

Clinical and Genetic Heterogeneity of the 15q13.3 Microdeletion Syndrome

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Key Words

Copy number variations · Microdeletion 15q13.3 · Segmental duplications

Abstract

The 15q13.3 microdeletion is a recurrent CNV, presumably mediated by NAHR between segmental duplications in chromosome 15. The 15q13.3 deletion and duplication are associated with a wide range of clinical manifestations, such as intellectual deficits, seizures, autism, language and developmental delay, neuropsychiatric impairments, and behavioral problems illustrating incomplete penetrance and expressivity. This study comprises an evaluation of 106 symptomatic patients carrying the heterozygous deletion, as well as of 21 patients carrying the duplication, who have been described in previous studies. The analysis shows considerable heterogeneity for the manifestation of different key symptoms and familiar occurrence. Furthermore, 8 new patients are introduced. Convolved familiar connections give new insights into the complexity of symptomatic manifestation. In previous studies, different opinions have been expressed as to the nature and precise location of the deletion breakpoints. Here, we show that not *CHRNA7* and *CHRFAM7A*, but rather *FAM7A* or *GOLGA8*, serve as breakpoint regions concerning our patients. The deletion is described as heterogeneous in

size. However, we assume that not only different breakpoints but also the imprecision of aCGH analysis on chromosome 15 due to segmental duplications accounts for the variability in size.

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The 15q13.3 microdeletion syndrome (OMIM 612002), which has been first described by Sharp et al. [2008], is a recurrent CNV, presumably mediated by NAHR between segmental duplications (BP3–BP5, BP4–BP5) in chromosome 15. The 15q13.3 deletion and duplication are associated with a wide range of neurodevelopmental disorders, such as intellectual deficits, seizures, autism, language and developmental delay, neuropsychiatric impairments, and behavioral problems showing incomplete penetrance and variable expressivity. The typical 1.6-Mb deletion harbors 7 genes: *ARHGAP11B*, *MTMR10*, *MTMR15*, *TRPM1*, *KLF13*, *OTUD7A*, and *CHRNA7*. *CHRNA7* encodes for the neuronal alpha7 nicotinic acetylcholine receptor and, therefore, is regarded as the major candidate gene responsible for the clinical features expressed.

In this study, we describe 6 so far unpublished patients carrying the typical 15q13.3 microdeletion between BP4 and BP5, including a family with 4 affected members who

