Short Report

Dysfunction of \textit{SHANK2} and \textit{CHRNA7} in a patient with intellectual disability and language impairment supports genetic epistasis of the two loci


Synaptopathies constitute a group of neurological diseases including autism spectrum disorders (ASD) and intellectual disability (ID). They have been associated with mutations in genes encoding proteins important for the formation and stabilization of synapses, such as \textit{SHANK1–3}. Loss-of-function mutations in the \textit{SHANK} genes have been identified in individuals with ASD and ID suggesting that other factors modify the neurological phenotype. We report a boy with severe ID, behavioral anomalies, and language impairment who carries a balanced \textit{de novo} triple translocation 46,XY,t(11;17;19)(q13.3;q25.1;q13.42). The 11q13.3 breakpoint was found to disrupt the \textit{SHANK2} gene. The patient also carries copy number variations at 15q13.3 and 10q22.11 encompassing \textit{ARHGAP11B} and two synaptic genes. The \textit{CHRNA7} gene encoding \(\alpha_7\)-nicotinic acetylcholine receptor subunit and the \textit{GPRIN2} gene encoding G-protein-regulated inducer of neurite growth 2 were duplicated. Co-occurrence of a \textit{de novo} \textit{SHANK2} mutation and a \textit{CHRNA7} duplication in two reported patients with ASD and ID as well as in the patient with t(11;17;19), severe ID and behavior problems suggests convergence of these genes on a common synaptic pathway. Our results strengthen the oligogenic inheritance model and highlight the presence of a large effect mutation and modifier genes collectively determining phenotypic expression of the synaptopathy.

Conflict of interest
The authors declare no conflict of interests.

Synaptopathies constitute a new group of brain disorders that are linked to disruption of synaptic networks (1, 2). They are directly associated with dysfunction of proteins important for the formation and stabilization of synapses. These synaptic diseases cross the traditional boundaries of neurology and psychiatry and involve autism spectrum disorders (ASD), intellectual disability (ID), schizophrenia, and Alzheimer disease. Synaptopathies can arise from mutations in genes encoding proteins of the synaptome, including synaptic cell adhesion molecules and postsynaptic proteins, such as \textit{SHANK1–3} (3–8). \textit{SHANK3} is deleted in patients with Phelan-McDermid syndrome (9), and \textit{SHANK3} mutations have been identified in patients with ASD, ID, and...
schizophrenia (5, 8, 10, 11). In 11q13, submicroscopic deletions and intragenic mutations covering \textit{SHANK2} have been detected in individuals with ASD and ID (3, 4, 6). Recently, microdeletions involving \textit{SHANK1} have been described in males with high functioning ASD (7).

Recent studies revealed that inheritance of ASD and ID is effectively oligogenic rather than monogenic. This has been illustrated by identification of an increasing burden of large and multiple small rare copy number variations (CNVs) in individuals with more severe phenotypes (12). It has been proposed that the different mutations and imbalances collectively converge on common pathways involved in neurodevelopment and synaptogenesis (2, 13).

We describe a boy with severe ID, behavioral anomalies, and language impairment carrying a balanced 11;17;19 translocation that caused disruption of \textit{SHANK2}. Co-occurrence of CNVs encompassing the synaptic genes \textit{GPRIN2} and \textit{CHRNA7} in this patient corroborates the oligogenic inheritance model.

\textbf{Patient and methods}

\textbf{Case report}

The male propositus was the second child of healthy non-consanguineous parents. There was no family history of miscarriages, ID or alcohol exposure \textit{in utero}. During pregnancy, the mother had cervical cancer which was treated after birth. The propositus was born at 39 weeks of gestation by cesarean section; birth weight was 3000 g (−0.23 SD), length 49 cm (−0.83 SD), and occipitofrontal circumference (OFC) 36 cm (+0.7 SD). Perinatal adaptation was uneventful. At the age of 2 months, a ventricular septal defect was diagnosed. He was referred to neurological examination at age 1.5 year when psychomotor retardation was obvious. The patient had coarse face, facial dysmorphism (Fig. 1), a broad thorax, and clinodactyly of second and third toes. Physical development was normal. Ultrasound showed no visceral or brain malformations. Ophthalmologic and auditory tests were normal. He presented ataxia and was able to stand up with support. The Brunet-Lézine developmental test showed delay in his movements, coordination, communication and social skills. His psychomotor development corresponded to 9 months, and his developmental quotient was 48. The Bayley scales of infant development resulted in the developmental index 84. Electroencephalogram showed an increased irregular activity. Laboratory investigations disclosed increased alkaline phosphatase, lactate dehydrogenase and serum magnesium levels. He was able to sit independently at 8 months of age; at age 2.5 years he began to walk with support and 1 year later he walked independently.

