3 c/s Sharps-Waves complexes, with sometimes rapid spikes and incisura on the vertex, lasting 1-4 seconds. High voltage spikes and Sharps-Waves complexes were recorded on the frontal regions of left hemisphere during sleep. Video-EEG showed clonic movements during spikes. She fulfills the criteria for myoclonic epilepsy. Eye examination showed myopic astigmatism. Brain MRI, electrocardiogram, hearing evaluation, pelvic ultrasound, and bone age are normal.

CMS5803 and CMS7833– maternally-inherited 1.5 Mb deletion BP4-BP5

This proband was born of mentally retarded parents by Cesarean delivery at term. He had low muscle tone, began using words and pulled to standing at 14 months. His IQ at age five years was 56 on the Stanford-Binet and 44 on the WISC-R. At age 8 years four months, his height was 122 cm (5th-10th centile) and head circumference was 48.2 cm (<3rd centile). He had a short philtrum, full lips with everted lower lip, and stiff fingers.

The mother, CMS7833, was born from a father-daughter mating. At age 33 years, she had a height of 152.4 cm (5th centile) and head circumference of 52 cm (3rd centile). She has a round face, depressed nasal bridge, smooth philtrum, everted lower lip, short fourth metacarpals, and stiff fingers. The Stanford-Binet IQ testing was 34 at age 23 years and 46 at age 28 years. She suffers from seizures which are under good control.

CMS5826 – 1.5 Mb deletion BP4-BP5, inheritance unknown

The proband is one of fraternal twins, both of whom experienced developmental delay. The mother had three pregnancies with other mates: an early abortion; a male infant with slow development and emotional problems, and a male infant who died in a fire. The pregnancy was complicated by asthma treated with steroids and bronchodilators, gestational diabetes controlled with diet, and a seizure disorder treated with phenobarbitol. Delivery was at 32-33 weeks by Cesarean section because of placenta previa. His birth weight was 2 kg. The patient walked at 14-18 months, said his first word at 12 months, but had only 10 words by three years, and was toilet trained at four years. Testing gave an IQ of 62. At age 5 years 1 month, examination he had

a head circumference of 53 cm (30th centile). Craniofacial features shared by his twin sister and his mother: long face, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, and posteriorly rotated ears with thick helices. He also had short fifth fingers. Brain MRI was normal. He scored in the mild autism range using the Childhood Autism Rating Scale. He has had five admissions to psychiatric facilities because of aggressive behavior and rage.

Testing of his affected twin sister showed that she did not carry the 15q13.3 deletion.

CMS7906 – 1.5 Mb deletion BP4-BP5, inheritance unknown

This individual is one of eight children to parents of undocumented intellectual abilities. She had a single seizure at age 12 years. The WAIS full scale IQ was 52. At age 37 years she had a height of 159.4 cm (30th centile) and head circumference of 54 cm (35th centile). She has a full and round face, upslanting palpebral fissures, normal interpupillary measurement, widely spaced teeth, limited elbow extension, short fourth metacarpals, and normal neurological findings. One older sister receives services for mental retardation. This patient has two children, both now in foster care and requiring special education.

543/06 – Paternally-inherited deletion of BP3-BP4

The girl was born at full term by caesarean delivery. Her weight, height, and OFC were 3.15 kg, 50 cm, and 33 cm respectively. The APGAR score was 6 at 1 min. and 9 at 5 min. She was examined for possible congenital heart defects causing transient cyanosis but investigations were negative. At two years of age ultrasonography showed ureteral ectasia of the right kidney. Aged 11 years and 8 months she weighed 50.7 kg (97th centile), height 144 cm (25th centile). The OFC was 54 cm (50-75th centile). She had developmental delay, some dysmorphic features including flattened midface, anteverted nostrils, iris coloboma of the right eye, prognathism, and synophrys. The proband's father carries the same 15q deletion and shows no phenotypic abnormalities.

References for Supplementary Material

Bailey JA, Yavor AM, Massa HF, Trask BJ, Eichler EE (2001) Segmental duplications: organization and impact within the current human genome project assembly. Genome Res 11:1005-17.

