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SHORT COMMUNICATION

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Phenotypic and molecular characterisation of a de novo 5q deletion that includes the *APC* gene

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Abstract We report on a 12-year-old female patient with mild dysmorphic signs, including bilateral epicanthal folds, low-set dysplastic ears, a short nose with anteverted nostrils, conically shaped fingers, generalised increase of subcutaneous fat, multiple fine venous teleangiectasia on her back, mild pectus carinatum, and a general muscular hypotonia. Cytogenetic analysis and fluorescence in situ hybridisation (FISH) studies using region-specific BAC and YAC clones indicated a de novo interstitial deletion of the long arm of chromosome 5, resulting in monosomy 5q21.1-q23.1. Molecular analysis of polymorphic markers helped to narrow down the breakpoints and demonstrated that the derivative chromosome 5 is of paternal origin. By using the same panel of polymorphic markers, a reinvestigation of a similar, already published, 5q deletion case [Raedle et al. (2001) Am J Gastroenterol 96:3016–3020] was performed, allowing a more detailed genotype–phenotype correlation. Phenotypic classification was also carried out. Several known genes, including *APC* and *MCC*, were found to map to the common deleted genomic segment. Genetic counselling based on the molecular analysis data was performed for the index family.

Keywords Chromosome 5q · Deletion · FISH · BAC · *APC* gene

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Introduction

Cytogenetic and molecular genetic investigations on deletions that include chromosome band 5q22 are rare. Fourteen cases with an interstitial deletion of chromosome 5q that included the entire *APC* gene at 5q21-q22 have been reported. Raedle et al. (2001) described a male patient (patient WS) with a 5q21-q22 deletion associated with adenomatous polyposis coli and carcinoma of the rectum due to a deletion of the *APC* gene. Both inherited and sporadic cases of colon cancer are strongly associated with mutations of the *APC* gene. In addition, the patient showed mild mental retardation and minor dysmorphic signs such as a hypoplastic midface, low set ears, a bulbous tip of the nose, and short fan-like toes. Bennett et al. (1997) reported on a 21-year-old female patient with a 5q22-q23.2 deletion associated with mental retardation, schizophrenia, and minor dysmorphic features; however, the deletion in this case does not include the *APC* gene.

In this communication, we report on another patient (patient SL) with an interstitial deletion of chromosome 5q similar to the case described by Raedle et al. (2001). To compare and characterise the deletions of both probands at the molecular level, we performed molecular analysis using fluorescence in situ hybridisation (FISH) with previously mapped bacterial artificial chromosome (BAC) clones and microsatellite marker analysis.

Materials and methods

Clinical report on patient SL

The proband was born in 1993 as the second child to a 20-year-old woman. The clinical history of the mother was unremarkable. The father had pronounced retardation of growth and sexual development, which was treated by artificial initiation of puberty by high-dosage sex hormone therapy (1,000 μg testosterone 5× per week

from 11 to 16 years). The brother of the proband was born in 1991 and shows a normal development.

The patient (SL) was referred to our laboratory for genetic analysis because of psychomotor retardation and delay in mental development at the age of 6 years. She was born following an unremarkable pregnancy at the 38th week of gestation by spontaneous vaginal delivery. APGAR scores were recorded as 9/10/10. Birth weight was 2,560 g (10–50th centile), length 48 cm (50th centile) and head circumference [occipital-frontal circumference (OFC)] 32 cm (10–50th centile). Neonatal investigations revealed a pulmonary fissure and a reflux of the urethra, which was surgically corrected at the age of 18 months. An early episode of pneumonia was successfully treated.

At the age of 6 months, a delay in her physical and mental development was diagnosed. At this time she had a weight of 6,100 g (25th centile), length of 62 cm (25th centile) and head circumference of 40 cm (3rd centile). Psychomotor and mental retardation became more obvious during the following months. Developmental milestones were as follows: she was able to sit from 9 months onwards, and started walking at 18 months. She started to speak a few words by 2 years. No apparent problems with hearing or vision were found.

Besides a general muscular hypotonia at the age of 7 years (see Fig. 1) the following dysmorphic signs were rather mild. Malrotated ears, epicanthus, tapered fingers, low-set hairline, increase of subcutaneous fat, and pectus carinatum. She had multiple fine venous teleangiecatses on the back and the ears, and supernumerary teeth. She currently attends a special-needs school were she reportedly performs well.

