Interstitial Deletion of 5q22.2q23.1 Including *APC* and *TSSK1B* in a Patient with Adenomatous Polyposis and Asthenoteratozoospermia

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Established Facts

- Interstitial 5q22 deletions are rare and typically accompanied by neurological and developmental complications.
- Most genes within the chromosomal band 5q22 have not been extensively studied nor associated with a clinical phenotype.

Novel Insights

- We present a case with a heterozygous deletion in the 5q22.2q23.1 locus that does not exhibit the developmental issues that typically arise from this type of deletion.
- The *TSSK1B* gene within this locus has recently been functionally tested in model organisms; here we can see the first report of haploinsufficiency of this gene potentially causing a human phenotype.
- *TSSK1B* can be a putative cause for male factor infertility.

Keywords

Array CGH · Asthenoteratozoospermia · Familial adenomatous polyposis · *TSSK1B*

Abstract

Interstitial 5q22 deletions are relatively rare and usually represented by severe clinical features such as developmental delay and growth retardation. Here, we report a 23-year-old male patient, referred to our laboratory for genetic confirmation of possible familial adenomatous polyposis. MLPA and the subsequent array CGH identified an approximately

8-Mb-sized deletion in the 5q22.2q23.1 locus. Further analysis of the deleted region and the genes within suggested a possible role for the *TSSK1B* (testis-specific serine/threonine kinase 1) gene in the patient's reproductive capacity. Semen analysis confirmed that the patient's reproductive capability was impaired, and that he suffered from asthenoteratozoospermia. Analysis of the azoospermia factor region on the Y chromosome revealed no microdeletions. Further sequencing tests could not find an alternative explanation for the patient's infertility. This case demonstrates a possible role of *TSSK1B* in male reproduction.

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Interstitial 5q22q23 deletions are relatively rare and usually accompanied by severe clinical features such as developmental delay, growth retardation, and dysmorphic features [Bennett et al., 1997]. Deletions in this region often involve the *APC* tumor suppressor gene (OMIM 611731) and lead to familial adenomatous polyposis (FAP). FAP is an inherited autosomal dominant condition leading to multiple intestinal polyps and predisposition to colon cancer. Although the first reported 5q interstitial deletion was in 1979 [Kadotani et al., 1979], the *APC* locus was discovered later by Kinzler et al. [1991], who mapped it to the 5q22.2 band. Because of the timing of this discovery, all the clinical cases of 5q deletions up to that moment did not examine the manifestation of FAP in the patient, and therefore do not contribute any information about the link between 5q22 deletions and FAP. Patients with chromosomal deletions that include *APC* are most often diagnosed when their dysmorphic features are revealed in infancy or when they fail to thrive and show other signs of delayed development [Lee et al., 2012; Yamaguchi et al., 2014]; those that remain asymptomatic usually experience gastrointestinal manifestations such as weight loss, rectorrhagia and bowel obstruction.

Because of the thorough characterization of the molecular basis for FAP and the severity of its pathological symptoms, clinical focus is most often concentrated on the *APC* gene itself or physical consequences of a large chromosomal deletion, revealing little to no information about the function of other genes in the 5q22.2 band. Although a number of chromosomal aberrations have been described [Bennett et al., 1997; Tzschach et al., 2006; Heald et al., 2007; Casper et al., 2014], analysis of the consequences tends to focus on the resulting dysmorphic features or FAP without imparting function to single genes. Large deletions within the chromosome itself present with a complex phenotype, combining several clinical features from those mentioned above and can be a challenge for the diagnosing clinician.

Case Report and Methods

The patient is a 23-year-old Bulgarian male without prior clinical history. Upon his first admission, he was complaining of frequent rectorrhagia with no abnormalities in defecation frequency or loss of appetite. A sigmoidoscopy was performed with results pointing towards FAP, for which he was directed to a specialized gastroenterological department for further testing. Family history was unavailable due to ethical reasons, as the patient had been in a foster family since an early age, and there was no

Fig. 1. **A** Fibrocolonoscopy images showing different polyps. **B** The proband, a 23-year-old Bulgarian male, with no discernible dysmorphic features.

contact with his biological parents. Fibrocolonoscopy revealed a large number of polyps in the rectum and the sigmoid – pale to dark red – with hyperplasia (Fig. 1A). There were no FAP-associated lesions outside of the gastrointestinal tract. At this point, the patient was referred to our genetic laboratory to confirm the FAP diagnosis.

Genomic DNA was isolated from peripheral blood using standard protocols and was used to screen the *APC* gene for larger deletions and duplications using MLPA. The MLPA *APC* kit was obtained from MRC-Holland (PO43, Amsterdam, The Netherlands), and analysis was performed according to the manufacturer's instructions.

In order to determine the boundaries of the deletion, genomic DNA was analyzed by array CGH on a high-resolution 1M oligonucleotide array according to the manufacturer's protocol (Agilent Technologies, Santa Clara, CA, USA). Copy number variation (CNV) detection was conducted using CytoGenomics software (version 2.9.2.4, Agilent Technologies). The following analysis settings were used: aberration algorithm ADM-2, threshold: 6.0, window size: 0.5 Mb, filter: 5 probes, and $\log_2$ ratio = 0.29.

