

ARTICLE

CHRNA7 triplication associated with cognitive impairment and neuropsychiatric phenotypes in a three-generation pedigree

Claudia Soler-Alfonso¹, Claudia MB Carvalho^{2,3}, Jun Ge², Erin K Roney², Patricia I Bader⁴, Katarzyna E Kolodziejska², Rachel M Miller⁴, James R Lupski^{2,5}, Pawel Stankiewicz², Sau Wai Cheung², Weimin Bi² and Christian P Schaaf^{6*},^{2,6}

Although deletions of *CHRNA7* have been associated with intellectual disability (ID), seizures and neuropsychiatric phenotypes, the pathogenicity of *CHRNA7* duplications has been uncertain. We present the first report of *CHRNA7* triplication. Three generations of a family affected with various neuropsychiatric phenotypes, including anxiety, bipolar disorder, developmental delay and ID, were studied with array comparative genomic hybridization (aCGH). High-resolution aCGH revealed a 650-kb triplication at chromosome 15q13.3 encompassing the *CHRNA7* gene, which encodes the alpha7 subunit of the neuronal nicotinic acetylcholine receptor. A small duplication precedes the triplication at the proximal breakpoint junction, and analysis of the breakpoint indicates that the triplicated segment is in an inverted orientation with respect to the duplication. *CHRNA7* triplication appears to occur by a replication-based mechanism that produces inverted triplications embedded within duplications. Co-segregation of the *CHRNA7* triplication with neuropsychiatric and cognitive phenotypes provides further evidence for dosage sensitivity of *CHRNA7*.

European Journal of Human Genetics (2014) 22, 1071–1076; doi:10.1038/ejhg.2013.302; published online 15 January 2014

Keywords: dosage sensitivity; copy number variation; cholinergic nervous system; autism spectrum disorder

INTRODUCTION

Copy number variants (CNVs) of chromosome 15q13.3 have been associated with multiple neurological and neuropsychiatric phenotypes.¹ In general, there are two different sizes of recurrent nonallelic homologous recombination (NAHR)-mediated deletions and duplications at this locus: the larger CNVs utilize BP4 and BP5 as substrate pairs, and typically involve a 1.6-Mb genomic interval, whereas the smaller CNVs arise between two *CHRNA7*-low copy repeat (LCR) copies, and are 350–680 kb in size. Although the larger deletion and duplication harbor six RefSeq genes (*MTMR15*, *MTMR10*, *TRPM1*, *KLF13*, *OTUD7A*, and *CHRNA7*) and one miRNA (*hsa-mir211*), the smaller deletion and duplication CNV encompass only the entire *CHRNA7* gene and the first exon of a non-coding RNA-variant of *OTUD7A*. The BP4 and BP5 microdeletion has been found in patients with developmental delay/intellectual disability (DD/ID), epilepsy, autism, schizophrenia and other neurocognitive disorders.^{1,2} *CHRNA7* was considered the likely culprit gene to cause neurological phenotypes in patients with the 15q13.3 deletion, which was subsequently confirmed by the identification of the small (680 kb) *CHRNA7* deletion, manifesting a similar phenotypic spectrum as the larger deletion.³ Follow-up studies have strongly associated 15q13.3 deletions with both DD/ID⁴ and schizophrenia.⁵

The reciprocal microduplications of 15q13.3, which are very common in the general population with an estimated frequency of

1 in 180,⁶ have been more challenging to interpret, as they do not manifest the high penetrance of neuropsychiatric phenotypes seen in deletion cases. Van Bon *et al*² first reported four patients with cognitive impairment and psychiatric disorders coinciding with BP4 and BP5 duplications. This was followed by the description of 55 cases of smaller-sized *CHRNA7* duplications (350–680 kb in size).⁷ The latter report provided clinical data and pedigree analysis of 11 families and suggested that the duplication may be associated with DD, ID, and a variety of neuropsychiatric phenotypes, but with high variability in expressivity and reduced penetrance. Additional studies showed that 15q13.3 duplications were not associated with schizophrenia,⁵ but significantly associated with DD and ID.⁴ Furthermore, the small *CHRNA7* duplication was found as a risk factor for attention deficit hyperactivity disorder.⁸

Here, we report triplication of *CHRNA7*, segregating in four affected family members in three generations (Figure 1). We present a detailed clinical and behavioral characterization of the affected individuals.

