Diagnostic Whole Genome Sequencing and Split-Read Mapping for Nucleotide Resolution Breakpoint Identification in CNTNAP2 Deficiency Syndrome

Christopher M. Watson,1,2* Laura A. Crinnion,1,2 Antigoni Tzika,1 Alison Mills,1 Andrea Coates,1 Maria Pendlebury,1 Sarah Hewitt,1 Sally M. Harrison,2 Catherine Daly,2 Paul Roberts,1 Ian M. Carr,2 Eamonn G. Sheridan,1,2 and David T. Bonthron1,2

1Yorkshire Regional Genetics Service, St. James’s University Hospital, Leeds, United Kingdom
2School of Medicine, University of Leeds, St. James’s University Hospital, Leeds, United Kingdom

Whole genome sequencing (WGS) has the potential to report on all types of genetic abnormality, thus converging diagnostic testing on a single methodology. Although WGS at sufficient depth for robust detection of point mutations is still some way from being affordable for diagnostic purposes, low-coverage WGS is already an excellent method for detecting copy number variants (“CNVseq”). We report on a family in which individuals presented with a presumed autosomal recessive syndrome of severe intellectual disability and epilepsy. Array comparative genomic hybridization (CGH) analysis had revealed a homozygous deletion apparently lying within intron 3 of CNTNAP2. Since this was too small for confirmation by FISH, CNVseq was used, refining the extent of this mutation to approximately 76.8 kb, encompassing CNTNAP2 exon 3 (an out-of-frame deletion). To characterize the precise breakpoints and provide a rapid molecular diagnostic test, we resequenced the CNVseq library at medium coverage and performed split read mapping. This yielded information for a multiplex polymerase chain reaction (PCR) assay, used for cascade screening and/or prenatal diagnosis in this family. This example demonstrates a rapid, low-cost approach to converting molecular cytogenetic findings into robust PCR-based tests.

INTRODUCTION

Biallelic mutations of the CNTNAP2 gene, encoding contactin-associated protein 2, was first demonstrated in a disorder labeled cortical dysplasia-focal epilepsy (CDFE) [Strauss et al., 2006]. Affected children, all from an Old Order Amish pedigree, suffered from intractable seizures with developmental regression and hyperactivity. They were homozygous for the CNTNAP2 frameshift mutation c.3709delG. In 2009, patients were then reported with autosomal recessive Pitt–Hopkins-like syndromes (PTHLS), resulting from mutations either in CNTNAP2 (PTHLS1) or NRXN1 (PTHLS2) [Zweier et al., 2009]. Both of these genes encode proteins that are members of the neurexin superfamily, a group of transmembrane proteins that are expressed in the nervous system and are involved in cell–cell interactions [Poliak et al., 1999].

All the reported cases due to biallelic loss of function mutations in CNTNAP2 appear to have a similar, severe neurodevelopmental phenotype (OMIM 610042). Although the parents of these affected children appeared normal, it has also been suggested that heterozygous CNTNAP2 variants may have a phenotypic effect. Such variants have been identified through large-scale association stud-
ies, and linked to a variety of phenotypes including language and autistic spectrum disorders. (For a recent review, see Rodenas-Cuadrado et al. [2014].) However, since no phenotypes have yet been reported in the heterozygous parents of children with CDFE/PHLS1, it may be that the penetrance of heterozygous CNTNAP2 variants is low.

Pitt–Hopkins syndrome (PHTHS, OMIM 610954) results from haploinsufficiency for the basic helix-loop-helix transcription factor encoded by TCF4 [Amiel et al., 2007; Zweier et al., 2007]. It is characterized by severe intellectual disability, epilepsy, stertotypical movements, breathing abnormalities, and typical facial dysmorphism [Peippo and Ignatius, 2012]. This characteristic facial dysmorphism of PHTHS was lacking in the patients described as having PTHLS.

