Original Article

# Examination of Y-Chromosomal Microdeletions and Partial Microdeletions in Idiopathic Infertility in East Hungarian Patients

*Attila Mokánszki, Anikó Ujfalusi, Éva Gombos, István Balogh*

Department of Laboratory Medicine, Division of Clinical Genetics, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

**Abstract**

**Purpose:** The aim of this study was to establish the Y chromosome microdeletion and partial AZFc microdeletion/duplication frequency firstly in East Hungarian population and to gain information about the molecular mechanism of the heterogeneous phenotype identified in males bearing partial AZFc deletions and duplications. **Materials and Methods:** Exactly determined sequences of azoospermia factor (AZF) region were amplified. Lack of amplification was detected for deletion. To determine the copy number of *DAZ* and *CDY1* genes, we performed a quantitative analysis. The primers flank an insertion/deletion difference, which permitted the polymerase chain reaction products to be separated by polyacrylamide gel electrophoresis. **Statistical Analysis Used:** Mann–Whitney/Wilcoxon two-sample test, Kruskal–Wallis test, and two-sample t-probe were used for statistical analysis. **Results:** AZFbc deletion was detected only in the azoospermic cases; AZFc deletion occurred significantly more frequently among azoospermic patients, than among oligozoospermic males. The frequency of gr/gr deletions was significantly higher in the oligozoospermic patients than in the normospermic group. The b2/b3 deletion and partial duplications were not different among our groups, while b1/b3 deletion was found only in the azoospermic group. In infertile males and in normozoospermic controls, similar Y haplogroup distribution was detected with the highest frequency of haplogroup P. The gr/gr deletion with *P* haplogroup was more frequent in the oligozoospermic group than in the normozoospermic males. The b2/b3 deletion with E haplogroup was the most frequent, found only in the normozoospermic group. **Conclusions:** Y microdeletion screening has prognostic value and can affect the clinical therapy. In case of Y chromosome molecular genetic aberrations, genetic counseling makes sense also for other males in the family because these types of aberrations are transmittable (from father to son 100% transmission).

**Keywords:** *Azoospermia factor region, DAZ and CDY1 genes, gr/gr deletion, Y chromosome haplotype, Y chromosome microdeletion/partial microdeletion*

## Introduction

One of the most frequent molecular genetic causes of spermatogenetic impairment in infertile males is regarded to be Y chromosome microdeletions. Higher incidence of microdeletions is observed in azoospermic men than in oligozoospermic men and so deletion frequency variation from 2% to 10% is typical, which reflects the makeup of the study population.[1-3] Clinically significant microdeletions occur in specific location of the Y chromosome long arm (azoospermia factor [AZF] regions). Complete AZF deletions originate

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.JHRS_12_18

***Address for correspondence:*** Dr. Attila Mokánszki, Department of Laboratory Medicine, Division of Clinical Genetics, Faculty of Medicine, University of Debrecen, Nagyerdei Krt. 98, Debrecen, H-4032, Hungary.
E-mail: mokanszki.attila@med.unideb.hu



**How to cite this article:** Mokánszki A, Ujfalusi A, Gombos É, Balogh I. Examination of Y-chromosomal microdeletions and partial microdeletions in idiopathic infertility in East Hungarian patients. J Hum Reprod Sci 2018;11:329-36.

from nonallelic homologous recombination. This takes place between highly homologous repeated sequences with equal orientation, and it leads to loss of the genetic material.[4-6]

The length of AZFa region is 1100 kb, in which the single-copy USP9Y and DDX3Y genes are contained. During the complete deletion of AZFa, 792 kb is removed.[7] There are 24 genes in AZFb and AZFc region, most of them are in multiple copies. Complete deletion of AZFb (6.2Mb, including 32 copies of genes) is caused by homologous recombination between the palindromes P5/proximal P1.[8] In AZFc region, there are 12 genes in a variable number of copies, making altogether 32 repeats.[6]

It was *DAZ* (deleted in azoospermia) the first AZFc examined gene that is expressed in the testis.[9] The *DAZ* gene sequences have been derived from chromosome 3 (*DAZL1* transposition) and it is distinguishable in four copies (*DAZ1*, *DAZ2*, *DAZ3,* and *DAZ4*). The *DAZ* gene family encodes testis-specific RNA-binding proteins, which are likely to be involved in the translational control of other germ line genes.[10] *CDY1* is the other significant AZFc testis-specific gene, present in two copies (*CDY1a* and *CDY1b*) and evolutionary originated from the *CDYL* gene on chromosome 6 (retrotransposition to Y chromosome).[11] CDY proteins are histone acetyltransferases playing a significant role in spermatid maturation.[12]