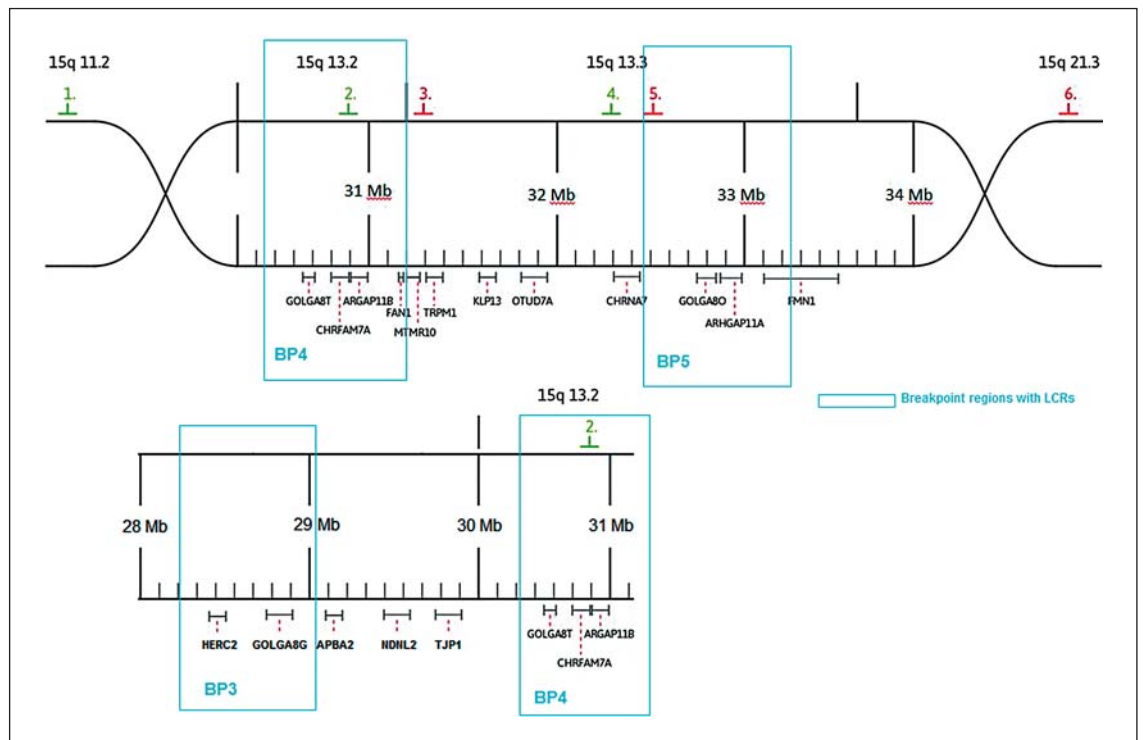


Fig. 1. Schematic overview of the region 15q13.3. Location of FISH probes is given by colored numbers.

are described further below. In addition, 1 patient with a 15q13.3 duplication and 1 patient carrying a 3.4-Mb deletion between BP3 and BP5 are described.

Moreover, in order to explain the variety of clinical manifestations and the severity of symptoms, we reviewed the data of 106 symptomatic patients with heterozygous 15q13.3 deletion and 21 patients with duplications which have been reported.

In recent studies, different opinions have arisen as to the nature and precise location of the deletion breakpoints. We investigated the breakpoint location using FISH in order to confirm in how far these options prove true for our patients.

Methods

DNA Extraction and Array CGH

Peripheral blood leukocytes were used as a source of DNA. Array-CGH analysis was performed using an array slide Sure Print 4x180K (Agilent Technologies, Santa Clara, Calif., USA). Patient DNA was labeled with Cy3, reference DNA with Cy5. Hybridization was carried out using Cot-1 DNA (1.0 µg/ml). Test DNA samples were hybridized with gender-matched reference DNA (Agilent). Purification, hybridization and washing steps were performed according to the manufacturer's instructions. Sure Scan

microarray Scanner G2600D (Agilent), Feature Extraction software (Agilent) and Agilent Cytogenomics Software Edition 2.0.6.0 were used.

FISH and Breakpoint Analysis

Slides with metaphases of cultivated peripheral lymphocytes had been stored at -70°C . Before hybridization, slides were processed in an alcohol series (70, 80 and 100%), followed by pepsin treatment (15 min, 37°C). Fluorescence-labeled BACs (Illumina®, BlueFish) were used as FISH probes. Probe preparation, hybridization and washing steps were performed according to Illumina's instructions.

BAC probes used were RP11-11H9, 22,067,176–22,300,706, chr.15q11.2; RP11-40J8, 30,546,758–30,724,265, chr.15q13.2; RP11-348B17, 31,281,641–31,502,115, chr.15q.13.3; RP11-265I17, 32,293,149–32,457,541, chr.15q13.3; RP11-280K19, 32,654,212–32,821,799, chr.15q13.3, and RP11-232J12, 53,792,861–53,948,902, chr.15q21.3 (see fig. 1, hg19).

Results

Clinical Assessment

The patients were initially observed on the occasion of neuropsychiatric examination because of intellectual deficits and/or developmental delay. Table 1 summarizes the cytogenetic and clinical findings.

Fig. 2. Pedigree of patients 1–4. Filled circles and squares indicate tested symptomatic deletion carriers. Circle with vertical line indicates a deletion carrier by evidence, who was not tested. P1–P4 were tested. aCGH results are shown in online supplementary material 2. Patients III/4 and III/5 were tested, but clinical evaluation was not possible. Patient III/9 was tested (no carrier), and patients III/7 and III/8 were not tested.