There is evidence that TCF4 can transactivate the CNTNAP2 and NRXN1 promoters, suggesting the existence of a regulatory network in the related PTHS and PTHLS syndromes [Forrest et al., 2012]. In Drosophila, both CNTNAP2 and NRXN1 orthologs may play a role in synaptic organization [Zweier et al., 2009] and CASPR2 (encoded by CNTNAP2) has been implicated in neuronal migration [Strauss et al., 2006]. The best characterized role for CASPR2 is, however, its interaction with CNTN2 (TAG-1) in the axon initial segment and juxtaparanodal regions of myelinated neurons. This complex is required for clustering of voltage-gated potassium channels at the juxtaparanodes [Polialk et al., 1999, 2003].

CNTNAP2 is the largest human gene, spanning 2.3 Mb (~1.5%) of Chromosome 7 [Nakabayashi and Scherer, 2001]. It has 24 exons, and 13 of its introns are larger than 60 kb. Mutation screening for intragenic deletions or duplications is therefore possible using array-based molecular karyotyping approaches. Indeed, in each of the two CNTNAP2-mutated families described by Zweier et al. [2009], a large intragenic deletion was detected using Affymetrix single nucleotide polymorphism (SNP) arrays. In two siblings of European origin, originally suggested to have Pitt–Hopkins syndrome [Orrico et al., 2001], a homozygous deletion of exons 2–9, spanning at least 1.15 Mb was identified. Despite the absence of parental consanguinity, genotyping demonstrated that the mutant allele was derived from a common ancestor. A second unrelated Pitt–Hopkins-like case was found to have compound heterozygous CNTNAP2 mutations, comprising a 180-kb in-frame deletion of exons 5–8 and the splice site mutation IVS10-1G>T.

Array comparative genomic hybridization (aCGH) is currently the method of choice for performing genome-wide screening for large structural abnormalities. The enthusiastic uptake of this technology as a first-line investigation for children with learning disability or dysmorphism has led to a surge of molecular diagnoses over recent years [McMullen et al., 2009; Miller et al., 2010]. More recently, though, the use of next-generation sequencing (NGS) methods for the detection of copy number variants has also been increasing [Wood et al., 2010]. In particular, low coverage short-read whole genome sequencing (CNVseq) is a reliable and cheap approach which has diagnostic sensitivity for germline CNVs comparable to that of standard aCGH reagents [Hayes et al., 2013]. We have recently introduced the CNVseq method into routine diagnostic use. Here, we show that NGS libraries prepared for routine diagnostic use can also be cheaply resequenced to define precise molecular breakpoints, leading to facile accurate PCR-based diagnosis for carrier detection and prenatal diagnosis.

**MATERIALS AND METHODS**

DNA was extracted from peripheral blood lymphocytes of the proband, her brother, affected maternal aunt, mother, and father using a standard salting out method. Ethical approval for this study was given by the Leeds East Research Ethics Committee (07/H1306/113).

**Array Comparative Genomic Hybridization Analysis**

Diagnostic aCGH analysis was performed using a BlueGnome ISCA 8 x 60k OligoArray (v2.0) (BlueGnome Ltd., Cambridge, UK) following manufacturer’s protocols. A mixed pool of five healthy sex matched individuals was used as a reference control (Promega U.K., Southampton, UK). Analysis was performed using BlueFuse Multi software (v3.1) (BlueGnome Ltd., Cambridge, UK).

**Copy Number Variation sequencing (CNVseq)**

CNVseq was performed by a method based on that of Wood et al. [2010]. Approximately 1 µg of genomic DNA was sheared using a CovarisS2 (Covaris, Inc., Woburn, MA) and an Illumina-compatible sequencing library was generated using NEBNext® Ultra™ reagents (New England Biolabs, Ipswich, MA). The library insert size was ~200 bp and the final enrichment PCR consisted of eight rounds of thermocycling. All other steps were followed as per the manufacturer’s protocol. The final libraries were pooled with 10 additional diagnostic CNVseq samples and the pool was split across two lanes of an Illumina HiSeq rapid mode flow cell for single-read 50-bp sequencing. Demultiplexing reads generated from the sample pool were undertaken using 6-bp indexes present within the adaptor of each sequenced DNA fragment. To analyze each patient, raw sequence reads were aligned to an indexed human reference genome (hg19) using bwa v0.6.2 [Li and Durbin, 2009]. Duplicate reads were discarded using Picard v1.85 (http://picard.sourceforge.net). Coordinates of uniquely mapped test and reference reads were extracted from BAM files [Li et al., 2009] and counted into genomic windows containing equal numbers of reference reads. A read count adjustment was performed to compensate for the effect of local GC% variation, and the adjusted ratios of test to reference read counts were used as input to the R module DNA copy v1.32.0 [Venkatraman and Olshen, 2007], which segments the data into regions of equal copy number. To visualize individual mapped read positions, a BED file containing the genomic start coordinate of each read was generated and displayed using the UCSC genome browser (http://genome.ucsc.edu/) [Kent et al., 2002].