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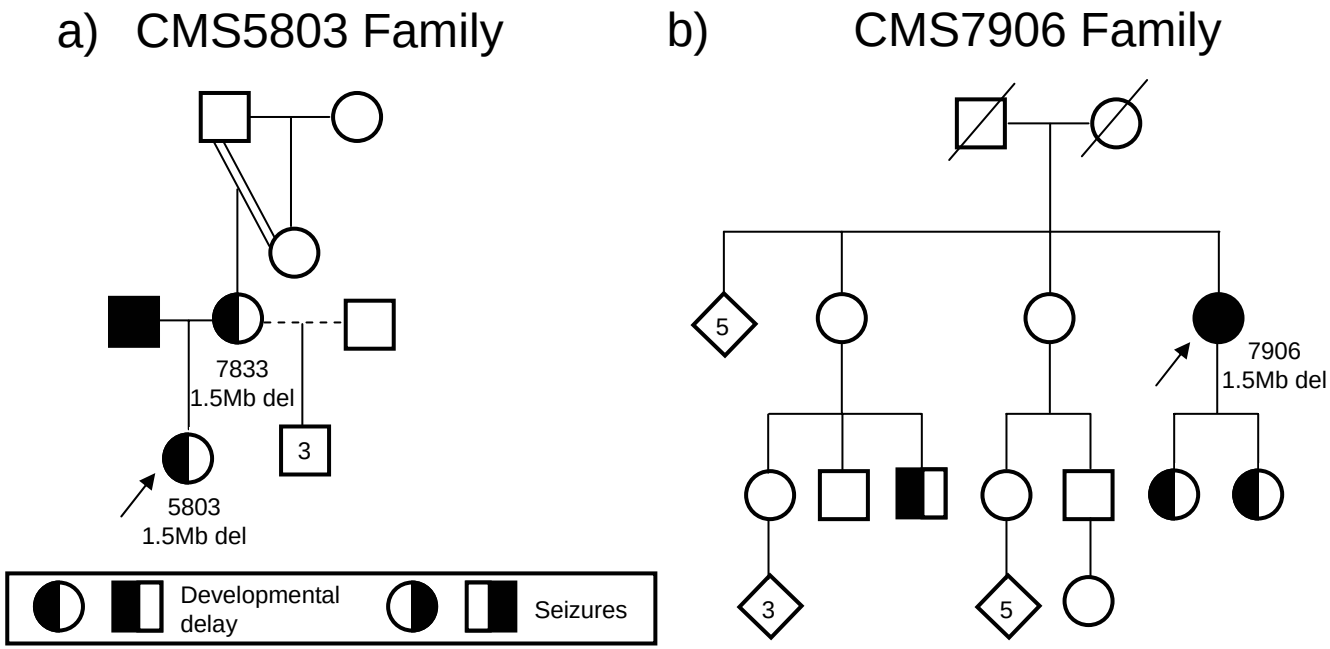
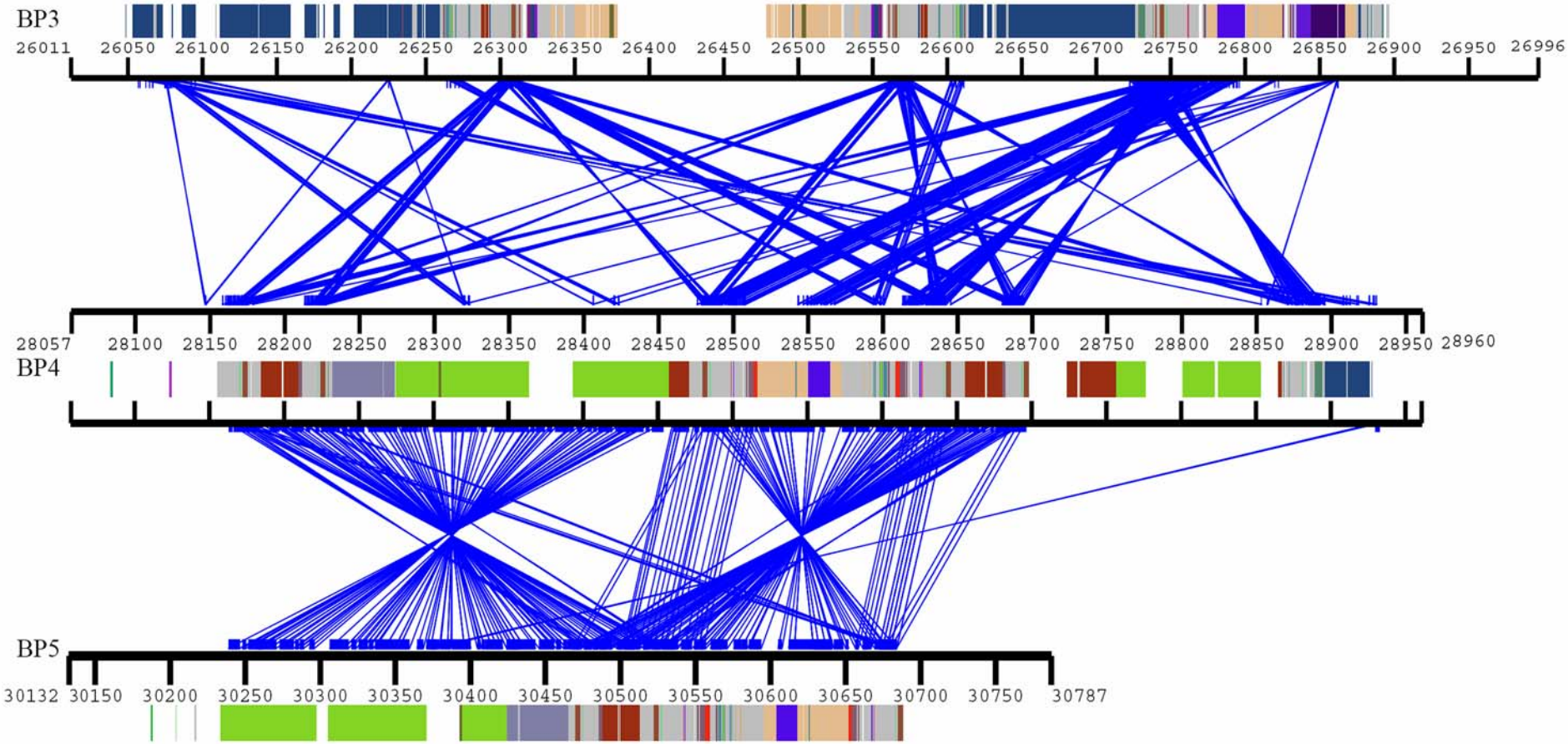
Sharp AJ, Selzer RR, Veltman JA, Gimelli S, Gimelli G, Striano P, Coppola A, Regan R, Price SM, Knoers NV, et al. (2007a) Characterization of a recurrent 15q24 microdeletion syndrome. Hum Mol Genet 16:567-72.