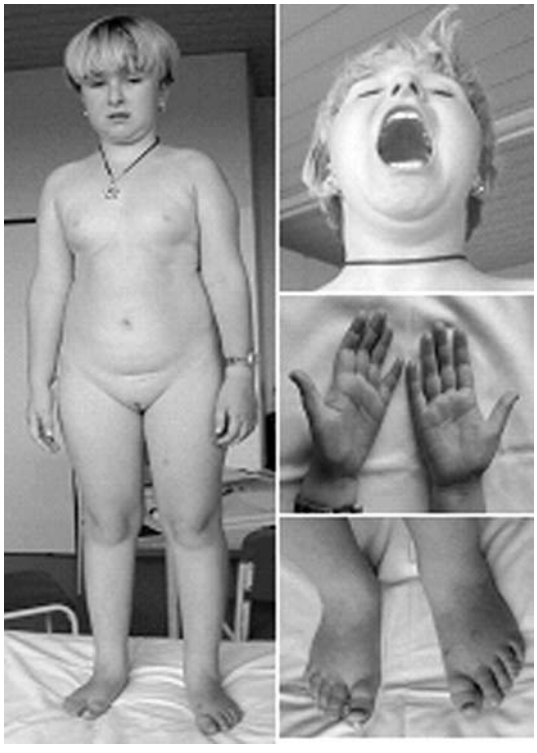

Fig. 1 The patient at age 7 years. Frontal view of the patient: increase of subcutaneous fat, muscular hypotonia, pectus carinatum, and short stature. Dysmorphic signs include low-set hairline, malrotated ears, epicanthus, mildly anteverted nostrils, tapered fingers, and supernumerary teeth

Because of the deletion of one copy of the entire *APC* gene, a coloscopy and a gastroscopy was performed at the age of 10 years. No polyps of the mucosa were found. Histological analysis revealed a normal mucosa of the colon and the duodenum. Table 2, details a comparison of manifestations of our patient and previously reported patients with similar interstitial deletions.

Methods

Peripheral blood was cultured and harvested according to standard methodology. Genomic DNA for molecular analysis was extracted from peripheral blood lymphocytes using standard techniques (Sambrook et al. 1989). FISH, using mapped and sequenced BACs and YACs specific for the long arm of chromosome 5, was performed according to Petek et al. (1997). Using dye-labelled primers, genotyping of the propositus and her parents was performed using 16 polymorphic microsatellite markers. Amplified products were separated and analysed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA). Microsatellite analysis was also performed on patient WS (Raedle et al. 2001) and his parents.

Results

Cytogenetic studies for the proband showed a female karyotype with an interstitial deletion of the long arm of chromosome 5 at the 400–550 band level. Maternal and paternal karyotypes were both normal. A detailed breakpoint characterisation was performed by application of site-specific BAC-FISH probes according to Petek et al. (1997). We hybridised digoxigenin labelled DOP-PCR products of ten BAC and two YAC clones (see Table 1). The breakpoints were mapped to 5q21.1 and 5q23.1, respectively. Detailed results of the FISH analysis are summarised in Fig. 2. Due to a phenotypic indication, FISH analysis was performed on the father's chromosomes; however, all results were negative.

Genotyping of the propositus and her parents was performed using 16 polymorphic microsatellite markers. Microsatellite analysis was also performed on patient WS (Raedle et al. 2001) and his parents. In both cases the interstitial deletion on chromosome 5q had occurred in the paternal chromosome as a de novo event. The pedigree of both families, with detailed results of marker analysis is shown in Fig. 3. The actual sizes of the deletions encompass about 18 Mb (patient SL) and 10 Mb (patient WS; Radle et al. 2001).

Table 1 Summary of fluorescence in situ hybridisation (FISH) analysis performed on patient SL

Informative STS markers[a]	BAC/YAC clones	Cytogenetic localisation	Signals on der(5q) relative to deletion Patient (SL) 10 years 46,XX,del(5)(q21.1-q23.1)
D5S1467	YAC 904_b_1	5q15	Proximal
D5S2466	YAC 955_e_6	5q15	Proximal
AFM184YB6	RP11-203J7	5q21.1-q21.2	Deleted
D5S1721	RP11-11P8	5q21.2-q21.3	Deleted
D5S1346	RP11-252I13	5q21.3	Deleted
D5S2553	CTB-2220M12	5q23.1	Deleted
D5S592	RP11-104C13	5q23.1	Deleted
RH92503	RP11-11P11	5q23.1	Deleted
RH67297	RP11-136L3	5q23.1	Deleted
D5S467	CTC-441N14	5q23.1-q23.2	Deleted
-	RP11-403M22	5q23.2	Distal
D5S2720	RP11-93O7	5q23.2	Distal