**Breakpoint Identification**

To determine the precise deletion breakpoints, the CNVseq libraries were resequenced to generate longer 190-bp reads. Each patient library was sequenced on a single lane of an Illumina HiSeq rapid mode single-read flow cell. Due to the increased read length, cutadapt v1.1 was used to remove contaminating adaptor sequences prior to alignment (http://code.google.com/p/cutadapt/) [Martin, 2011]. Reads that remained unmapped to the reference sequence were extracted from the duplicate-cleaned coordinate-
sorted BAM files and converted to FASTQ format using bam2fastq (http://www.hudsonalpha.org/gsl/information/software/bam2fastq). Unmapped reads were mapped to a FASTA reference sequence encompassing the CNVseq-defined deletion, using SplazerS (http://www.seqan.de/projects/splazers/) [Emde et al., 2012]. A split read alignment was performed which exported the read IDs and the CIGAR string formatted alignment. Putative split read alignments were identified and BLAT was used to determine the genomic coordinates of both the 5' and 3' sequences (http://genome.ucsc.edu) [Kent, 2002].

Molecular Assay for Cascade Testing

A PCR amplicon was optimized to amplify across the breakpoints identified by SplazerS. The primers used to amplify the breakpoint of the deleted allele were dTGTAAAACGACGGCCAGTTAAGCCCATCCATATAATTTC (common forward) and dCAGGAAAACACGTTAGCAATATACAATTCTCAAAGGAAG (reverse deletion), which generated a 470-bp PCR product. A second reverse primer dCAGGAAAACACGCTATGACAACGTTCATCCTTCCATAAG (reverse normal) was designed to work in combination with the common forward primer to amplify a smaller 393-bp PCR product specific to the normal allele. Both primer sets contained universal tags (underlined) for Sanger sequencing in our routine diagnostic laboratory workflow. MegaMix PCR reagents (Microzone Ltd., Haywards Heath, UK) were used with the thermocycling conditions outlined in Supplementary Table SI (in supporting information online). Each PCR reaction consisted of 11 μl MegaMix, 2 μl of 10 μM common forward primer, 1 μl of 10 μM reverse deletion primer, 0.5 μl of 10 μM reverse normal primer. To facilitate robust diagnostic testing, the amplicons were optimized to work within a multiplex reaction. PCR products were resolved on a 1.5% tris-borate EDTA agarose gel. Sanger sequencing on an ABI3730 was used to confirm identity of both PCR amplicons; manufacturer’s protocols were followed throughout (Life Technologies Ltd., Paisley, UK).

RESULTS

A sister and brother (Fig. 1, Patients III:4 and III:5) in a UK family of Pakistani ancestry were assessed at ages 19 and 14 years respectively, on account of a long standing severe neurodevelopmental phenotype. This was presumed to be an autosomal recessive disorder, in view of the sibling recurrence and the consanguinity between the healthy parents. Both children had been previously diagnosed with ataxic cerebral palsy, but also had severe cognitive impairment and generalized tonic-clonic seizures from the age of 2 years. They both displayed a broad-based gait and hyporeflexia.

The daughter (Patient III:4) had been born by caesarean (for breech presentation and cephalopelvic disproportion) after an uneventful pregnancy. She was described as an “awkward” baby. Her motor milestones were delayed (sitting at 10 months, standing at 18 months, walking at 4 years). Surgery to correct strabismus was performed at age 1 year. She had extensive metabolic investigations at age 2 years, at which time her head circumference was 45 cm (−1.6 SD, WHO charts). She was noted to have decreased muscle bulk but muscle histology was normal. She was diagnosed with ataxic cerebral palsy aged 2.5 years. Seizures began at 2 years and were managed with carbamazepine. After puberty, the seizures were catamential. As a young adult, she presented with a thin habitus; her eyes were deep-set but there was no other significant facial anomaly. She had a broad-based gait, but with minimal ataxia and no spasticity, and was hyporeflexic. She used speech, with a vocabulary estimated by her parents to be that of a 5-year-old, and displayed obsessive-compulsive behavior.