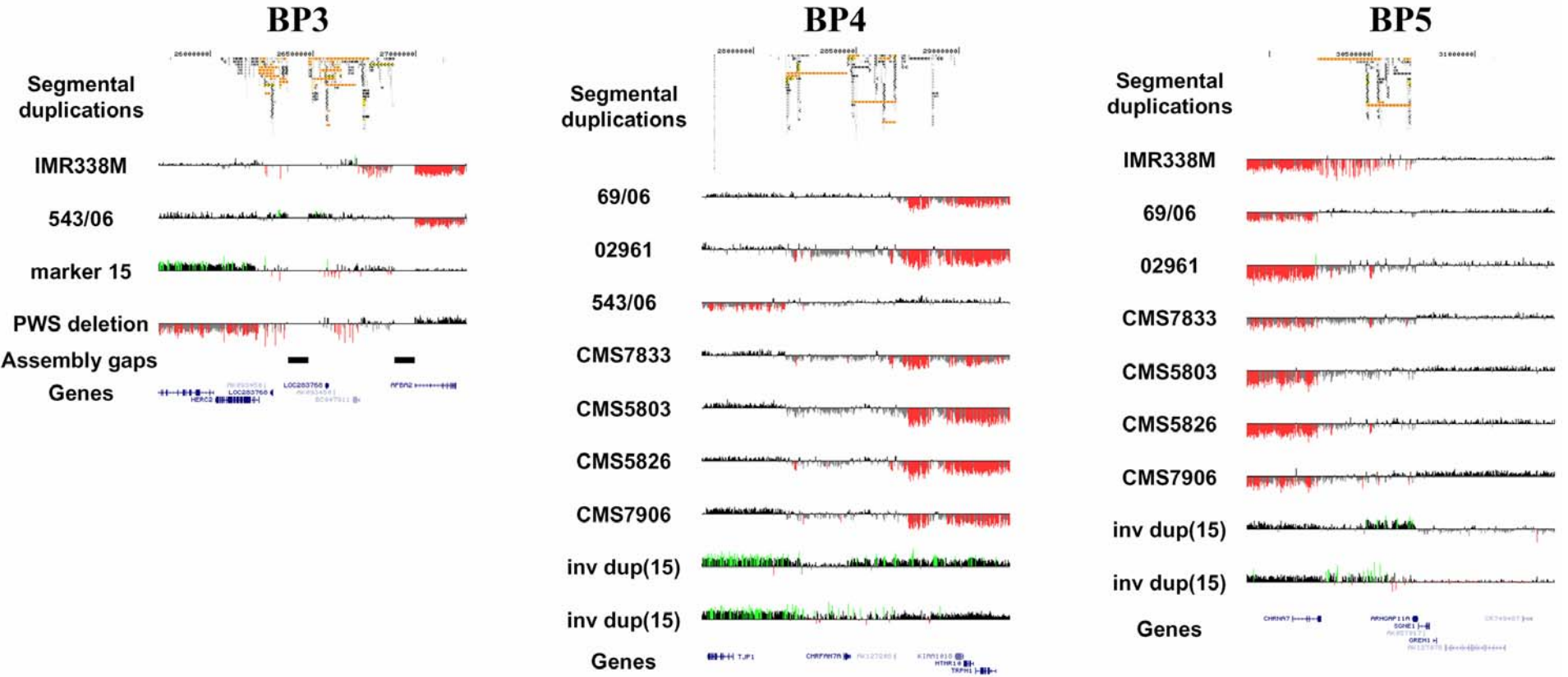
Sharp AJ, Itsara A, Cheng Z, Alkan C, Schwartz S, Eichler EE (2007b) Optimal design of oligonucleotide microarrays for measurement of DNA copy-number. Hum Mol Genet 16:2770-9.