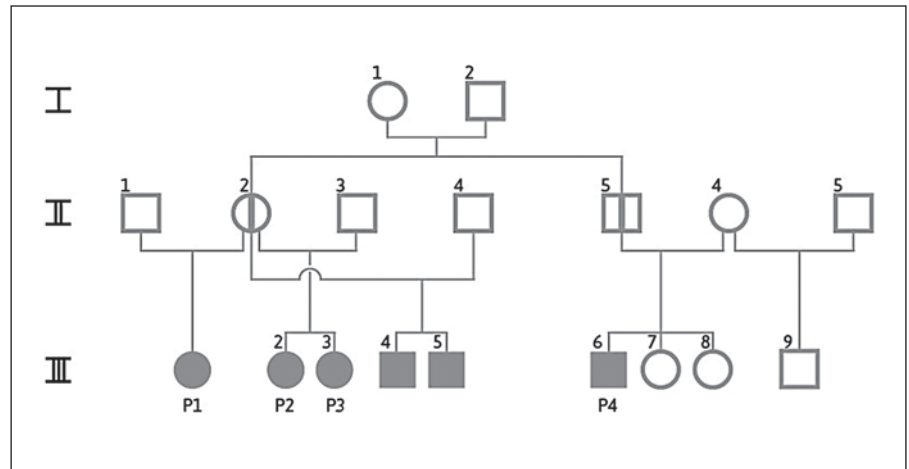


Table 1. Overview of newly introduced patients

	Patient							
	1, female	2, female	3, female	4, male	5, male	6, male	7, male	8, male
Aberration size	1.97 Mb	1.85 Mb	1.78 Mb	1.47 Mb	1.85 Mb	3.47 Mb	2.26 Mb	1.97 Mb
Intellectual deficits	+	IQ 63	+	IQ 56	IQ 78	+	+	+
Development delay	+	+	NA	+	-	+	+	+
Parental phenotype noticeable	+	+	+	+	-	-	-	-
OFC	P>97	P3–10	P50	P50	NA	P50	P10	P50
Weight	P10	P50	P>97	P97	NA	P50	P3–10	P10
Language problems	-	+	-	+	NA	+	+	+
Behavioral problems	ADHD	ADHD	Stereotypies	ADHD	ADHD	ADHD	-	+
Epilepsy	-	-	-	-	+	-	-	-
Hypotonia	NA	NA	NA	NA	-	+	NA	+

For aCGH results see online supplementary material 2 and figure 2 for the pedigree of patients 1–4. NA = No data available; + = feature expressed; - = feature not expressed.

Description of Patients

Patient 1 (III/1)

The 13-year-old girl presented with attention deficit hyperactivity disorder (ADHD), intellectual deficits, developmental delay, recurrent respiratory infections, and scoliosis. Her weight and head circumference (OFC) vary around the 10th percentile (P), respectively P90. She attends a special school. Her mother (II/2, in fig. 2) also attended a school for special education and completed it successfully. Both parents have not been available for further investigation. Patient 1 is a half sister of patients 2 and 3, and the cousin of patient 4.

Patient 2 (III/2)

The female patient was introduced at the age of 9 and showed developmental and language delay as well as intellectual deficits (K-ABC-Test: IQ 63). A waking EEG showed general alterations, but so far, no seizures have occurred. Her OFC is between P3 and P10, weight and height are in normal ranges. ADHD is treated with methylphenidate. She is described as a cheerful but also aggressive and impulsive person and attends a special school. Both parents have not been available for further investigation.

Patient 3 (III/3)

The 10-year-old patient was diagnosed with a learning disorder, which was not further defined. The girl's body measurements show a high variation in time. Until the age of 2, her OFC was $P < 3$; currently, her weight is P97. She is obese, shows stereotyped behavior and attends a special school.