Clinical description

The proband (III-2) was a 10-year-old boy at the time he presented to the genetics clinic for evaluation of ID and autistic behaviors. The boy was born as a monozygotic twin conceived naturally by a 26-year-old mother and 30-year-old father. Both parents were

¹Department of Pediatrics, Division of Medical Genetics, University of Texas Health Science Center, Houston, TX, USA; ²Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; ³Centro de Pesquisas René Rachou – FIOCRUZ, Belo Horizonte, Brazil; ⁴Parkview Health Laboratories, Genetics Center, Fort Wayne, IN, USA; ⁵Department of Pediatrics, Texas Children's Hospital, Houston, TX, USA; ⁶The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA

*Correspondence: Dr CP Schaaf, The Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, 1250 Moursund Street, Suite 1350, Houston, TX 77030, USA. Tel: +1 832 824 8787; Fax: +1 832 825 1251; E-mail: Schaaf@bcm.edu

Received 26 July 2013; revised 30 October 2013; accepted 27 November 2013; published online 15 January 2014

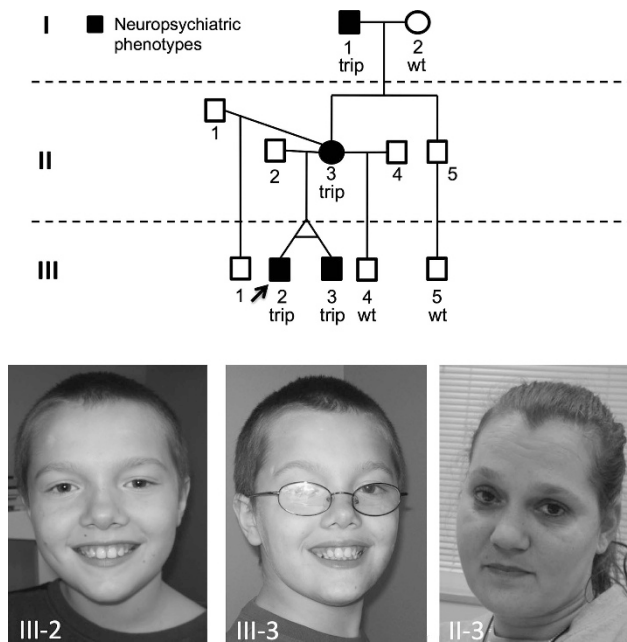


Figure 1 Three generation family with *CHRNA7* triplication. Trip, *CHRNA7* triplication; wt, wildtype (tested for *CHRNA7* copy number by FISH, with normal result). Patient photographs: individuals III-2 and III-3 at 10 years of age, individual II-3 at 37 years of age.

of Caucasian descent. The twins were born by spontaneous vaginal delivery at approximately 36 weeks of gestation. The proband's birth weight was 1.955 kg (10th–25th percentile). The birth head circumference and length were not available for review. He was hospitalized in the neonatal intensive care unit for 1 week because of mild feeding difficulties. Infancy and early childhood were significant for speech delay, with active intervention speech therapy started at 2 years of age, and cognitive impairment. Motor development was reported as normal. At the time of examination, he was in the fourth grade, enrolled in general and special education classes. He was able to follow two-step commands, but required constant repetition and redirection. A short attention span had been noted, as well as low frustration tolerance. He had transition difficulties and did not tolerate changes in his routine. His emotional profile was significant for anxiety and depression. A formal developmental and neuropsychological assessment was performed at 10 years and 7 months of age and was consistent with dysthymia, anxiety disorder, pervasive developmental disorder not otherwise specified (PDD-NOS) and cognitive abilities in the borderline range (full scale IQ of 75 on the Wechsler Intelligence Scale for Children IV).

Upon physical examination, his head circumference was 55 cm (80th percentile), height was 145 cm (90th percentile) and weight was 41 kg (90th percentile). The patient was mildly dysmorphic with a triangular face, hypertelorism (inner canthal distance 4 cm, >97th percentile), bilateral prominent antihelices, a broad philtrum and a high-arched palate (Figure 1, III-2). Examination of the extremities revealed bilateral clinodactyly of the fifth fingers, a single palmar crease on the left and generalized joint hypermobility. Neurological examination was abnormal for mild degree of dysarthria and generalized hypotonia. He exhibited poor balance and poor coordination when walking, but was not frankly ataxic. MRI of the brain performed at 10 years of age showed no anatomic abnormalities.

The proband's monozygotic twin brother (III-3) also presented to clinic for an evaluation of DD and learning problems. At the time of

evaluation, he was 10 years old and was able to follow one-, but not two-step commands. He was enrolled in special education classes. A short attention span, impulsive behavior and aggressiveness to his classmates had been reported. Like his brother, he did not tolerate changes in his routine. A formal neuropsychological evaluation revealed dysthymia, anxiety disorder, PDD-NOS, language processing deficits, and borderline intellectual functioning (full scale IQ of 84 on the Wechsler Intelligence Scale for Children IV), with significantly more salient non-verbal than verbal functions, impaired language functions, impaired memory functions and impaired cognitive flexibility. On physical examination, he manifested mild dysmorphic features similar to his twin brother (Figure 1, III-3), had mild dysarthria, some coordination difficulties, and generalized hypotonia. At 2 years of age, a head CT had been obtained after accidental head trauma, revealing a left middle cranial fossa arachnoid cyst, which was later confirmed by MRI at 10 years. Routine genetic evaluation and diagnostic testing for both twin brothers included chromosome analysis, FISH analysis for Sotos syndrome, *PTEN* sequencing, and Fragile X testing.