[a]Polymorphic markers used for microsatellite analysis in the present family

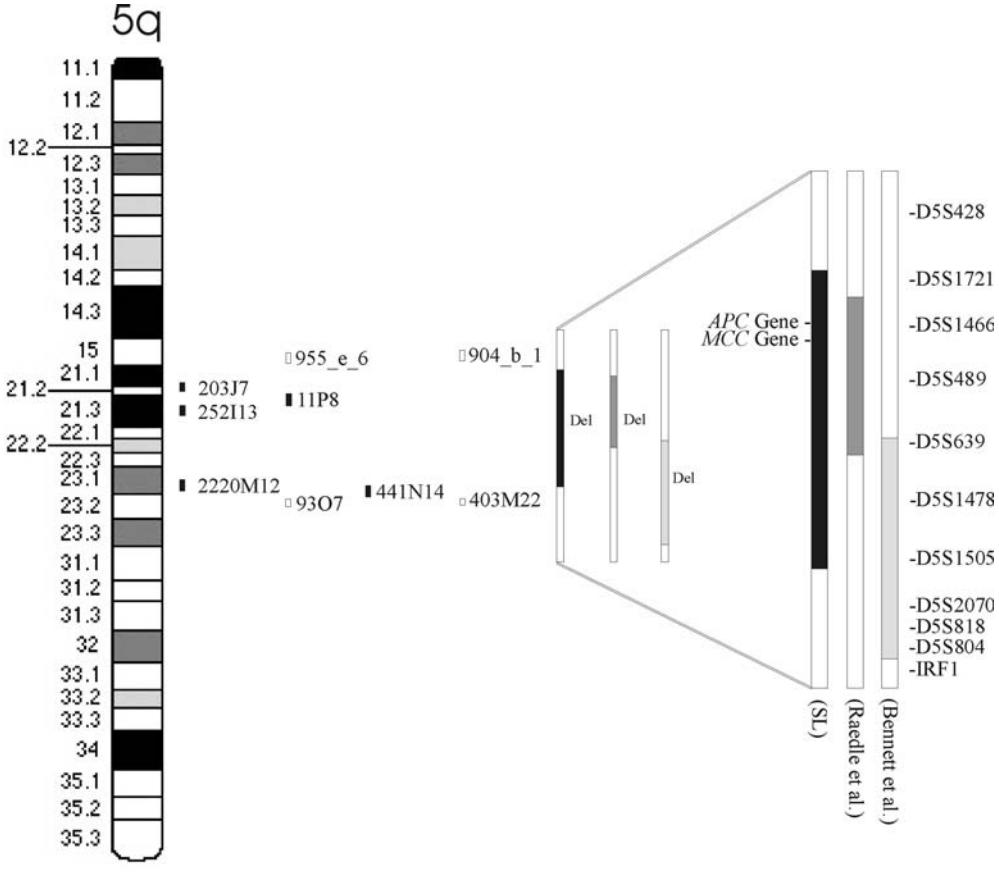

Fig. 2 Comparison of the YAC FISH, BAC FISH and polymorphic marker analysis results of patient SL and the patients published by Raedle et al. (2001) and Bennett et al. (1997)

Discussion

Rearrangements of chromosome 5, in particular constitutional interstitial deletions, are uncommon and the corresponding phenotype is not well defined. Only 37 cases with interstitial deletions of the middle portion of the long arm of chromosome 5 have been reported (Garcia-Minaur et al. 2005). Most reported cases of 5q deletions are de novo aberrations, except for three cases with an inherited deletion (Hockey et al. 1989; Cross et al. 1992; Hastings et al. 2000).

Patient SL was referred to our laboratory because of failure to thrive and mental retardation. Detailed phenotypic investigations defined mild dysmorphic signs,