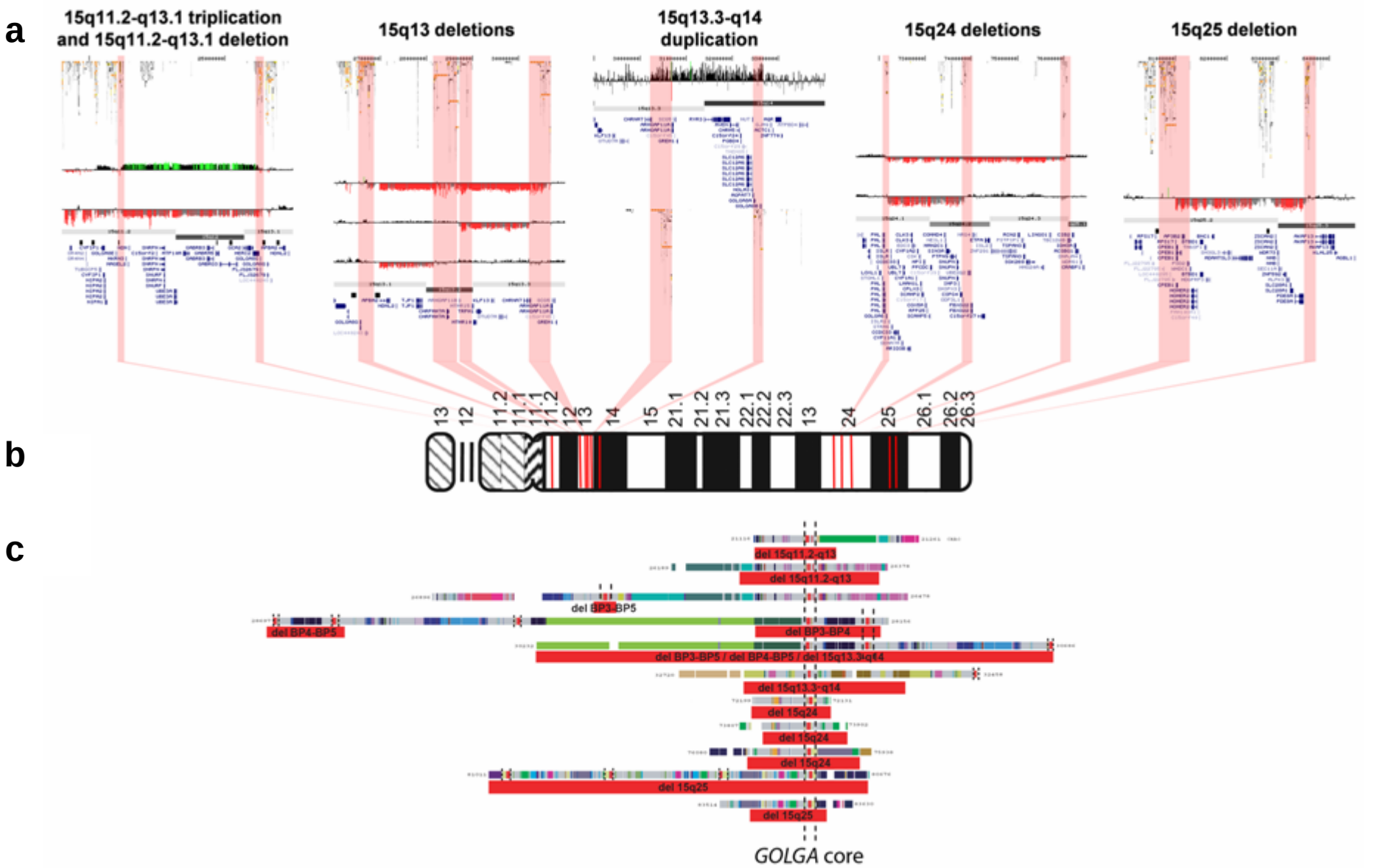
Supplementary Figure 1. Pedigrees of 15q13 deletions. (**a**) Pedigree for individuals CMS5803 and CMS7833 who were identified independently by qPCR and subsequently found to be related. (**b**) Pedigree for proband CMS7906.