The son (Patient III:5) had been noted to have a congenital right-sided convergent strabismus and ptosis. His mother had been happy with his development at age 3 months, but at 18 months he was not walking or vocalizing. He began to have seizures at age 2 years; a brain MRI scan was normal. When seen at age 5, he still had no speech. His gait was broad-based, with mild ataxia; these features persisted, and at age 14, he also exhibited restless behavior, unusual...
posturing, and stereotypical thrusting and waving movements of the upper limbs. He had frequent generalized tonic-clonic seizures, treated with valproate.

There was a complex family history (Fig. 1), with at least two other autosomal recessive disorders known to affect individuals in other sibships. (The urea cycle disorder N-acetyl glutamate synthase deficiency had been ruled out in Patient III:4 by detailed metabolic investigations performed at the age of 2 years.) A maternal aunt (II:5) also had an undiagnosed severe neurodevelopmental disorder including early onset seizures, and was reported to be unable to walk or talk. Both index cases had normal G-banded karyotypes, and subtelomeric FISH analysis of both parents had been negative, as had a BAC aCGH analysis (resolution ~0.8 Mb). Although the possibility of Pitt–Hopkins syndrome had been raised, both Patients III:4 and III:5 had been seen by several clinical geneticists over the years, and felt not to have significant facial dysmorphism. The daughter and son scored respectively 9/20 and 7/20 using the PTHS assessment criteria of Whalen et al. [2012], which emphasize craniofacial dysmorphic features; these scores would not merit TCF4 mutation testing.

Further aCGH was performed on both siblings using the Blue-Gnome ISCA 8 × 60k OligoArray v2.0 reagent. In both, this showed an apparently homozygous deletion of a 66-kb Chr.7q35 region, spanning 146,544,329–146,610,191. These coordinates lie within intron 3 of CNTNAP2 (Fig. 2), but the CNV was based on abnormal signals from only 8 probes, and was below the usual diagnostic reporting size. To verify and more closely delineate the putative deletion, whole-genome sequencing at low coverage (0.4×) with copy number analysis was next performed. Using our standard CNVseq analysis parameters of approximately 250 test sample reads per window (~9 kb), the DNA copy segmentation analysis verified the presence of the deleted segment (supplementary Fig. S1 in supporting information online). The positions of individual mapped reads flanking the deletion indicated a likely size of approximately 76.8 kb, encompassing CNTNAP2 exon 3 (at 146,536,803–146,536,996) and creating a predicted out-of-frame mRNA deletion. Figure 2 displays these data visually by the almost complete absence of mapped reads. The affected son (blue data points) was the more informative, refining the maximal extent of the deletion to chr7:146,534,699–146,611,541. Two reads from the affected daughter were mapped within the presumed deleted region as a result of either low-level contamination or ambiguous alignment. (When the sequence complexity of these reads is observed in the context of RepeatMasker repeat elements, incorrect alignment appears to be the most likely explanation.) The two deletion endpoints both lie within L1 family elements which, however, do not display close sequence homology to each other.

To characterize the deletion breakpoints, the CNVseq libraries were resequenced at greater coverage (12×) and using longer (190-bp) reads; see Figure 2. The increased read length enabled a search for reads that spanned the deletion breakpoint. As the sequencing libraries had been optimized for CNVseq, their