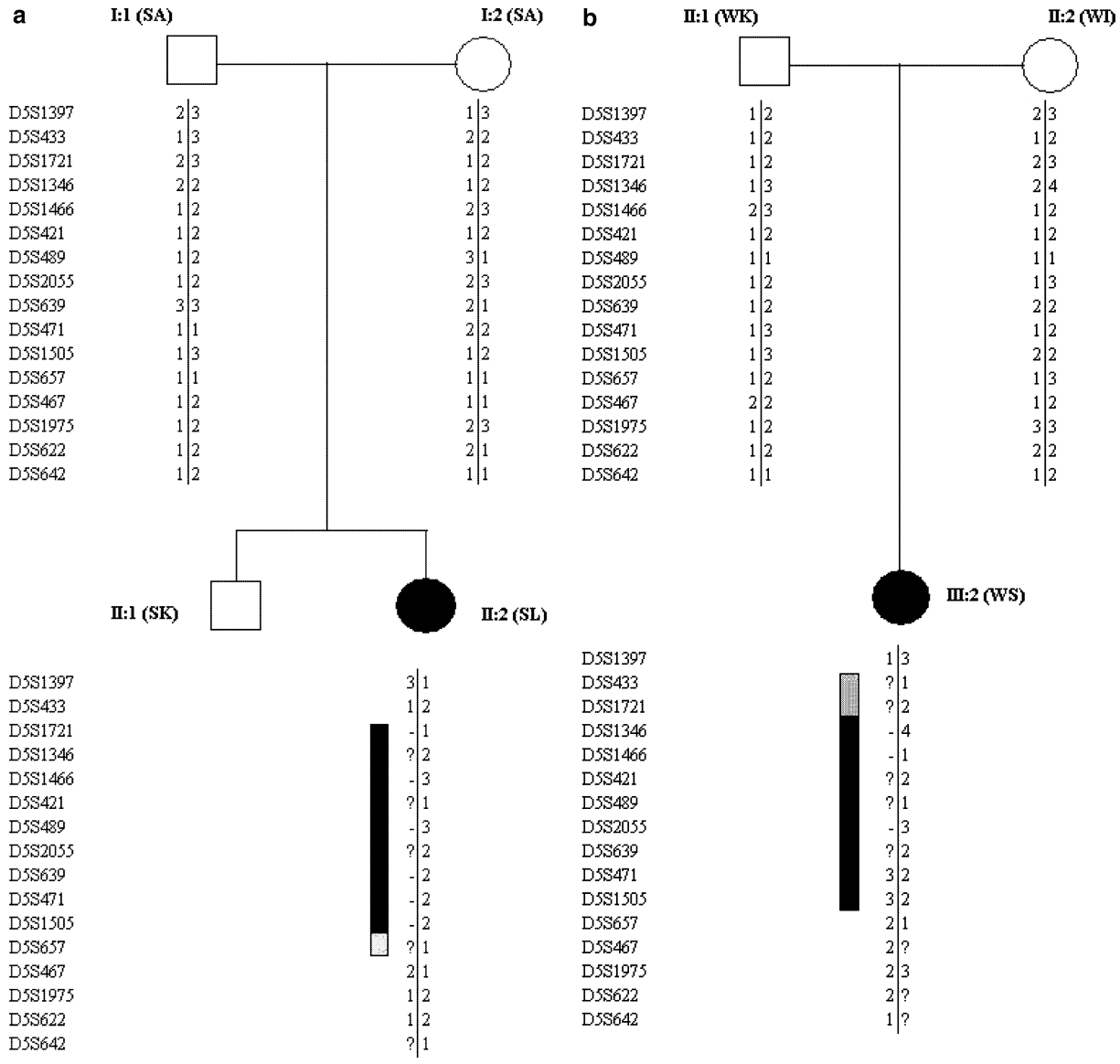

Fig. 3 **a** Pedigree of the investigated family. Individual II:2 (SL) carries the deletion (*black box*). Marker D5S657 is uninformative. Markers are listed next to individual I:1 (SA) and II:2 (SL). *Question mark* indicates uncertainty regarding FISH deletion in individual II:2 (SL). **b** Pedigree of the investigated family. Individual III:2 (WS) carries the deletion (*dark square*). Markers D5S433 and D5S1721 are noninformative to define the deletion. Markers are listed next to individual II:1 (WK) and III:2 (WS). *Question mark* indicates uncertainty regarding FISH deletion in individual III:2 (WS)

including bilateral epicanthal folds, low-set dysplastic ears, a short nose with anteverted nostrils, conically shaped fingers, multiple fine venous teleangiectasia on her back, mild pectus carinatum, a general muscular hypotonia, and a generalised increase of subcutaneous fat. Cytogenetic and molecular analysis revealed that the deletion (5q21.1-23.1) occurred in the paternal chromosome as a de novo event.

Only one other 5q deletion case with adipose features has been reported: Bennett et al. (1997) reported a woman with increased subcutaneous fat in addition to mild mental retardation and schizophrenia. The breakpoints were identified cytogenetically as 5q22-23.2.

About 60 genes have been mapped within the deleted region. Presumably the most clinically relevant gene in this region is the *APC* gene at 5q22.2. Early linkage studies confirmed the location of the *APC* gene to 5q (Bodmer et al. 1987; Leppert et al. 1987; Nakamura et al. 1998). Reports of LOH for 5q in sporadic tumours (Solomon et al. 1987; Vogelstein et al. 1988) supported the role of the *APC* gene as a tumour suppressor. The *MCC* gene, at 5q22.2, also plays a role in the cause of colorectal cancer. The Dmx-like 1 (*DMXL1*) gene at 5q23.1 is expressed in a variety of tissues and seems to have an important regulatory function. Regions 5q23.2 (D5S804) and 5q23.3 (D5S818) have been implicated in genetic (Straub et al.