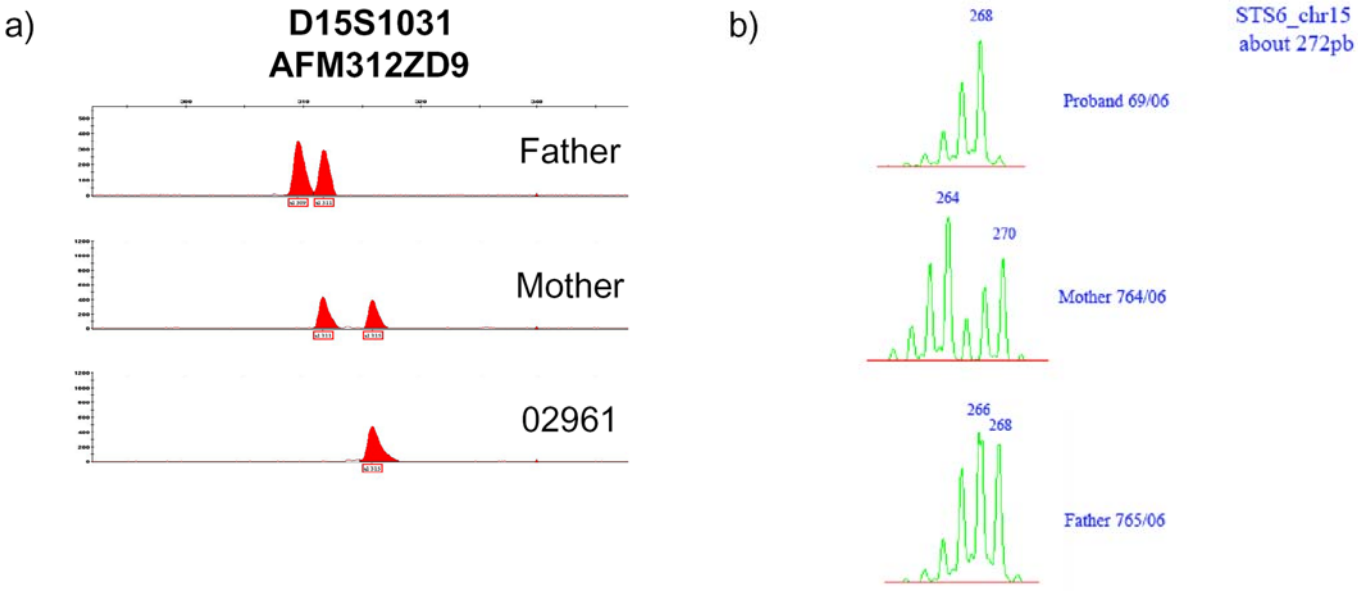
Supplementary Figure 2. Analysis of the duplication architecture of 15q13 breakpoint regions. For clarity, we used two different sensitivity thresholds, with homology between BP3, BP4, and BP5, as judged by pairwise WGAC alignments of identity ≥95% and ≥10kb in size (Bailey *et al*., 2001) represented by blue lines joining regions of 500 bp (BP3-BP4) or 2000 bp (BP4-BP5) of perfect identity. At each breakpoint, the underlying duplicon structure and orientation is shown by coloured arrows, with blocks of identical colour representing duplicons that share the same evolutionary origin (Jiang *et al*., 2007). The large, highly-identical duplicons which lie in an inverted orientation at BP4 relative to BP5 are clearly visible. In contrast, homology between BP3 and BP4/BP5 is much lower, consistent with the high frequency of BP4-BP5 rearrangements, relative to other 15q13 deletions.