The twins' mother (II-3) was 37 years old at the time of examination. She had a history of DD and did not walk until 2–3 years of age. Her medical history was significant for bipolar disorder, anxiety disorder, attention deficit disorder, learning disability, and seizures since her twenties. At the time of examination, she lived in an assisted living facility. Her physical examination was remarkable for bilateral prominent antihelices, bulbous nasal tip, mildly flat philtrum and a fine resting tremor (Figure 1, II-3). The twins' half siblings (III-1 and III-4) were reported as healthy with normal physical appearance, and normal development. Both attended regular schools with no learning difficulties. Individuals II-2 (the twins' father) and I-2 (maternal grandmother) were in good physical health, with no cognitive impairment and no history of neuropsychiatric disease. Clinical information on the twins' maternal grandfather (I-1) is very limited, but significant for alcoholism, drug addiction, and abusive behaviors to family members. Reportedly, he had multiple family members with a history of alcoholism.

METHODS

The index case was referred to the Medical Genetics Laboratories at Baylor College of Medicine (BCM), Houston, TX, USA, for clinical array comparative genomic hybridization (aCGH) analysis. DNA was extracted from whole blood using the Puregene DNA extraction kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's instructions. The Institutional Review Board of BCM approved the study, and family members were recruited after informed consent was obtained. Photographs of the patients were collected after obtaining informed consent for publication of the photographs in the medical literature.

aCGH analysis and fluorescence *in situ* hybridization

Custom aCGH (CMA V8.1 OLIGO) was performed as previously described.^{9,10} Dual-color FISH analysis was performed on cultured lymphoblast cells with the test probe directly labeled by rhodamine (red) and the control probe directly labeled by FITC (green). For each sample, 50 interphase nuclei were examined to evaluate copy number. For FISH analyses using fosmid probes on proband, twin brother and an unaffected control, >10 metaphase cells were examined in addition to 50 interphase cells.

Whole-genome high-resolution oligonucleotide aCGH analysis was performed on the proband's (III-2) DNA for confirmation of the triplication and fine structure mapping of the sizes of the triplication, using the NimbleGen array with 4.2 M oligonucleotides (NimbleGen Systems, Madison, WI, USA), in accordance with the manufacturer's instructions.¹⁰

Breakpoint identification by PCR and sequencing

To further determine the size, genomic extent and gene content of this rearrangement, we designed a tiling-path oligonucleotide microarray spanning 2.8 Mb of the 15q13.3 region (all coordinates based on hg19): chr15:g.30 200 000–33 000 000 that includes BP4 (chr15:g.30 370 111–30 935 159) and BP5 (chr15:g.32 444 018–32 914 165). The custom 4 × 44k Agilent Technologies microarray was designed using the Agilent eArray website (<http://earray.chem.agilent.com/earray/>). We selected 14 765 probes, which represents an average distribution of 1 probe per 190 bp. Probe labeling and hybridization were performed as described.¹¹

Reverse and forward primer pairs (relative to the reference genome) were designed at the apparent boundaries of each duplicated or triplicated segment as defined by aCGH analysis. Long-range PCR was performed using TaKaRa LA *Taq* (Clontech, Mountain View, CA, USA). PCR sample-specific products for one of the junctions were obtained using the following primers: R2.1 5'-TGCGATGCAATTGATAGAGC-3' and R1.1 5'-TCACCCATTGCTAGT GCAAC-3'. Products were sequenced by the Sanger sequencing methodology. PCR and sequencing results were independently confirmed by repeated experiments.

Real-time quantitative PCR (qPCR)

To assess the copy numbers of regions of interest within 15q13.3, we performed qPCR using PrimeTime Assays (Integrated DNA Technologies, Coralville, IA, USA). The primers and probes for the qPCR reactions were as follows: *CHRNA7* exon2 (chr15:g.32 323 092–32 323 210) forward 5'-CTCC TTAAGTGTCCCTGCAAG-3', reverse 5'-GAAGTAGACGGTGAGTGGTTG-3', probe/56-FAM/TTGTAAAGC/Zen/TTCTCTGGAAGCTCGCC/3IABkFQ; *CHRFAM7A* (chr15:g.30 669 904–30 670 043) forward 5'-AGTAGCTGGGAC TACAGGTG-3', reverse 5'-ATGTTCCATTTTGTAGTATTTTATGTGTAC-3', probe/56-FAM/CCGGGATGG/Zen/TCTCGATCTCCTGA/3IABkFQ; duplication region proximal of *CHRNA7* triplication (chr15:g.32 024 776–32 024 877)

forward 5'-TCTAGTCACTTGGAACGCC-3', reverse 5'-TGCAATTGATA GAGCCACCTG-3', probe/56-FAM/ATGCCCCAG/ZEN/TTGAAATCCACGC/ 3IABkFQ; and the internal control RPPH1 (chr14:g.20 811 249–20 811 332), forward 5'-TCTGAATTGGGTTATGAGGTCC-3', reverse GCTTGAACA GACTCACGG, probe/56-FAM/TCACCTCAG/Zen/CCATTGAACTCACTTCG/ 3IABkFQ. Each of the reactions contained each primer at 500 nM, 250 nM probe, 10 ng genomic DNA and 1X TaqMan Universal PCR Master Mix, no AmpErase UNG (Life Technologies, Carlsbad, CA, USA). The reactions were performed in the StepOnePlus Real Time PCR System (Life Technologies) under the following conditions: AmpliTaq Gold Activation for 10 min at 95 °C, then 40 cycles of denature (15 sec, 95 °C) and annealing/extension (1 min, 60 °C). DNA samples III-2, III-3 and III-4 were run in parallel with control genomic DNA for which copy number of all tested regions had previously been demonstrated to be 2. All reactions were run in quintuplicate.