At 5 years, his weight was 19.3 kg (+0.1 SD), length 105 cm (−1.0 SD), and OFC 51 cm (0 SD). The patient was not toilet trained. He was anxious and exhibited a habit disorder with stereotypic movements. He had decreased sensitivity to pain and often caused self-injury. He had speech disability and serious sleeping disturbances.

\textbf{Array CGH analysis}

Genomic DNA was extracted from peripheral blood of the child and his parents after obtaining consent for this study. Array comparative genomic hybridization (aCGH) in the parent–child trio was performed using the Human Genome CGH Microarray 244A platform (Agilent Technologies, Waldbronn, DE). Experimental procedures were performed as described previously (14).

\textbf{Fluorescence in situ hybridization}

Metaphase spreads from peripheral blood lymphocytes were prepared by standard procedures and routine
karyotyping was performed on the child and parents. Subtelomere probes for 11p15.5, 11q25, 19p13.3, and 19q13.43 (Abbot Molecular Inc, Des Plaines, IL, USA) were used in fluorescence in situ hybridization (FISH). FISH for delineation of the breakpoints was performed with bacterial artificial chromosomes (BACs; RPCI-11 libraries 753 and 737) and fosmids (WIBR-2 library; BACPAC Resource Center, Oakland, CA). DNA preparation, labeling, hybridization and evaluation of the slides were performed as described previously (15).

Microsatellite analysis
We verified declared relationships by genotyping both parents and the patient at 15 microsatellite loci.

Quantitative real-time polymerase chain reaction
Quantitative RT PCR on genomic DNA of the translocation patient was carried out as described previously (15).

Results
The 5-year-old patient was referred at 18 months because of marked psychomotor and developmental delay. Cytogenetic analysis revealed an apparently reciprocal 11;17 translocation: 46,XY,t(11;17)(q13.3;q25.1)dn (Fig. 2a). Chromosome analysis was normal in both parents, indicating that the translocation occurred de novo (paternity confirmed). Array CGH revealed that the translocation is balanced. We performed FISH with BAC and fosmid clones and located the 11q13.3 breakpoint within intron 13 of the SHANK2 gene (Fig. 2b,c).

For the 17q25 region, we identified three clones which yielded split signals on 17q25.1, der(17) and another chromosome not resembling der(11) (Fig. 2d,e), suggesting that a third chromosome was involved in the rearrangement (Fig. 2a). The 17q25.1 breakpoint interrupted the UBE2O gene (Fig. 2e). UBE2O is predominantly expressed in skeletal muscle and heart (16) and encodes an ubiquitin-conjugating enzyme involved in erythroid differentiation (17). Neither UBE2O nor any other gene near this breakpoint region is a plausible candidate for the translocation patient’s neurodevelopmental phenotype.

The breakpoint of the third rearranged chromosome [der(19)] as determined by subtelomeric FISH (data not shown) was mapped in 19q13.42 between the genes RPS9 and LILRB3 (Fig. 2f,g). Both genes are weak candidates for the patient’s phenotype (18, 19). However, a position effect on genes in proximity to the breakpoint, such as PRKCG mutated in different ataxias (20, 21) and TTYH1 that is widely expressed in neurons (22) and required for embryonic development (23), cannot be excluded. The karyotype of the patient was corrected to 46,XY.t(11;17;19)(q13.3;q25.1;q13.42)dn (Fig. 2a).

Finall, we evaluated 27 known CNVs identified in the patient with t(11;17;19) by array CGH (Table S1, Supporting information). At 15q13.3, he carried a 489-kb duplication of the CHRNA7 gene and a 152-kb deletion including ARHGAP11B (Fig. 3a), both transmitted from father as determined by array CGH (data not shown). ARHGAP11B deletions accompany the majority of CHRNA7 microduplications (24), similar to the translocation patient. Individuals with 15q13.3 microdeletion and/or duplication are at increased risk for various neurological phenotypes (24). The third CNV was a maternally inherited 1.15-Mb duplication at 10q11.22 encompassing GPRIN2 besides other genes (Fig. 3b). This CNV has been associated with ASD (https://gene.sfari.org/autdb/CNVHome.do). We verified the three CNVs in the patient by quantitative real-time PCR (data not shown).