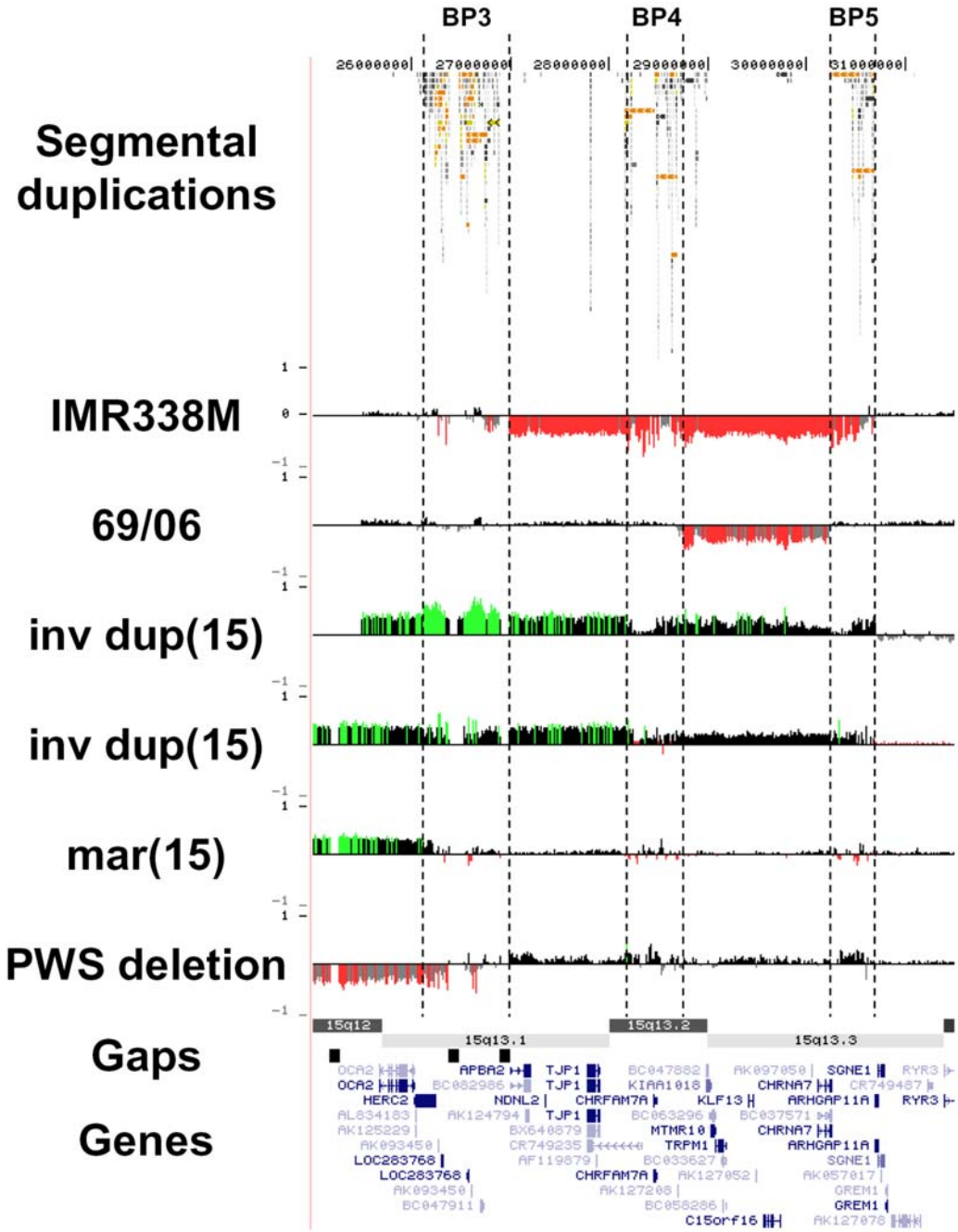
Supplementary Figure 3. High-resolution breakpoint mapping of 15q13 rearrangements. Each panel shows oligonucleotide array data for a 1.5 Mb region surrounding BP3, BP4, and BP5 (BP3, chr15:25,750,000-27,250,000; BP4, chr15:27,750,000-29,250,000; BP5, chr15:29,900,000-31,400,000). For each individual, deviations of probe log2 ratios from zero are depicted by grey/black bars, with those exceeding a threshold of 1.5 standard deviations from the mean probe ratio colored green and red to represent relative gains and losses, respectively. Tracks above each plot indicate segmental duplications (grey/yellow/orange bars representing duplications with 90-98%/98-99%/99-100% sequence identity, respectively).