RESULTS

CMA performed on DNA obtained from peripheral blood of the index patient (III-2) revealed a triplication at chromosome region 15q13.3, suggested by a log ratio of 0.7–1.0. The estimated size of the triplication on the clinical array was a minimum of 0.227 Mb, chr15:g.32 218 274–32 445 252[3] (hg19) and a maximum of 1.195 Mb with adjacent oligonucleotides showing normal copy number (Figure 2a). The triplication encompasses the entire *CHRNA7* gene and the first non-coding exon of a transcript variant of the *OTUD7A* gene. Interphase FISH analysis performed on cultured lymphocytes of the index patient showed four signals for probe RP11-265I17 with three signals clustered together and well separated from the fourth signal, suggesting a triplication rather than a homozygous duplication. FISH analysis on metaphase cells showed no involvement of translocation or insertion of the triplicated region. Subsequent

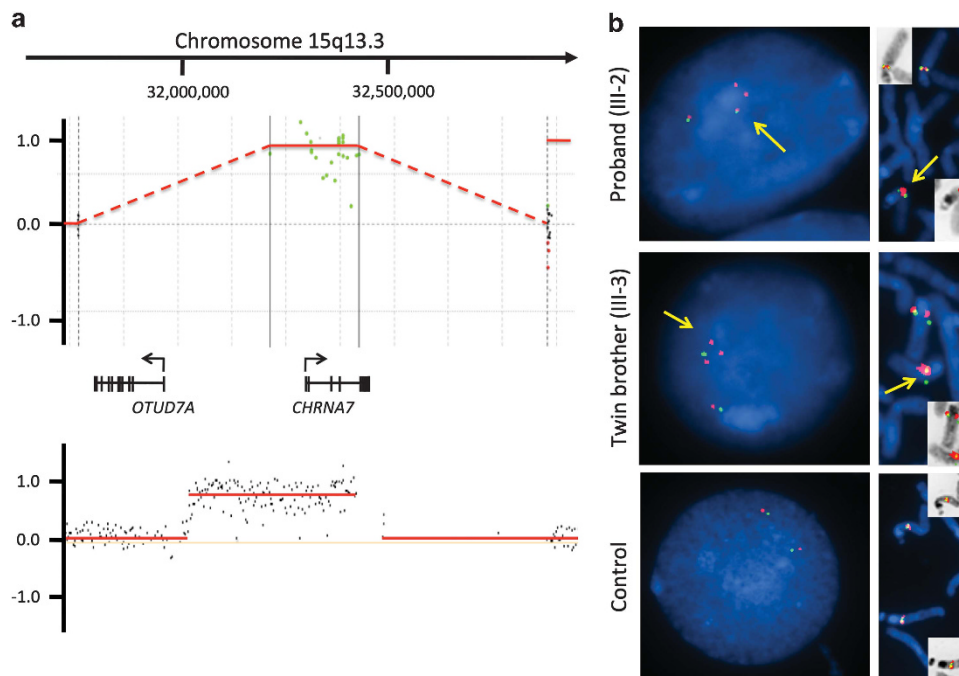


Figure 2 aCGH and FISH analyses. (a) Upper panel: clinical CMA V.8.1 OLIGO (individual III-2), lower panel: NimbleGen 4.2M array, both showing copy number gain in 15q13.3, encompassing the *CHRNA7* gene. Log ratios of 0.7–1.0 suggest triplication of the respective area. (b) FISH analysis, using test probe G248P89177H7 within the first 4 exons of the *CHRNA7* gene (red) and control probe G248P85751G8 outside the *CHRNA7* gene on 15q13.3 (green). Triplication of 15q13.3 in one copy of chromosome 15, as indicated by yellow arrows, is seen in the proband (III-2, upper panels) and his twin brother (III-3, middle panels), but not in a control individual (lower panels). Left panels: interphase cells, showing three red signals cluster with one green signal, whereas one separate red signal is next to another green signal. Right panels: metaphase cells, showing red signals that are stronger and bigger on one copy of chromosome 15, whereas green signals have comparable size and intensity on both copies of chromosome 15.

interphase FISH of maternal cells revealed the same *CHRNA7* triplication pattern in the proband's mother. Additional investigations revealed two additional triplication carriers in this family: the proband's twin brother (III-3, by chromosome microarray and FISH analysis) and the proband's maternal grandfather (I-1, by FISH analysis). However, CMA and FISH analyses showed that the triplication was not present in the proband's older half-sibling (III-4) who is phenotypically normal. The triplication was not present in the maternal grandmother (I-2), as determined by FISH analysis, either. Additional FISH analyses were performed on both the proband and the twin brother using a fosmid probe G248P89177H7, which is located within a region specific for *CHRNA7*, and a control probe G248P85751G8 outside of the *CHRNA7* gene on 15q13.3 (Figure 2b). The hybridization pattern showed clustering of three *CHRNA7* signals and one signal for the control probe in the majority of the interphase cells examined (36/50 interphase cells in subject III-2 and 38/50 interphase cells in subject III-3). Moreover, one chromosome 15 in metaphase cells consistently showed stronger and bigger signals than its homolog. These data are most consistent with the genomic change in this family representing a triplication of *CHRNA7* on one copy of chromosome 15, whereas the other copy of chromosome 15 is normal.