Ejaculate was detached in the laboratory and studied in the order: observation of native preparation, determining sperm concentration, assessment of swabs for vitality in mobility <40%, and evaluating sperm morphology according to the criteria of ESHRE/NAFA, using Sperm Class Analyzer (SCA), a system of quantitative and qualitative analysis of parameters of human sperm.

Molecular testing for microdeletions in the azoospermia factor region were performed as described in the guidelines and standards of the European Academy of Andrology [Krausz et al., 2014].

Sanger sequencing was performed to examine previously reported regions associated with male infertility, i.e., *DAZL* (OMIM 601486), *SEPT12* (OMIM 611562), *SLC26A8* (OMIM 608480), *SYCE1* (OMIM 611486), and subsequently, *TSSK1B* (OMIM 610709).

Fig. 2. Array-CGH analysis, genomic overview of the chromosomal region 5q21.1q23.3, and sperm defects observed in our patient. **A** Array-CGH profile of chromosome 5 shows a 5q interstitial deletion. The 8-Mb 5q22.2q23.1 deletion (indicated by the red bar) is shown in greater detail. **B** Disease-causing OMIM genes are depicted as green bars within the overview of the genomic region encompassing the cytobands 5q21.1 to 5q23.3. The genes *APC* and *TSSK1B* are highlighted in red. In the lower part, the 8-Mb deletion of our patient is compared with overlapping deletions of patients

reported in the literature (all positions according to GRCh37/hg19). Note the case from Yamaguchi et al. [2014] does not encompass the *TSSK1B* gene. The cases indicated in orange present with severe developmental delay and/or dysmorphic features. The asterisks mark cases analyzed by array CGH. **C** Spermatozoa with pointed heads, a round-headed sperm with a short tail, and decapitated sperm as well as microcephalic sperm and a sperm head without DNA content (arrows).

Results

We found a 50% peak area decrease in all *APC*-specific gene probes by MLPA. As this corresponded to a heterozygous deletion of the entire *APC* gene sequence, our next step was to perform an array CGH to determine the boundaries of the deletion. The obtained results showed that the interstitial deletion in chromosome 5q was confined to the region 5q22.2q23.1, approximately 8 Mb long (Fig. 2A). The deletion spans the region between base pairs chr5:111,779,298–119,794,955 (GRCh37/hg19), karyotype: arr[hg19] 5q22.2q23.1(111779298_119794955)×1 [ISCN 2016], confirming the *APC* deletion and involving 29 other protein-coding genes in the deletion. Along with the well-described *APC* and *MCC* genes, another gene, *TSSK1B*, had been previously studied and attributed with a specific function. *TSSK1B* is highly expressed in testis and required during spermatid development [Hao et al., 2004]. Therefore, we decided to perform a semen analysis and test our patient for infertility, sperm count, morphology, and motility.

The final evaluation concluded that the patient suffered from asthenoteratozoospermia with a prevalence of cephalic defects, 97% atypical sperm morphology, and reduced sperm motility (Fig. 2C). The teratozoospermia index value at 1.71 shows more than 1 defect per single sperm, pointing towards impaired spermatogenesis. Since this phenotype is most often caused by microdeletions in the azoospermia factor region and large chromosomal deletions within the Y chromosome were not shown on the array CGH, we performed molecular testing for possible microdeletions in the Y chromosome. Our analysis did not reveal any deletions within the azoospermia factor markers sY84, sY86, sY127, sY134, sY254, and sY255. Testing for the most common mutations in genes associated with male infertility such as *DAZL*, *SEPT12*, *SLC26A8*, and *SYCE1* revealed no alterations. Finally, sequencing the *TSSK1B* gene revealed no abnormalities within the single present copy.

These results narrowed down the possibility that *TSSK1B* is a strong candidate gene for male infertility and is involved in the asthenoteratozoospermia phenotype of our patient.

Discussion

Chromosomal deletions of the 5q22 band resulting in FAP have been rarely reported throughout the literature. Existing reports have aimed to compare and contrast de-

letions between different patient cases; however, the pathological implications of those deletions have not been investigated in detail beyond the initial clinical diagnosis [Lee et al., 2012; Yamaguchi et al., 2014]. The size of the 5q interstitial deleted region varies greatly among patients, ranging from 1.7 Mbp [Yamaguchi et al., 2014] to 20 Mbp [Casper et al., 2014] and larger, with the majority of the cases presenting with various degrees of learning disability, failure to thrive, and minor to major dysmorphic signs [Rivera et al., 1990; Pilarski et al., 1999]. These symptoms are not confined to a single chromosomal band (Fig. 2B), which has prevented the classification of 5q deletion subtypes, although several attempts have been made [Rivera et al., 1990; Lindgren et al., 1992; Lee et al., 2012]. Notable findings from these studies show a lack of a genotype-phenotype correlation in cases that share an affected region as well as no association between the size of the deletion and the severity of the phenotype.