![FIG. 2. Visualization of the Chr7q35 deletion using the UCSC genome browser. The purple track displays the 66-kb aCGH-defined deletion located in intron 3 of CNTNAP2. Datapoints for the low coverage and medium coverage whole genome sequencing tracks represent the genomic start coordinate of each aligned sequence read. Red datapoints are from the affected daughter (Patient III:4) and blue datapoints are from the affected son (Patient III:5). The green track displays the genomic coordinates of the nucleotide resolved deletion. The RepeatMasker track shows repeat elements within the genomic interval. The centromeric and telomeric deletion breakpoints map to L1 LINE elements.](image-url)
insert size was only ~200 bp, with the consequence that ~66% of the new longer reads were adaptor-trimmed (Table I). Nevertheless, a total of 11 breakpoint-spanning reads were identified in the two libraries, demonstrating that in both affected siblings, 76,838 nucleotides were deleted. The number of nucleotides spanning the centromeric and telomeric breakpoints and the genomic coordinates of each read are recorded in Table II. The position of the breakpoint-mapping reads with respect to the human reference sequence and RepeatMasker-defined LINEs are shown in supplementary Figure S2 (in supporting information online).

To validate the deletion, a breakpoint PCR assay was designed. Sanger sequencing of the amplicon confirmed a clean deletion breakpoint, with no inserted nucleotides, in all three recruited family members (Fig. 3A). A multiplex PCR was optimized, incorporating a normal allele-specific reverse primer to work in conjunction with the deletion-specific reverse primer, providing a single assay to determine zygosity status for future diagnostic testing of the family (Fig. 3B). In heterozygous mutation carriers, the deletion-specific PCR product appears disproportionately weak due to preferential amplification of the smaller amplicon. The molecular assay further verified the clinical suspicion that the third affected individual (Patient II:5) was also homozygous for the causative CNTNAP2 deletion.

**DISCUSSION**

Despite the very large mutational target presented by CNTNAP2, biallelic mutations of this gene have so far been described only in an Old Order Amish kindred and in two other unrelated families presenting with a Pitt–Hopkins-like syndrome [Strauss et al., 2006; Zweier et al., 2009]. The phenotype of the present family conforms to those in these earlier reports, thus contributing to the validity of previous studies.

We have recently introduced copy number analysis by high-throughput short-read sequencing (CNVseq) into routine diagnostic use in our center. Using ~25 million reads, this method has comparable sensitivity to aCGH, and is currently equivalent in cost. Here, we demonstrate an additional advantage of CNVseq for diagnostic purposes, in that it may be readily extended, by resequencing the CNVseq library. With minimal additional experimental effort, this can lead quickly to a precise molecular definition of a CNV. We were thus able to design a definitive molecular assay for future testing within this family.

As the cost of sequencing decreases and the resolution of CNVseq continues to rise, it is likely that aCGH will be phased out, allowing both point mutation detection and CNV analysis to be performed concurrently. New technologies under development further

---

**TABLE I. Comparison of Next Generation Sequencing Raw Data**

| Patient   | Read length (bp) | Raw read count | Adaptor trimmed reads (%) | Reads identified as duplicates (%) | Mapped readsa | Unmapped reads |
|-----------|------------------|----------------|---------------------------|-----------------------------------|---------------|---------------|
| Daughter (III:4) | 50              | 30,022,879     | —                         | 3.03                              | 28,150,220    | 993,250       |
| Son (III:5)    | 50              | 26,010,700     | —                         | 3.62                              | 24,067,381    | 1,039,205     |
| Daughter (III:4) | 190             | 180,072,658    | 68.7                      | 4.14                              | 159,379,549   | 13,765,587    |
| Son (III:5)    | 190             | 178,602,141    | 62.7                      | 5.02                              | 144,836,781   | 26,098,996    |

aFollowing duplicate removal.