High-resolution oligonucleotide aCGH (4.2 M NimbleGen) confirmed the triplication and enabled further refinement of the genomic coordinates encompassed by the apparent triplication to experimentally allow for better breakpoint mapping (Figure 2a). The proximal breakpoint was amplified using PCR, followed by Sanger sequencing.

It maps to chr15:g.32024752. Using this oligonucleotide array, we were not able to map the precise coordinates of the distal breakpoint, as it falls within the low copy repeats of BP5.

Custom-tiled oligonucleotide aCGH ($4 \times 44k$ Agilent) that also covers the LCR region BP4 and BP5 enabled us to map precisely the breakpoint junctions and define the total size of the alteration as 650.4 kb because it includes part of BP5 (~233 kb). As a result of the presence of multiple copies of this LCR in the human genome it remains challenging to calculate accurately the copy number gain of that specific region in patients: calculated average \log_2 ratio of the 233-kb segment is 0.24 consistent with a duplication (considering four copies of BP5 in the reference genome and five copies in subjects with a duplication, expected \log_2 ratio is 0.32 contrasting with 0.6, which would be expected if a subject carried six copies). In addition, the array revealed a small duplication preceding the triplication at the proximal breakpoint junction (Figure 3a), which we were able to further characterize by long-range PCR using primers designed at both copy number transition sites (Figure 3b). That DUP-TRP breakpoint junction unveiled the presence of an inversion, which indicated that the triplicated segment is in an inverted orientation with respect to the duplication. Therefore, the rearrangement present in the patients described herein appears to be best classified as a DUP-TRP/INV-DUP type,¹² although we were not able to sequence the distal breakpoint junction to define whether an inverted repeat had mediated that rearrangement or not.

Real-time qPCR was performed in order to distinguish copy number of *CHRNA7* and *CHRFAM7A*, the latter of which is a

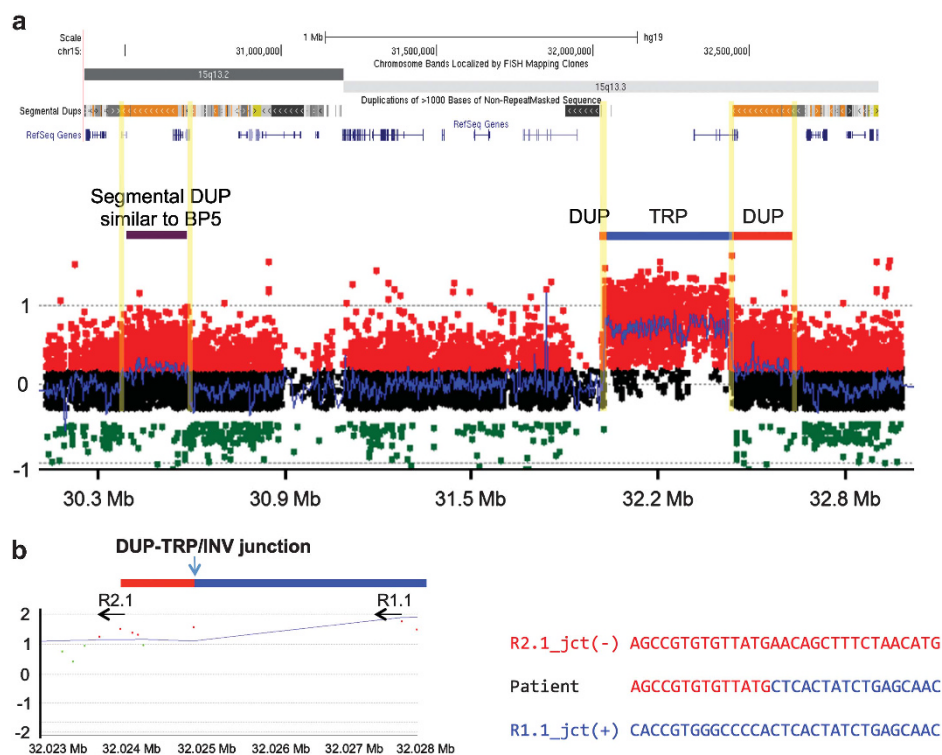


Figure 3 Agilent high-resolution tiling-path aCGH. (a) CNV results for individual III-2, shown in the context of the genomic human reference (hg19). LCRs present within the region color coded according to nucleotide similarity as presented in UCSC genome browser (orange >99%, light to dark grey 90–98% similarity). The triplicated segment is represented by a blue rectangle; duplicated segment is represented by red rectangles. Note that BP4 seems duplicated likely due to the high similarity between probes that span BP4 and BP5 (purple rectangle). (b) Close view of aCGH proximal DUP-TRP breakpoint showing location of primers used to amplify and sequence the junction (sequence is shown on the aCGH view to the right). Of note, primers R2.1 and R1.1 have the same orientation in the reference genome but produce a patient-specific PCR product that reveals that the triplication is inverted in orientation regarding the small duplication. Sequencing data is color-coded to show transition from the duplicated to the triplicated segment.