The most frequent deletion of the Y chromosome is AZFc, removing 3.5 Mb, including 21 copies of genes (homologous recombination between amplicons b2 and b4 in palindromes P3 and P1).[4] Codeletion of AZFb and AZFc involves homologous recombination between P5 and distal P1 (7.7 Mb, 42 copies removed) or between P4 and distal P1 (7.0 Mb, 38 copies removed).[8] The most frequent deletion type observed is the AZFc (~80%) followed by AZFb (1%–5%), AZFa (0.5%–4%), and AZFbc (1%–3%).[7]

AZFc sequence contains a total of 13 different ampliconic units.[4] This organization is functionally relevant because the amplicons accommodate genes needed for spermatogenesis. If gene dosage is varied, changes in amplicon copy number can lead to ultimate phenotypical modifications in the spermatogenesis. Although several partial AZFc deletions have been described, only one of them is of potential clinical interest: the gr/gr deletion.[6] Its clinical significance is widely disputed in spite of the fact that it removes half of the AZFc gene content. The reason for this is that carriers may exhibit highly variable spermatogenic phenotypes, from azoospermia to normozoospermia. The ethnic and geographic origin of the study population influences the effect of the deletion. The transmission to the male offspring of gr/gr deletion will happen in every case. In the next generation, the partial deletion expand to a complete AZFc deletion, but yet no sufficient data have been available to make final conclusions concerning this specific risk.[13] No effect on fertility status was observed in cases with b2/b3,[14] u3-gr/gr,[15] or g1/g3[16] deletion, removing a similar quantity of AZFc genes, in association with Y chromosome haplogroup N commonly present in northern Eurasian populations.

Considering the Y chromosome DNA sequences, some other possible partial deletions have been proposed in both the AZFb and AZFc regions.[5,17] More studies are needed to examine the frequency and the pathological significance of these partial deletions. Homologous recombination between AZFc amplicons can get generated, causing not only partial AZFc deletions, but also partial AZFc duplications.

The aim of this study was: (a) to establish the Y chromosome microdeletion and partial AZFc microdeletion/duplication frequency in East Hungarian population and (b) gain information about the molecular mechanism of the heterogeneous phenotype identified in males bearing partial AZFc deletions and duplications.

## Materials and Methods

### Patients

The study population included 347 infertile patients (101 nonobstructive azoospermic; age range: 24–39 years and 246 oligozoospermic males; age range: 22–41 years) and 111 normozoospermic control males (age range: 21–40 years) of East Hungarian origin. Cytogenetic analysis revealed 46, XY karyotype in all the examined males. Semen analysis was performed according to the WHO guidelines, and morphology was examined using strict criteria.[18] The sperm concentration of oligozoospermic patients was up to 15 × $10^6$/mL (range: 1–15) along with the above 32% progressive motility (range: 35–80). The normozoospermic controls have normal sperm parameters according to the WHO criterion (sperm concentration range: 45–164 × $10^6$/mL and progressive motility range 45%–87%).[18]

Prior to the study, all patients were given detailed information about the aim and method of investigation and their consents were obtained. All protocols have been approved by the author's respective Institutional Review Board for human subjects (IRB reference number: 2976/2012-EHR).

### DNA isolation

DNA isolations were performed from EDTA anticoagulated blood. Genomic DNA was isolated using QiaAmp DNA mini kit (Qiagen, Hilden) according to the manufacturer's protocols (Cat. No./ID: 51306).

### Y chromosome mirodeletion analysis

Exactly determined sequences of AZF region-sequence tagged site (STS) were amplified. These assays were performed by STS primers specific for the three AZF regions: two primer pairs were used in single polymerase chain reaction (PCR) for every region: in AZFa region: SY84 and sY86; in AZFb region: SY127 and sY134; and in AZFc region: SY254 and SY255 (both in *DAZ* gene). Lack of amplification was detected for deletion.[7]

DNA samples of males with normal spermatogenesis were applied for positive control, whereas DNA samples of females were applied for negative control. Reagent contaminations were verified with no-template control. *ZFX*/*ZFY* genes were used for internal control because it was present in females and males as well. The presence of chromosome Y was proved by the study of *SRY* gene on the short arm of chromosome Y.