**TABLE II. Characteristics of SplazerS Mapped Breakpoint Spanning Reads**

| Patient   | Read ID             | 5’ match (nts) | 3’ match (nts) | Trimmed read length (bp) | Strand | 5’ match start | 5’ match stop | 3’ match start | 3’ match stop |
|-----------|---------------------|----------------|----------------|--------------------------|--------|----------------|---------------|----------------|---------------|
| Daughter (III:4) | 1:1105:7121:93283  | 33             | 157            | 190                      | +      | 146,534,671   | 146,534,703   | 146,611,542   | 146,611,698   |
| Daughter (III:4) | 1:1204:20690:28490 | 84             | 106            | 190                      | +      | 146,534,620   | 146,534,703   | 146,611,542   | 146,611,647   |
| Daughter (III:4) | 1:1208:4536:25886  | 39             | 89             | 128                      | -      | 146,611,580   | 146,611,542   | 146,611,542   | 146,534,615   |
| Daughter (III:4) | 1:1209:4493:76571  | 86             | 104            | 190                      | +      | 146,534,618   | 146,534,703   | 146,611,542   | 146,611,645   |
| Daughter (III:4) | 1:2103:8417:52205  | 130            | 53             | 183                      | +      | 146,534,574   | 146,534,703   | 146,611,542   | 146,611,594   |
| Daughter (III:4) | 1:2214:7935:76888  | 88             | 61             | 149                      | -      | 146,611,629   | 146,611,542   | 146,611,542   | 146,534,643   |
| Son (III:5)    | 2:1107:5583:35287  | 43             | 147            | 190                      | +      | 146,611,584   | 146,611,542   | 146,534,557   | 146,534,655   |
| Son (III:5)    | 2:1109:2875:58010  | 66             | 89             | 155                      | +      | 146,534,638   | 146,534,703   | 146,611,542   | 146,611,630   |
| Son (III:5)    | 2:1111:13469:40356 | 27             | 121            | 148                      | +      | 146,534,677   | 146,534,703   | 146,611,542   | 146,611,662   |
| Son (III:5)    | 2:1202:17637:43981 | 59             | 119            | 178                      | +      | 146,534,645   | 146,534,703   | 146,611,542   | 146,611,660   |
| Son (III:5)    | 2:2110:6301:4685   | 129            | 61             | 190                      | +      | 146,534,575   | 146,534,703   | 146,611,542   | 146,611,602   |

Chromosome 7 coordinates are provided with respect to the strand from which the read was sequenced.
been required. We determined that the time and expertise needed to undertake the design, optimization and validation of such a custom reagent would have been considerably more burdensome than pursuing a WGS split-read mapping approach which utilized our laboratory’s standard WGS workflow. As the price of WGS continues to fall, it is increasingly likely that many laboratories will adopt our approach, rather than designing custom MLPA reagents.

By defining the molecular breakpoint in cases with inherited structural rearrangements, a simple, less resource intensive assay can be quickly designed for familial diagnostic testing. As demonstrated in this family, such an assay is quick to establish and the downstream DNA testing requirements are consequently far less than those required by aCGH or next generation sequencing. Our experimental strategy of performing aCGH or CNVseq as a first line investigation, prior to following up positive results with medium coverage WGS and split-read mapping has demonstrated its clinical utility and cost effectiveness.

REFERENCES

Amiel J, Rio M, de Pontual L, Redon R, Malan V, Bodaert N, Plouin P, Carter NP, Lyonnet S, Munnoch A, Colleaux L. 2007. Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. Am J Hum Genet 80:988–993.

Emde AK, Schulz MH, Weese D, Sun R, Vingron M, Kalscheuer VM, Haas SA, Reinert K. 2012. Detecting genomic indel variants with exact breakpoints in single and paired-end sequencing data using SplazerS. Bioinformatics 28:619–627.

Forrest M, Chapman RM, Doyle AM, Tinsley CL, Waite A, Blake DJ. 2012. Functional analysis of TCF4 missense mutations that cause Pitt-Hopkins syndrome. Hum Mutat 33:1676–1686.

Hayes JL, Tzika A, Thygesen H, Berri S, Wood HM, Hewitt S, Pendlebury M, Coates A, Willoughby L, Watson CM, Rabbitts P, Roberts P, Taylor GR. 2013. Diagnosis of copy number variation by Illumina next generation sequencing is comparable in performance to oligonucleotide array comparative genomic hybridisation. Genomics 102:174–181.

Kent WJ. 2002. BLAT—The BLAST-like alignment tool. Genome Res 12:656–664.

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. 2002. The human genome browser at UCSC. Genome Res 12:996–1006.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBO J 17:1:10–12.