human-specific fusion gene at 15q13.2 sharing exons 5-10 with *CHRNA7*. The analysis was carried out using genomic DNA of individuals III-2, III-3, III-4 and a male control known to have two copies of each *CHRFAM7A* and *CHRNA7*. Data as shown in Figure 4 suggest that both the proband and his twin brother have four copies of *CHRNA7* and one copy of *CHRFAM7A*. In addition, qPCR confirmed the presence of the small duplication proximal to the *CHRNA7* triplication in both twins. The proband's older half-sibling has normal copy number of *CHRNA7*, as had been suggested by CMA and FISH.

DISCUSSION

This is the first case report of *CHRNA7* triplication, with four individuals in three generations being affected. Although deletions of *CHRNA7* cause ID and neuropsychiatric phenotypes with relatively high penetrance,^{3,4,6} the pathogenicity of *CHRNA7* duplications remains unclear, given its high prevalence in the general population with multiple individuals that are clinically unaffected.⁷ Therefore, it has been uncertain whether *CHRNA7* is a dosage-sensitive gene with penetrant clinical phenotypes for both deletions and duplications.

In this report, the family members harboring the triplication are affected with cognitive abilities in the borderline intellectual functioning range, and various degrees of neuropsychiatric pathology. Both the proband and his twin brother are affected with PDD-NOS, dysthymia, and anxiety disorder. Their mother has been diagnosed with bipolar disorder, anxiety disorder, attention deficit disorder, learning disability, and seizures since her twenties. Although information about the maternal grandfather is limited, he was reported as affected with alcoholism, drug addiction, and displays abusive behaviors to others, including his family – neurobehavioral traits observed and delineated in association with the initial deletion described at this locus.¹ The variability of expressivity seen in the family members with *CHRNA7* triplication is similar to what has been reported in individuals with *CHRNA7* duplication and many other CNVs associated with neuropsychiatric phenotypes.¹³ On the other hand, at least in this family, and to the point studied, there is a

complete penetrance of behavioral phenotypes for the *CHRNA7* triplication. Whether this holds true for *CHRNA7* triplications in general remains to be seen and awaits the identification of additional cases and families. Nevertheless, precedence exist for penetrance of neuropsychiatric traits being related to further in increments of gene dosage; triplications at the *STS* locus appear to be more penetrant than duplications for a DD/ID-associated phenotype.¹⁴

Triplications are very rare chromosomal rearrangements.¹⁵ In general, the resulting clinical phenotype is consistent with, but more severe than, the duplication of the same locus.^{12,14,16–18} Triplications of genomic loci associated with neurocognitive and neuropsychiatric abnormalities have been described, among others, at 22q11.2,¹⁸ 7p11.2,¹⁹ Xq28 encompassing the *MECP2* gene,²⁰ and 7q11.23 at the Williams syndrome critical region.¹⁷ Triplications can, in general, be classified as types I and II, as well as being recurrent or non-recurrent, which relates to the molecular mechanism underlying their formation.²¹ Type I may result from a two-step NAHR involving flanking paralogous LCRs such as those producing triplications of the *STS* region on Xp22.31,¹⁴ whereas the type II triplication product displays an unusual DUP-TRP/INV-DUP pattern of rearrangement – it has been reported in 20% of the *MECP2* CNV gains at Xq28, and also observed at the *PLP1* locus at Xq22.^{12,22} Inverted repeats in the human genome appear to be a susceptibility architecture for type II triplication.²³ Remarkably, LCRs BP4 and BP5, which mediate most of the rearrangements involving *CHRNA7*, are constituted by a complex set of large duplicated segments with >99% identity, lying in an inverted orientation relative to each other (Figure 3a).²⁴ Polymorphic inversions mediated by BP4 and BP5 are frequently found in individuals from various populations²⁴ and have been hypothesized to predispose to microdeletions and microduplications of the region by NAHR. In fact, at least six classes of microduplications, varying in size from 350 kb to 1.6 Mb, were shown to arise involving pre-existing heterogeneous inverted BP4 and BP5 chromosomes.⁷ Here we describe a non-recurrent triplication of 650.4 kb, for which the breakpoint junctions do not involve LCRs (Figure 3b), implicating an alternate underlying mechanism of formation. Its molecular characterization enabled us to experimentally classify it as type II or DUP-TRP/INV-DUP, likely generated by a replication-based mechanism.²⁵