For the determination of the extension of the AZFb (P5/proximal P1) breakpoint, we used the following STS primers: SY105 (present) and sY121 (absent) for the proximal border and sY143 (absent) and sY153 (present) for the distal border. The analysis of sY160 determines the deletion of b2/b4 (AZFc).

### Screening for partial AZFc deletions

Gr/gr type of deletion was defined, when sY1291 was deleted, sY1161, sY1191, sY1201 and sY1206 were presented. B2/b3 deletion means deletion of sY1191 and amplification of sY1161, sY1201, sY1206, and sY1291. In case of b1/b3 deletion, sY1161, sY1191, and sY1291 were deleted and sY1201 and sY1206 were present.[6]

### *DAZ* and *CDY1* copy number determination

To determine the copy number of *DAZ* and *CDY1* genes, we performed a quantitative analysis, according to a previously reported method.[15,19] The *DAZ* dosage method consists of the simultaneous amplification of a fragment of intron 10 from AZFc *DAZ* copies and from its homolog *DAZL*, using a single primer pair (o1130/o1313). This intron is present in one copy per *DAZ* or *DAZL* gene (the number of *DAZ* copies is four, whereas there are two copies of *DAZL* in a normal 46, XY male). *DAZL* was an internal standard with a known number of copies. The primers flank an insertion/deletion difference, which permitted the PCR products to be separated by polyacrylamide gel electrophoresis. One of the primers (o1130) was labeled at its 5' end with FAM. The quantitative analysis for *CDY1* copies was analogous to *DAZ*. The PCR products were separated on an 310-Avant Genetic Analyzer (Applied Biosystems), and quantification was performed by Peak Scanner Software v1.0 (Thermo Fisher Scientific). Quantification was performed comparing the peak area corresponding to the *DAZ* locus and to its homolog *DAZL* and *CDY1* to *CDY2* as well.

### *DAZ* and *CDY1* copy type determination

Qualitative analysis for *DAZ* and *CDY1* was performed according to Machev *et al*.[15] We amplified the sequence family variant (SFV) at STS sY587 in intron 10, which distinguishes DAZ1/2 from DAZ3/4. For CDY1, we used a C/A SFV situated at 7750 bp 5′ of the CDY1 translation start codon (CDY7750), which distinguishes CDY1a from CDY1b.

### Y chromosome haplotyping

Y chromosome haplotyping was performed as previously published.[20] All men were haplotyped for YAP, 12f2, and 92R7 polymorphisms, and individuals with partial AZF deletions and duplications were further genotyped.[21]

### Statistical analysis

Statistical analysis were performed with commercial software SigmaStat (Systat Software Inc., San Jose, CA, USA) and SPSS (SPSS Inc., IBM, Chicago, DE, USA). We analyzed the normality of samples using Shapiro–Wilk test, and the samples' homogeneity was analyzed using Bartlett test. We tested the differences of the Y microdeletion and AZFc partial deletion frequency between our infertile and control populations using Mann–Whitney/Wilcoxon two-sample test, Kruskal–Wallis test (when normality does not exist), and two-sample t-probe (when normality exists). $P < 0.05$ was considered as statistically significant difference.

## Results

### Chromosome Y microdeletions

Chromosome Y microdeletions were found in 10 cases out of the 101 azoospermic male patients (9.9%), AZFbc in 4 patients (3.99%), and AZFc deletion in 6 patients (5.94%). Among the 246 oligozoospermic males, two full AZFc microdeletions were detected (0.8%), but microdeletions did occur among normozoospermic control men. AZFbc deletion was detected only in the azoospermic cases; AZFc deletion occurred significantly more frequently among azoospermic patients, than among oligozoospermic males ($P < 0.01$) [Figure 1].