McMullan DJ, Bonin M, Hehir-Kwa JY, de Vries BB, Dufke A, Rattenberry E, Steehouwer M, Moruz I, Pfundt R, de Leeuw N, Riess A, Altug-Teber O, Enders H, Singer S, Grasshoff U, Walter M, Walker JM, Lamb CV, Davison EV, Brueton L, Riess O, Veltman JA. 2009. Molecular karyotyping of patients with unexplained mental retardation by SNP arrays: A multicenter study. Hum Mutat 30:1082–1092.

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Cro nulla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID,
Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Wagg-oner DJ, Watson MS, Martin CL, Ledbetter DH. 2010. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86:749–764.

Nakabayashi K, Scherer SW. 2001. The human contactin-associated protein-like 2 gene (CNTNAP2) spans over 2 Mb of DNA at chromosome 7q35. Genomics 73:108–112.

Orrico A, Galli L, Zappella M, Lam CW, Bonifacio S, Torricelli F, Hayek G. 2001. Possible case of Pitt-Hopkins syndrome in sibs. Am J Med Genet 103:157–159.

Peippo M, Ignatius J. 2012. Pitt-Hopkins syndrome. Mol Syndromol 2:171–180.

Poliak S, Gollan L, Martinez R, Custer A, Einheber S, Salzer JL, Trimmer JS, Shragar P, Peles E. 1999. Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K+ channels. Neuron 24:1037–1047.

Poliak S, Salomon D, Elhanany H, Sabanay H, Kiernan B, Pevny L, Stewart CL, Xu X, Chiu SY, Shragar P, Furley AJ, Peles E. 2003. Juxtaparanodal clustering of Shaker-like K+ channels in myelinated axons depends on Caspr2 and TAG-1. J Cell Biol 162:1149–1160.

Rodenas-Cuadrado P, Ho J, Vernes SC. 2014. Shining a light on CNTNAP2: Complex functions to complex disorders. Eur J Hum Genet 22:171–178.

Schneider GF, Dekker C. 2012. DNA sequencing with nanopores. Nat Biotechnol 30:326–328.

Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH. 2006. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. N Engl J Med 354:1370–1377.

Venkatraman ES, Olshen AB. 2007. A faster circular binary segmentation algorithm for the analysis of array CGH data. Bioinformatics 23:657–663.

Whalen S, Héron D, Gaillon T, Moldovan O, Rossi M, Devillard F, Giuliano F, Soares G, Mathieu-Dramard M, Afenjar A, Charles P, Mignot C, Burglen L, Van Maldergem L, Piard J, Aftimos S, Mancini G, Dias P, Philip N, Goldenberg A, Le Merrer M, Rio M, Josifova D, Van Hagen JM, Lacombe D, Edery P, Dupuis-Girod S, Putoux A, Sanlaville D, Fischer R, Drévilleon L, Briand-Suleau A, Metay C, Goossens M, Amiel J, Jacquette A, Giurgea I. 2012. Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: Clinical score and further delineation of the TCF4 mutational spectrum. Hum Mutat 33:64–72.

Wood HM, Belvedere O, Conway C, Daly C, Chalkley R, Bickerdike M, McKinley C, Egan P, Ross L, Hayward B, Morgan J, Davidson L, MacLennan K, Öng TK, Papagiannopoulos K, Cook I, Adams DJ, Taylor GR, Rabbits P. 2010. Using next-generation sequencing for high resolution multiplex analysis of copy number variation from nanogram quantities of DNA from formalin-fixed paraffin-embedded specimens. Nucleic Acids Res 38:e151.

Zweier C, Peippo MM, Hoyer J, Sousa S, Bottani A, Clayton-Smith J, Reardon W, Saraiva J, Cabral A, Gohring I, Devriendt K, de Ravel T, Bijlsma EK, Hennekam RC, Orrico A, Cohen M, Drewekes A, Reis A, Nurnberg P, Rauch A. 2007. Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). Am J Hum Genet 80:994–1001.

Zweier C, de Jong EK, Zweier M, Orrico A, Ousager LB, Collins AL, Bijlsma EK, Oortveld MA, Ekici AB, Reis A, Schenck A, Rauch A. 2009. CNTNAP2 and NRXN1 are mutated in autosomal-recessive Pitt-Hopkins-like mental retardation and determine the level of a common synaptic protein in Drosophila. Am J Hum Genet 85:655–666.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.