To the best of our knowledge, the last review of genes and their functions within this region was conducted by Ofner et al. [2006]. So far, only 4 genes (*APC*, *MCC*, *TRIM36*, and *HSD17B4*) located within the deleted region in our patient have been associated with a disease in OMIM, although research is ongoing. Furthermore, the fertility status of most patients with a 5q deletion was never published because of their early age, and their condition was never followed past the initial diagnosis. In previously published reports of 5q deletions, only a single case [Torrezan et al., 2012] has documented a possible inheritance of a *TSSK1B* deletion from a suspected male carrier; however, the patient's father could not be tested to confirm that the deletion was inherited. Regarding *TSSK1B* encompassing CNVs in the normal population, there is only a single DGV entry of a copy loss (nsv599396, total sample size 17,421; PMID 21841781) [Cooper et al., 2011].

In the patient presented here, we did not observe severe pathological symptoms that are exhibited in typical 5q22 deletion cases besides FAP. This can be explained by the possibility that the deletion we present does not completely overlap with deletions in patients with severe dysmorphic/developmental issues (Fig. 2B). While the common clinical phenotype for patients with deletions in the long arm of chromosome 5 covers a wide array of features such as developmental delay, failure to thrive, psychomotor retardation, flat nasal bridge, and others [Lee et al., 2012], the phenotype presented by our patient is represented almost exclusively by the heterozygous deletion of the *APC* gene and the consequent FAP diagnosis.

The genetic background of testicular infertility factors, specifically asthenozoospermia, has long been limited and poorly described. The majority of testicular infertility factors and their genetic background have been linked to genes residing in the Y chromosome and chromosomal aberrations involving the Y chromosome [O'Flynn O'Brien et al., 2010]. There has been progress in the field, as recent research has aimed to relate sperm dysmorphology phenotypes to specific genes, such as *DAZL*, *SYCE1*, *SLC26A8*, *SEPT12*, and others that have a function related to spermatogenesis [Ray et al., 2017]. Even so, the field of male infertility genetics is still relatively new, and novel contributions are crucial to the ongoing improvement of genetic testing, diagnosis, and counseling [Lamb et al., 2016].

Testis-specific serine/threonine kinase 1 is an intronless gene, part of the TSSK family, and a branch of the serine/threonine kinase superfamily. It was first identified by Bielke et al. [1994], who in their study attributed to the gene an open reading frame of 1092 bp coding for a 364-aa protein. Human *TSSK1B* was investigated using Northern and dot blots, which showed 85% similarity to murine *Tssk1b*. The gene was mapped on chromosome 5q22, in a region that shows no synteny to the mouse chromosomal locus of *Tssk1b* [Hao et al., 2004]. Further studies have pointed out that the specific expression of *Tssk1b* is localized almost exclusively in the testes, which implicated a function related to reproduction and spermatogenesis [Hao et al., 2004]. Temporal expression studies further demonstrated that *Tssk1b* is expressed exclusively in the late stages of sperm maturation [Kueng et al., 1997]. The function of *Tssk1b* was confirmed in vivo by Xu et al. [2008] using chimeric mice to determine if a targeted deletion in *Tssk1b* and *Tssk2* can be transmitted in the germline. Their experiments showed that the chimeric mice only produced wild-type offspring, even though sperm cells contained both the wild-type and the mutated allele. Further examination of the testis and epididymis in the chimera showed that only a low number of sperm could reach maturation [Xu et al., 2008]. In their study, Xu et al. [2008] suggested that the infertility is caused by haploinsufficiency. Although the exact mechanism of the pathological manifestation has not been studied, the functional implications of an allele deletion in this gene would seem to result in altered sperm morphology. In humans, a microdeletion encompassing *TSSK1B* may thus constitute a risk factor for male infertility.

So far, *TSSK1B* has been exclusively studied in animal models, and the functional implications have not been explored in humans. Here, we present what we consider

the first case of correlation between a deleted copy of *TSSK1B* and asthenozoospermia, possibly confirming the link between gene and function in vivo.

Conclusion

This is one of the few cases describing a young adult with a large interstitial deletion in chromosome 5, with symptoms manifesting at an age when the patient can be tested for fertility. To the best of our knowledge, no patients with deletions in the 5q22.2 band have been examined for infertility, and only one case of a possible, yet unconfirmed inheritance of a *TSSK1B* deletion from a male carrier has been observed. Even the mildest developmental alterations observed in addition to the FAP phenotype should lead to further investigation of this chromosomal region beyond the *APC* gene, using the methods listed above to define any chromosomal alterations. We would also like to encourage the further study of the role of *TSSK1B* in male reproduction and its part in causing asthenozoospermia.

In addition, we demonstrate the usefulness of both MLPA and array CGH in clinical practice. MLPA might be considered as the first-line analysis, and if a deletion of the entire target gene is found, the results could be confirmed and the breakpoints localized by array CGH.

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Statement of Ethics

The patient signed informed consent forms for genetic testing and medical photography. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflicts of interest.

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