Patient 4 (III/6)

The examination of the patient (13 years old) revealed language and psychomotor delay as well as mild intellectual deficits (HAWIK-IV: SW 56). Noticeable features are multiple dyslalia and ADHD, which is treated with methylphenidate (10–15 mg/day). The boy's EEG is unremarkable, his OFC is normal, height P90, and his weight P97. The patient's character is described as friendly but at times aggressive and impulsive. He attends a school for the handicapped. Both parents attended a special school. His father (II/5) is imprisoned for assault. His 2 sisters (III/7, III/8) also are visiting a special school, and his half brother (III/9) shows a delay in development and language, but does not carry the deletion. The patient is the cousin of patients 1, 2 and 3. The parents have not been available for investigation.

Patient 5

The patient (14 years old) presented with abnormal social behavior and ADHD. Due to his poor school performance and the discrepancy between his intellectual performance (HAMIK-IV, IQ 78) and the family's mean, a medical examination was initiated. After an epileptic seizure, he has been treated successfully with Orfiril®. ADHD is treated with methylphenidate (5–10 mg/day). His parents have not been available for investigation.

Patient 6

As an infant, patient 6 manifested developmental and language delay. He showed signs of hypotonia. At the time of examination, the 19-year-old patient presented with intellectual deficits and ADHD. The treatment with methylphenidate was stopped after the patient started high-performance sport, which proved to be an effective treatment of the ADHD symptoms. His mother is not a deletion carrier. His father has not been available for investigation.

Patient 7

The patient was examined at the age of 7 and revealed mild intellectual deficits (K-ABC-Test, SW: 63). His body

measurements are all within P10. He is described as restless and inattentive. He attends a special school. His parents have been unavailable for investigation.

Patient 8

The patient was born with a heart defect (ventricular septal defect and atrial septal defect). He shows dysmorphic features such as low-set ears, hypertelorism, strabismus, and hypotonia. His psychomotor development is delayed. The patient's birth length and OFC were P50, his weight was P10. He inherited the duplication from his father who shows an unremarkable phenotype. An uncle (on the maternal site) with epilepsy and malignant hyperthermia has been reported.

Breakpoint Analysis

In previous studies, the heterogeneity of the deletion size has been a topic of interest. Shinawi et al. [2009] assumed FAM7A1/2 to function as a breakpoint, whereas Szafranski et al. [2010] suspected *CHRNA7* and *HRFAM7A* to be responsible for the recurrent deletion in the 15q13.3 locus. Antonacci et al. [2014] suggested the gene family *GOLGA8* as possible candidates. An attempt was made to validate these theories by using FISH. Probe 4 marks *CHRNA7*, probe 5 marks *FAM7A*, probe 2 marks *HRFAM7A*, and probe 3 marks a region distally to *HRFAM7A* (fig. 1). In our patients, *FAM7A* is detectable in both chromosomes 15. This finding indicates that the distal breakpoint is located between *CHRNA7* and *FAM7A*. The analysis of patients 7 and 4 show that the proximal breakpoint is located distally to *HRFAM7A* (figs. 3, 4). In patient 7, probe 3 bridges the breakpoint. Therefore, *CHRNA7* and *HRFAM7A* do not mediate the recurrent deletion regarding our patients.

Evaluation of Symptomatic Patients Described in Previous Studies

For patients and studies see table 2 and online supplementary material 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000443343). Statistical analysis was performed by Fisher's exact test, using software 'R'. Results with $p < 0.05$ were assessed as significant.

Discussion

The family we present shows interesting features. On the one hand, the degree of relationship varies; on the other hand, the symptoms differ as well (fig. 2). However,

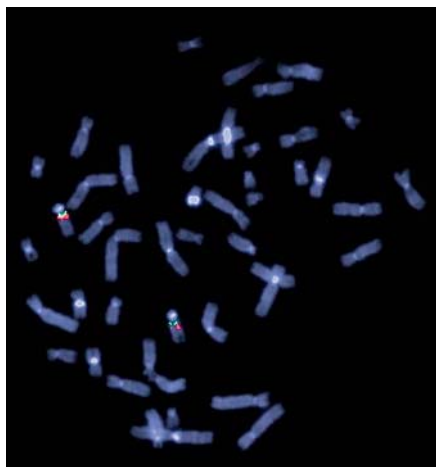


Fig. 3. Metaphase FISH using BAC RP11-40J8 (probe 2, labeled in green) and RP11-348B17 (probe 3, labeled in red) showing the proximal breakpoint of patient 7. Probe 3 bridges the breakpoint (weak red signal).