Supplementary Figure 4. Breakpoints of multiple chromosome 15 rearrangements coincide with the location of a duplication family containing the *GOLGA* gene. (**a**) High-resolution oligonucleotide array data from nine different structural rearrangements of chromosome 15 (*left to right*): a triplication of 15q11.2-q13.1 (Sharp *et al.* 2007b); a deletion of 15q11.2-q13.1 associated with Angelman syndrome (Sharp *et al.* 2007b); BP3-BP4-BP5 deletions of 15q13; a duplication of 15q13.3-q14 associated with epilepsy; deletions of 15q24 (Sharp *et al.* 2007a); a deletion of 15q25 associated with congenital diaphragmatic hernia (Mefford *et al.* 2007). In each image, the locations of duplication blocks containing the *GOLGA* gene (Jiang *et al.* 2007) are indicated by red shaded regions. Tracks show segmental duplications, cytogenetic band, assembly gaps, and RefSeq genes. (**b**) *GOLGA*-containing duplications blocks that coincide with the breakpoints of deletion/duplication events are highlighted (*red bars*). (**c**) Diagram showing the localization of rearrangement breakpoints within *GOLGA*-containing duplication blocks. Red bars below each duplication block indicate the interval in which rearrangement breakpoints occur. Although the presence of structural polymorphisms and cross-hybridization between paralogous sequences means we are unable to precisely determine the breakpoints by oligonucleotide array CGH, in every case data showed that the intervals in which the breakpoints occur overlap a *GOLGA* core. The “core element”, which contains the *GOLGA* gene and is shared by all duplication blocks, is highlighted by vertical dash lines. Note that some blocks contain multiple *GOLGA* sequences.



Supplementary Figure 5. Microsatellite analysis of two BP4-BP5 deletions of 15q13.3 (**a**) Patient 02961, data from D15S1031 showing the deletion is of paternal origin. (**b**) Patient 69/06, data from STS6 showing the deletion is of maternal origin.



Supplementary Figure 6. Breakpoints of multiple different rearrangements of proximal 15q map to large blocks of segmental duplications at BP3, BP4, and BP5. Image shows the deletions identified in IMR338 (BP3-BP5) and 69/06 (BP4-BP5). Results obtained in two unrelated patients with inv dup(15) chromosomes show both are composed of two copies of the region 15cen-BP4 and a single copy of region BP4-BP5. This was subsequently confirmed by FISH (data not shown). We also tested one patient carrying a marker chromosome 15 (four copies of the region 15cen-BP3), and one patient with a class II Prader-Willi deletion (one copy of the region BP2-BP3), providing further evidence of recurrent chromosomal breakpoints in 15q. Image shows a 6.5 Mb region of 15q12-15q13.3 (chr15:25,000,001-31,500,000). For each individual, deviations of probe log2 ratios from zero are depicted by grey/black bars, with those exceeding a threshold of 1.5 standard deviations from the mean probe ratio colored green and red to represent relative gains and losses, respectively. Tracks above the plot indicate segmental duplications (grey/yellow/orange bars representing duplications with 90-98%/98-99%/99-100% sequence identity, respectively).

Supplementary Table 1. Paralogous duplication architecture of 15q13 breakpoint regions.

BP3 vs BP4								
query chr	**query begin**	**query end**	**sequence chr**	**sequence begin**	**sequence end**	**% similarity**	**size, bp**	**orientation**
chr15	26741971	26774623	chr15	28479130	28510479	97.9	32653	+
chr15	26741971	26774623	chr15	28646106	28614749	97.9	32653	-
chr15	26774338	26796514	chr15	28542772	28564834	96.5	22177	+
chr15	26741971	26761640	chr15	28177108	28157403	98.1	19670	-
chr15	26721326	26739796	chr15	28899881	28881354	97.2	18471	-
chr15	26741971	26758355	chr15	28229170	28212797	98.3	16385	-
chr15	26742555	26758355	chr15	28697597	28681775	98.3	15801	-
chr15	26296502	26311689	chr15	28630557	28646098	92.3	15188	+
chr15	26296502	26311689	chr15	28494673	28479138	92.4	15188	-
chr15	26562875	26578062	chr15	28646098	28630557	92.3	15188	-
chr15	26562875	26578062	chr15	28479138	28494673	92.3	15188	+
chr15	26296527	26311689	chr15	28213652	28229162	92.5	15163	+
chr15	26296527	26311689	chr15	28161556	28177100	92.6	15163	+
chr15	26562875	26578037	chr15	28229162	28213652	92.5	15163	-
chr15	26562875	26578037	chr15	28177100	28161556	92.6	15163	-
chr15	26053471	26068259	chr15	28924396	28909241	94.0	14789	-
chr15	26296527	26311080	chr15	28682629	28697566	92.5	14554	+
chr15	26563560	26578037	chr15	28697492	28682629	92.5	14478	-
chr15	26259846	26272336	chr15	28606493	28595032	93.0	12491	-
chr15	26602225	26614712	chr15	28595032	28606493	92.9	12488	+
chr15	26068653	26080186	chr15	28910056	28904701	93.1	11534	-
chr15	26746272	26757525	chr15	28881355	28870054	93.4	11254	-
chr15	26296492	26307641	chr15	28870044	28881332	91.9	11150	+
chr15	26566923	26578072	chr15	28881332	28870044	91.9	11150	-
chr15	26086390	26095350	chr15	28904329	28894760	94.9	8961	-
chr15	26817800	26825337	chr15	28565018	28572641	95.2	7538	+
chr15	26279366	26282334	chr15	28595017	28591488	93.2	2969	-
chr15	26592237	26595168	chr15	28591488	28594978	93.2	2932	+
chr15	26884121	26886058	chr15	28600359	28598489	90.5	1938	-
chr15	26282356	26283033	chr15	28587233	28586559	94.7	678	-
chr15	26591538	26592215	chr15	28586559	28587233	94.5	678	+
					Mean	**94.0%**	**13.5 kb**	
					Median	**93.1%**	**14.8 kb**	
					Total		**418.8 kb**	