Discussion
We report a boy with severe ID, language impairment, and behavioral anomalies and a balanced de novo 11;17;19 translocation disrupting the SHANK2 gene. SHANK2 interruption is likely deleterious and probably the main cause for the clinical features of the patient. De novo loss-of-function SHANK2 alleles have been associated with ASD, mild to moderate ID, and/or language impairment (3, 4, 6). Although ASD and ID are clinically distinct disorders, there is a strong correlation between the two: individuals with ID can have ASD and vice versa (25). The phenotypic overlap between ID and autism is also mirrored at the genetic level. Mutations in genes encoding proteins involved in synaptogenesis and neuronal circuit formation have been linked to synaptopathies. It has been proposed that scaffolding proteins at the postsynaptic density, like SHANK1–3, are sensitive to stoichiometric imbalances caused by gene dosage changes suggesting that alteration of synaptic homeostasis underlies ASD and other brain disorders (1). But how can mutations in the same subset of proteins cause a continuum of phenotypes? Strong Mendelian mutations, such as SHANK2 interruption have a large effect and are probably the main cause of moderate/severe ID (26). Similarly, a translocation disrupting the SHANK3 gene has been reported in a patient with expressive language delay and ID (27) and a female with Phelan-McDermid syndrome (28). The clinical outcome, however, may be modulated by additional weaker mutations in other components of the postsynaptic signaling complexes (2), for example, a position effect in patients with a chromosomal rearrangement. This is in keeping with the ‘multiple hit model’ as has been proposed for ASD and ID (6, 13): two or more mild mutations that together have an additive effect on the components/protein composition of the postsynaptic density could induce the development of a cognitive disorder (2, 26). This phenomenon, called genetic epistasis, has been reported in three ASD-affected patients with de novo SHANK2 deletions. Two of them had autism and ID and carried a deletion of ARHGAP11B, encoding a Rho GTPase activating protein, along with a CHRNA7 duplication (4). We detected a similar genetic constellation in the patient with t(11;17;19) and SHANK2 disruption. Co-occurrence
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The same CNVs in three patients with **SHANK2** haploinsufficiency strongly suggests that the **SHANK2** alteration together with imbalances of **ARHGAP11B** and **CHRNA7** collectively converge on the same pathway leading to the observed neurological phenotypes. This is corroborated by the function of the \( \alpha_7 \)-nicotinic acetylcholine receptor subunit (\( \alpha_7 \)-nAChR) encoded by **CHRNA7**. The pre- and postsynaptic location of \( \alpha_7 \)-nAChRs and their calcium permeability enable the receptors to modulate transmitter release and synaptic plasticity (29).

In the patient with t(11;17;19), we additionally identified a 1.15-Mb duplication at 10q11.22 that influences neurological phenotypes (30, 31).
Fig. 3. Array comparative genomic hybridization (CGH) profile of chromosome 15 (a) and chromosome 10 (b) in the translocation patient. A value of zero indicates a balanced chromosomal status. The outlines to the right (red dots) indicate the two duplicated regions [15q13.3 in (a) and 10q11.22 in (b)]; the outline to the left (green dots) [15q13.3 in (a)] indicates the deleted region. Extensions of duplicated and deleted segments are marked by gray shaded areas through the Agilent software. Synaptic genes located in a deleted or duplicated interval are boxed and highlighted in red. See text for further explanations. Data are according to the Human February 2009 (GRCh37/hg19) assembly.

**GPRIN2** is located in the duplicated interval and encodes the G-protein-regulated inducer of neurite outgrowth 2 (32).

We conclude that **SHANK2** interruption in the boy with t(11;17;19) and a neurodevelopmental disorder represents the large effect mutation. However, this alteration probably acts together with **CHRNA7**, **GPRIN2** and possibly also **ARHGAP11B**, all converging on the same synaptic pathway and determining the clinical outcome. This oligogenic inheritance model highlights the presence of common neuronal networks and strengthens the existence of genetic modifiers which significantly contribute to abnormal neuronal function in patients with ASD and ID.

**Supporting Information**

The following Supporting information is available for this article:

Table S1. Copy number variations identified in the patient with t(11;17;19).

Additional Supporting information may be found in the online version of this article.
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Acknowledgements

The authors are grateful to the child and his parents for their interest and participation in this study. We thank Karin Ziegler and Dr. Sigrid Fuchs for chromosome analysis and help with FISH experiments. The results summarized here form part of the MD thesis of Bettina Chilian at the University of Hamburg. This work was supported by grants of the Deutsche Forschungsgemeinschaft (KU 1240/5-1 to K. K. and RO 3660/1-1 to G. R.). [Correction added on 29 October 2013, after first online publication: The correct grant information has been included.]

References