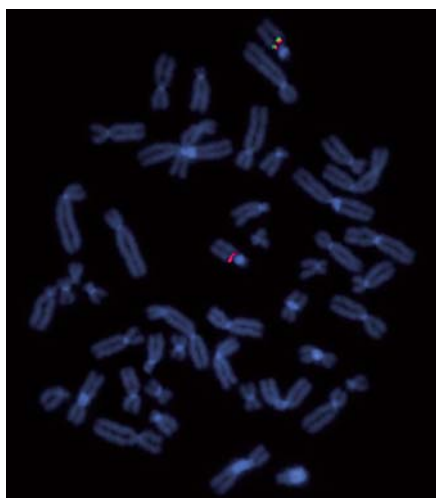


Fig. 4. Metaphase FISH using BAC RP11-265I17 (probe 4, labeled in green) and RP11-280K19 (probe 5, labeled in red) showing the distal breakpoint of patient 4.

there is no demonstrable connection between the degree of relationship and symptoms. Patients 2 and 3, who are sisters, are remarkably different. They vary considerably in body measurements, character and the severity of symptoms, although they are exposed to similar environmental factors and carry the same deletion size. Therefore, other genetic factors are bound to influence the degree of clinical manifestations. A possible explanation could be an altered expression of her genes in the genome,

Table 2. Summary of features of patients described in previous studies

Feature	Percentage	n/N
Size		
<1.0 Mb	24.53	26/106
1–1.6 Mb	63.21	67/106
>1.6 Mb	12.26	13/106
Intellectual deficits		
No	20.22	18/89
Undefined	11.24	10/89
Mild	40.45	36/89
Severe	28.09	25/89
Developmental delay	41.77	33/79
Epilepsy	30.61	30/98
Autism	27.71	23/83
Paternal	26.47	18/68
Maternal	54.41	37/68
De novo	19.12	13/68
Parental phenotype noticeable	66.67	34/51
Male	55.56	45/81
Female	44.44	36/81
OFC		
P<25	26.09	18/69
P25–75	53.62	37/69
P>75	20.29	14/69
Weight		
P<25	14.29	8/56
P25–75	64.29	36/56
P>75	21.42	12/56
Language problems	75.34	55/73
Hypotonia	39.02	16/41
Behavioral problems	63.64	49/77

For a list of patients see online supplementary material 1. n/N = Affected/total patients.

which may have diverse effects [Le Pichon et al., 2013]. This approach has been further confirmed by Henrichsen et al. [2009], who have shown that CNV regions are expressed at lower and more variable levels and modify the expression of neighboring genes. Chaignat et al. [2011] stated that CNVs alter the expression timing of genes during development in mice. Additionally, different temporal patterns of expression can be found in different individuals. Furthermore, altered expression or mutations of different genes coding for different steps of the same neurobiological pathway could intensify neurological symptoms. Poot et al. [2011] suggest different mechanisms which can lead to phenotypic pleiotropy of CNVs, for example, the interaction of CNVs, genetic epistasis or allelic exclusion.

Three out of 4 family members manifest ADHD; all show developmental delay. As the origin of ADHD apparently is multifactorial, it is not surprising to find an accumulation in one family. Remarkably, all children are affected by the deletion. Particular familial genetic constellations possibly increase the probability to manifest symptoms. Furthermore, the intrauterine surroundings of the deletion-carrying mother may have affected the phenotype of her children. However, no shared CNVs correlating with the occurrence of symptoms could be identified, and no second pathogenic CNV could be identified in any of our patients.