The nervous system is exquisitely sensitive to the dosage of many proteins. Interestingly, there exist multiple examples for which both the deletion and duplication cause overlapping, if not similar, clinical phenotypes, a classic example is the myelinopathy phenotypes associated with *CMT1A* duplication and *HNPP* deletion.²⁶ It has been speculated that the similar cognitive and social behavior phenotypes caused by both loss and gain of function mutations is the manifestation of a failure in neuronal homeostatic response because of downstream effect of various molecular changes.²⁷

However, there have also been many examples of 'mirror traits' associated with gain *versus* loss at a locus. Perhaps the best classified is microcephaly *versus* macrocephaly at the 16p11.2 locus, associated with loss *versus* gain, respectively, and at the 1q21.1 locus, associated with gain *versus* loss, respectively.²⁸ Remarkably, such observations have been used to support a prominent theory in the social anthropology literature by Crespi²⁹ proposing that autism and schizophrenia represent opposing extreme phenotypes of social behavior.

This case report of the *CHRNA7* triplication family supports the idea of dosage sensitivity for *CHRNA7* encoding the alpha7 nicotinic acetylcholine receptor, which forms homopentameric ligand-gated cation channels, highly expressed at the synapse and elsewhere in the

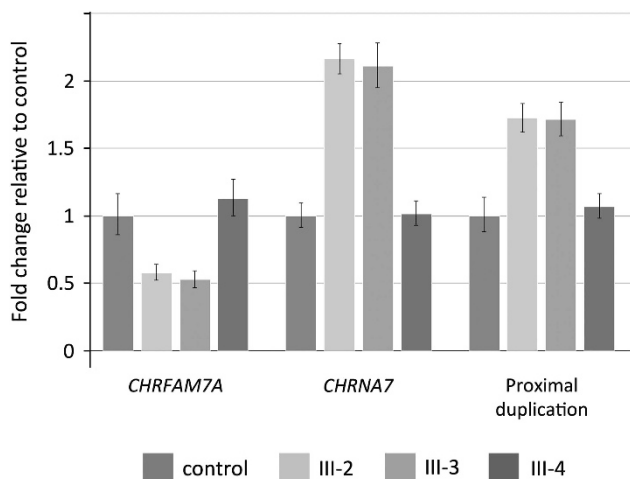


Figure 4 Real-time quantitative PCR of genomic DNA. Three primer and probe sets were used, unique to *CHRNA7*, *CHRFAM7A*, and the small duplication proximal to *CHRNA7*, respectively. Data were normalized to an internal control, *RPPH1* on chromosome 14, and are shown relative to a control individual known to carry two copies of each *CHRNA7* and *CHRFAM7A*. Reactions were run in quintuplicate. Error bars indicate SD.

brain.³⁰ Although overexpression of other nicotinic acetylcholine receptor subunits has been shown to modify and cause abnormal behaviors,³¹ this has not conclusively been proven for CHRNA7. Given the frequency of CHRNA7 duplications, even a modest penetrance of 5–10% would entail significant pathogenic potential in the population overall. Studies investigating this, as well as potential genetic and environmental modifiers, are even more important, given that the alpha7 nicotinic receptor represents an excellent target for therapeutic intervention.³² The study of large cohorts of individuals for sequence variants, mutations and CNVs of CHRNA7 gene are warranted to be able to elucidate a more accurate role in health and disease.

URLs

Leiden Open Variation Database (LOVD), <http://www.lovd.nl/>; CHRNA7-specific variations listed in LOVD, <http://databases.lovd.nl/shared/genes/CHRNA7>.

CONFLICT OF INTEREST

Drs Carvalho, Lupski, Stankiewicz, Cheung, Bi and Schaaf are faculty members of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the chromosomal microarray analysis offered in the Medical Genetics Laboratory.

ACKNOWLEDGEMENTS

We are indebted to the family who participated in this study. Dr Schaaf's work is generously supported by the Joan and Stanford Alexander Family. Dr Schaaf is also a recipient of a Doris Duke Clinical Scientist Development Award. NIH grant HD037283 supported Dr Ge's work in the laboratory of Dr Arthur Beaudet. In addition, this work was supported in part by US National Institute of Neurological Disorders and Stroke grant NS058529 to JRL.