BP3 vs BP5								
query chr	**query begin**	**query end**	**sequence chr**	**sequence begin**	**sequence end**	**% similarity**	**size, bp**	**orientation**
chr15	26741971	26774623	chr15	30520486	30551861	97.9	32653	+
chr15	26742555	26774623	chr15	30687000	30656194	97.9	32069	-
chr15	26774338	26796514	chr15	30623921	30601869	96.5	22177	-
chr15	26296502	26311689	chr15	30536045	30520494	92.4	15188	+
chr15	26296502	26311080	chr15	30672033	30686969	92.3	14579	-
chr15	26741971	26758355	chr15	30468167	30484537	98.3	16385	+
chr15	26562875	26578062	chr15	30520494	30536045	92.3	15188	+
chr15	26296527	26311689	chr15	30483679	30468175	92.5	15163	-
chr15	26562875	26578037	chr15	30468175	30483679	92.5	15163	+
chr15	26563560	26578062	chr15	30686895	30672033	92.3	14503	-
chr15	26259846	26272336	chr15	30560161	30571668	93.0	12491	+
chr15	26602225	26614712	chr15	30571668	30560161	92.9	12488	-
chr15	26817800	26825337	chr15	30601678	30594055	95.4	7538	-
chr15	26592237	26595168	chr15	30575217	30571722	93.2	2932	-
chr15	26279407	26282334	chr15	30571722	30575217	93.2	2928	+
chr15	26884121	26886058	chr15	30566295	30568166	90.7	1938	+
chr15	26282356	26283033	chr15	30579473	30580147	94.7	678	+
chr15	26591538	26592215	chr15	30580147	30579473	94.5	678	-
					Mean	**94.3%**	**13.0 kb**	
					Median	**93.1%**	**14.5 kb**	
					Total		**234.7 kb**	
BP4 vs BP5								
query chr	**query begin**	**query end**	**sequence chr**	**sequence begin**	**sequence end**	**% similarity**	**size, bp**	**orientation**
chr15	28479703	28697597	chr15	30687000	30468739	99.6	217895	-
chr15	28157403	28297673	chr15	30540153	30400001	99.4	140271	-
chr15	28297674	28393148	chr15	30400000	30304524	99.8	95475	-
chr15	28400001	28457303	chr15	30289536	30232699	99.7	57303	-
chr15	28157403	28176535	chr15	30667922	30687000	98.0	19133	+
chr15	28722450	28741566	chr15	30505878	30486859	95.0	19117	-
chr15	28212797	28228597	chr15	30671215	30687000	98.2	15801	+
chr15	28870054	28881355	chr15	30536045	30524730	93.4	11302	-
chr15	28870054	28881355	chr15	30672033	30683340	93.4	11302	+
chr15	28870079	28881355	chr15	30483679	30472406	93.5	11277	-
chr15	28393145	28400000	chr15	30296419	30289537	99.8	6856	-
					Mean	**97.3%**	**55.1 kb**	
					Median	**98.1%**	**19.1 kb**	
					Total		**660.8 kb**	

Pairwise sequence similarity between breakpoints, ordered by size at BP3 (breakpoint 3), BP4 (breakpoint 4), and BP5 (breakpoint 5). Direct orientation indicated by (+), inverted orientation indicated by (-).

Supplementary Table 2. Results of inversion testing of the interval BP4-BP5 in eight HapMap individuals

Coriell ID	**HapMap population origin**	**Orientation of BP4-BP5 region relative to the reference assembly (hg17)**
GM12156	CEPH	Homozygous non-inverted
GM18507	Yoruba	Homozygous non-inverted
GM18517	Yoruba	Heterozygous
GM18555	China	Heterozygous
GM18956	Japan	Heterozygous
GM19129	Yoruba	Heterozygous
GM19240	Yoruba	Heterozygous
GM12878	CEPH	Homozygous inverted