The breakpoint analysis shows that the distal breakpoint is positioned distally to *CHRNA7*. Possible locations would be within the gene families *FAM7A* or *GOLGA8*, as has already been suggested, or within the locus control regions [Shinawi et al., 2009; Anttonaci et al., 2014]. The proximal breakpoint is located distally to *CHRFAM7A*. In patients 1–4, who belong to one family, the deletion sizes detected by the aCGH-analysis software differ. These differences are brought about by gaps in the distribution pattern of oligonucleotides in the aCGH within and around the breakpoint regions. Slight changes in the log ratio of marginally located oligonucleotides result in a considerable shift of deletion size detected by the analysis software. The obvious difference in size of the recurrent deletion 15q13.3 is most likely an artifact of the processing software. We assume the deletion to be identical in size for all family members (see online suppl. material 2).

Different CNV studies have revealed an association of the 15q13.3 microdeletion with schizophrenia, epilepsy and autism. Several publications have been issued presenting new cases and theories about breakpoints and factors which alter symptoms [e.g. Freedman et al., 2001; Dibbens et al., 2009; Mikhail et al., 2011]. Efforts were made to merge this information in order to find new connections.

The results leave room for speculations. Deletions are inherited in 80.88%. Maternal descent is described disproportionately more often for deletions (54.41%, $p = 0.001$) and duplications (53.33%, $p = 0.027$).

An explanation might be given by Sinkus et al. [2011]. They studied the impact of *CHRNA7* promoter polymorphisms, which decrease the level of transcription by ~25% and influence the cortisol level in mother and child. The cortisol level of the child is lowered further, if the mother as well as the child are polymorphism carriers. The impact of the intrauterine surrounding may also have an influence on other factors. The OFC is reduced in patients if the deletion is inherited by the mother ($P < 25$ in 50%, $p = 0.002$). Therefore, we assume that if the aberration

is inherited from the mother, the intrauterine surrounding will intensify the symptoms. As we only included symptomatic patients in this assessment, this would explain why the majority of aberrations are apparently of maternal origin (54%, $p = 0.001$). In this context, it is striking that the gender distribution of the deletion is equal. Our data confirms the findings of Lowther et al. [2015]. In their comprehensive review of the 15q13.3 deletion, they combined symptomatic and asymptomatic patients. As we only included symptomatic patients, the proportions of distributional patterns of features are similar but the relative numbers differ. Regarding the increased maternal descent of deletions, they state a reduced reproductive fitness in males as a possible explanation.

The size of the deletion does not consistently correlate with the degree of symptoms. For example, comparing the degree of intellectual deficits with the size of the deletion shows no linear connection. Patients with the typical 1.6-Mb deletion show severe deficits more frequently than patients with smaller or larger deletions (41.07%, $p = 0.00045$). Intellectual deficits are reported significantly more often in patients with deletions than duplications (79.78 and 55.56%, $p = 0.037$). As expected, normal intelligence is described most often in patients with deletions <1 Mb (45%, $p = 0.0037$).

Deletions with a size of 1–1.6 Mb are most frequent (63.21%, $p = 0.0002$). Duplications <1 Mb (66.67%, $p = 0.0004$) are most commonly described. Regarding deletions, intellectual deficits (79.78%), language disorder (75.34%), a noticeable phenotype of the parents (66.67%), and behavioral problems (63.64%) are described much more often than epilepsy (30.61%) or autism (27.71%) ($p = 0.02$ – $3.931E-09$). Comparing duplications and deletions, duplications are associated more often with autism ($p = 0.039$) and the phenotype of the parents is noticeably less frequent ($p = 0.027$).

Epilepsy is significantly associated with mild intellectual deficits (59.09%, $p = 0.0029$). Language disorder is associated with intellectual deficits in 79.24% of the cases ($p = 0.003$).

To conclude, it can be said that the clinical phenotype of the microdeletion 15q13.3 is of multifactorial origin. Even close family members exposed to similar environmental factors differ significantly in their clinical manifestations. Genetic counseling in families with 15q13.3 microdeletions or notably microduplications remains thus complex and occasionally inadequate. Further investigations in genetic alterations regarding the nondeleted allele or promotor regions of affected genes may be more promising when it comes to discovering influencing factors.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.