Table 2 Comparison of phenotypes

	Our patient (SL)	Raedle et al. (2001)	Bennett et al. (1997)	Gracia-Minaur et al. (2005)
Age/karyotype	12 years 46,XX,del(5)(q21.1-q23.1)	21 years 46,XY,del(5)(q21.3-q23.1)	40 years 46,XX,del(5)(q23.1-q23.3)	11.5 years 46,XY,del(5)(q22.3-q23.3)
Type 5q15-q22				
Mental handicap	+	+	+	+
Hypertelorism	+	?	+	+
High forehead	–	+	–	–
Macrognathia	–	–	–	–
Down-turned corners of mouth	–	?	–	+
High arched palate	+	+	–	–
Deformed limbs	–	–	–	–
Horseshoe kidney	?	?	?	+
APC	– (at 10 years)	+ (>100 colonic polyps)	–	–
Type 5q22-q31				
Severe developmental delay	–	–	+	+
Low birth weight	–	–	+	+
Failure to thrive	+	+	+	+
Hypotonia	+	?	?	–
Hypertelorism	+	?	+	+
Down-slanting palpebral fissures	–	+	+	+
Flat nasal bridge	+	–	+	+
Abnormal ears	+	+	+	+
Micrognathia	+	+	+	+
Wide neck	+	?	+	–

1997) and cytogenetic (Bennett et al. 1997) studies, respectively, for schizophrenia. Recent linkage studies of schizophrenia showed a multipoint H-LOD of 3.35 in this genomic region at marker D5S804 (5q23.2; Straub et al. 1997). A 5q deletion in a 34-year-old woman in combination with development of schizophrenia was described by Bennett et al. (1997). The deletion contained some regions (band 5q23.1, D5S639–D5S1505, see Fig. 2) overlapping with the case SL. The patient described by Bennett et al. (1997) was also moderately mentally retarded and showed discreet dysmorphic features such as delays in speech and motor development, low-set ears, low posterior hairline and tapered fingers (see Table 2). Medical records of the family of SL revealed that the paternal grandmother of the proband suffered from schizophrenia, and the father of SL was also mildly affected. Because the father shows symptoms of schizophrenia and has an increasing number of cutaneous lipomae we also investigated chromosomal segment 5q21.1-5q22.2 in the father. Furthermore, to rule out an inversion on the father's chromosome 5q, which could be responsible for the interstitial deletion of the proband, high resolution banding as well as FISH-analysis was performed. No chromosomal abnormalities were found (data not shown).

The case described by Raedle et al. (2001) showed many characteristics similar to those of our patient. The deletion (5q21.3-q22.1) included the *APC* gene, and a coloscopy revealed multiple colonic polyps. He also shows mild mental retardation and some phenotypic anomalies in common with our patient (see Table 2). However, the patient described by Raedle et al. (2001) was not affected by schizophrenia or other major psychosis.

Garcia-Minaur et al. (2005) concluded that there is a general phenotype for proximal deletions that encompasses the q15-q22 region (Lindgren et al. 1992). Individuals with distal deletions encompassing the q22-q31 region also share a consistent phenotype (Garcia-Minaur et al. 2005; Felding and Kristoffersson 1980; Lindgren et al. 1992). A list of the clinical symptoms of the four patients with regard to phenotype (type 5q15-q22 and type 5q22-q31) is presented in Table 2. The deletion in our patient and in the patient described by Raedle et al. (2001) overlap in both regions. Although the phenotypes of the patients vary, most characteristics of the phenotype of patient SL conform to the second type. Patients described by Bennett et al. (1997) and Garcia-Minaur et al. (2005) can also clearly be classified as phenotype type 5q22-q31.

Herrera et al. (1986) and Hockey et al. (1989) also described mentally retarded individuals with 5q deletions, multiple developmental abnormalities and familial adenomatous polyposis. De Michelena et al. (1990) presented a patient with a reciprocal translocation (1;11)(p22;q21) and a large interstitial deletion 5q15-q31, who also showed multiple congenital abnormalities and developmental delay. However, the deleted segment in SL is much smaller. This could indicate that genes responsible for normal mental function are included in the region.

A more detailed phenotype description and molecular characterisation of further patients with interstitial 5q deletions could help to define potential 5q deletion syndromes.

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