- Ben-Shachar S, Lanpher B, German JR *et al*: Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. *J Med Genet* 2009; **46**: 382–388.
- van Bon BW, Mefford HC, Menten B *et al*: Further delineation of the 15q13 microdeletion and duplication syndromes: a clinical spectrum varying from non-pathogenic to a severe outcome. *J Med Genet* 2009; **46**: 511–523.
- Shinawi M, Schaaf CP, Bhatt SS *et al*: A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. *Nat Genet* 2009; **41**: 1269–1271.
- Cooper GM, Coe BP, Girirajan S *et al*: A copy number variation morbidity map of developmental delay. *Nat Genet* 2011; **43**: 838–846.
- Sahoo T, Theisen A, Rosenfeld JA *et al*: Copy number variants of schizophrenia susceptibility loci are associated with a spectrum of speech and developmental delays and behavior problems. *Genet Med* 2011; **13**: 868–880.
- Helbig I, Mefford HC, Sharp AJ *et al*: 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet* 2009; **41**: 160–162.
- Szafrański P, Schaaf CP, Person RE *et al*: Structures and molecular mechanisms for common 15q13.3 microduplications involving CHRNA7: benign or pathological? *Hum Mutat* 2010; **31**: 840–850.
- Williams NM, Franke B, Mick E *et al*: Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: the role of rare variants and duplications at 15q13.3. *Am J Psychiatry* 2012; **169**: 195–204.
- Boone PM, Bacino CA, Shaw CA *et al*: Detection of clinically relevant exon copy-number changes by array CGH. *Hum Mutat* 2010; **31**: 1326–1342.
- Carvalho CM, Zhang F, Liu P *et al*: Complex rearrangements in patients with duplications of MECP2 can occur by fork stalling and template switching. *Hum Mol Genet* 2009; **18**: 2188–2203.
- Shinawi M, Liu P, Kang SH *et al*: Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet* 2010; **47**: 332–341.
- Carvalho CM, Ramocki MB, Pehlivan D *et al*: Inverted genomic segments and complex triplication rearrangements are mediated by inverted repeats in the human genome. *Nat Genet* 2011; **43**: 1074–1081.
- Sebat J, Levy DL, McCarthy SE: Rare structural variants in schizophrenia: one disorder, multiple mutations; one mutation, multiple disorders. *Trends Genet* 2009; **25**: 528–535.
- Liu P, Erez A, Nagamani SC *et al*: Copy number gain at Xp22.31 includes complex duplication rearrangements and recurrent triplications. *Hum Mol Genet* 2011; **20**: 1975–1988.
- Reddy KS, Logan JJ: Intrachromosomal triplications: molecular cytogenetic and clinical studies. *Clin Genet* 2000; **58**: 134–141.
- Bi W, Sapir T, Shchelochkov OA *et al*: Increased LIS1 expression affects human and mouse brain development. *Nat Genet* 2009; **41**: 168–177.
- Beunders G, van de Kamp JM, Veenhoven RH, van Hagen JM, Nieuwint AW, Sijm AM, Sijm EA: A triplication of the Williams-Beuren syndrome region in a patient with mental retardation, a severe expressive language delay, behavioural problems and dysmorphism. *J Med Genet* 2010; **47**: 271–275.
- Yobb TM, Somerville MJ, Willatt L *et al*: Microduplication and triplication of 22q11.2: a highly variable syndrome. *Am J Hum Genet* 2005; **76**: 865–876.
- Girirajan S, Williams SR, Garbern JY, Nowak N, Hatchwell E, Eisea SH: 17p11.2p12 triplication and del(17)q11.2q12 in a severely affected child with dup(17)11.2p12 syndrome. *Clin Genet* 2007; **72**: 47–58.
- del Gaudio D, Fang P, Scaglia F *et al*: Increased MECP2 gene copy number as the result of genomic duplication in neurodevelopmentally delayed males. *Genet Med* 2006; **8**: 784–792.
- Liu P, Carvalho CM, Hastings PJ, Lupski JR: Mechanisms for recurrent and complex human genomic rearrangements. *Curr Opin Genet Dev* 2012; **22**: 211–220.
- Shimajima K, Mano T, Kashiwagi M *et al*: Pelizaeus-Merzbacher disease caused by a duplication-inverted triplication-duplication in chromosomal segments including the PLP1 region. *Eur J Med Genet* 2012; **55**: 400–403.
- Dittwald P, Gambin T, Gonzaga-Jauregui C *et al*: Inverted low-copy repeats and genome instability—a genome-wide analysis. *Hum Mutat* 2013; **34**: 210–220.
- Sharp AJ, Mefford HC, Li K *et al*: A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet* 2008; **40**: 322–328.
- Hastings PJ, Lupski JR, Rosenberg SM, Ira G: Mechanisms of change in gene copy number. *Nat Rev Genet* 2009; **10**: 551–564.
- Schaaf CP, Wisniewska J, Beaudet AL: Copy number and SNP arrays in clinical diagnostics. *Annu Rev Genomics Hum Genet* 2011; **12**: 25–51.
- Zoghbi HY, Warren ST: Neurogenetics: advancing the "next-generation" of brain research. *Neuron* 2010; **68**: 165–173.
- Lupski JR: Brain copy number variants and neuropsychiatric traits. *Biol Psychiatry* 2012; **72**: 617–619.
- Crespi BJ: Revisiting Bleuler: relationship between autism and schizophrenia. *Br J Psychiatry* 2010; **196**: 495; author reply 495–496.
- Lin H, Vicini S, Hsu FC *et al*: Axonal alpha7 nicotinic ACh receptors modulate presynaptic NMDA receptor expression and structural plasticity of glutamatergic presynaptic boutons. *Proc Natl Acad Sci USA* 2010; **107**: 16661–16666.
- Vinals X, Molas S, Gallego X *et al*: Overexpression of alpha3/alpha5/beta4 nicotinic receptor subunits modifies impulsive-like behavior. *Drug Alcohol Depend* 2012; **122**: 247–252.
- Kem WR: The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). *Behav Brain Res* 2000; **113**: 169–181.