### AZFc deletion frequency

We found the following partial AZFc deletions [Figure 1]: gr/gr (17/458, 3.7%), b2/b3 (12/458, 2.62%), and

b1/b3 (2/458, 0.44%). The gr/gr and b2/b3 deletions were found in both the infertile and normospermic groups, although at different frequencies. The frequency of gr/gr deletions was significantly higher in the oligozoospermic patients (13/246, 5.3%) than in the normospermic group (2/111; 1.8%) ($P < 0.01$). There were no significant differences between azoospermic (2/101, 1.98%) and normozoospermic male gr/gr deletion frequencies. In contrast, the frequency of gr/gr deletion was significantly higher in oligozoospermic group than in azoospermic group ($P < 0.05$). The b2/b3 deletion was not different among our groups: azoospermic: 3/101 (2.97%), oligozoospermic: 6/246 (2.44%), and normozoospermic males: 3/111 (2.7%). B1/b3 deletion was found only in the azoospermic group 2/101 (1.98%).

The gr/gr and b2/b3 deletions can be detected with a wide range of sperm count, from azoospermia to normozoospermia, but gr/gr deletion was observed only among males with decreased sperm concentration (<25 × $10^6$/mL). We found no association between sperm concentration, motility, and morphology of carrier patients and males without deletion. The b1/b3 deletion was specific for azoospermia in our study population.

## AZFc partial duplications

Based on the *DAZ* and *CDY1* gene content, it was possible to screen partial duplications of AZFc region, which is characterized by a higher number of both *DAZ* and *CDY1* gene copies than the reference AZFc sequence. We found six copies of *DAZ* and three copies of *CDY1*, indicating a partial duplication of the region containing both gene families. The frequency of partial duplications in our study population was 2.4% (11/458). Their frequency was 2.97% (3/101) in azoospermic, 1.63% (4/246) in oligozoospermic, and 3.6% (4/111) in normozoospermic males. There were no significant differences among the studied groups. It was not significantly different between individuals with and without partial AZFc duplication, neither among males with decreased sperm count nor among controls.

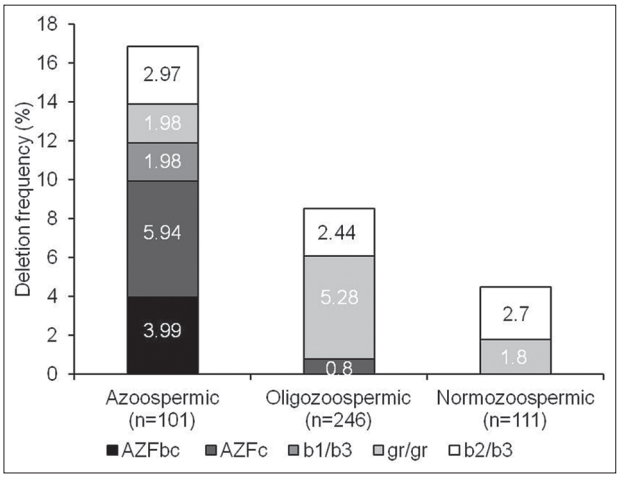


**Figure 1:** Chromosome Y microdeletion and AZFc partial deletion frequency in azoospermic, oligozoospermic, and normozoospermic patients. AZFbc and AZFc microdeletion was observed in azoospermic and severe oligozoospermic men, b1/b3 deletion was detected in only azoospermic males, gr/gr deletion was significantly higher in oligozoospermic males than in the other two groups, while b2/b3 deletion frequency was similar in the three groups

## *DAZ* and *CDY1* gene copy variations

Characterizing the AZFc deletions, we defined the type of missing DAZ (DAZ1/DAZ2/DAZ3/DAZ4) and CDY1 (CDY1a/CDY1b) gene copies [Figure 2]. In the gr/gr deletion group, we found three types of deletion pattern: DAZ1/DAZ2+CDY1a ($n$ = 6, 35.3%), DAZ1/DAZ2+CDY1b ($n$ = 7, 41.2%), and DAZ3/DAZ4+CDY1b ($n$ = 3, 17.65%). In the b2/b3 deletion group, we found two types of deletion pattern: DAZ1/DAZ2+CDY1a ($n$ = 6, 50%) and DAZ3/DAZ4+CDY1b ($n$ = 6, 50%). In the two cases with b1/b3 deletion, there was *DAZ1/DAZ2* deletion pattern with *CDY* copies present. In all AZFc deletion cases, we found half copy number of *DAZ* and *CDY1*, but in one case, gr/gr pattern with normal *DAZ* and *CDY1* dosage (deletion followed by duplication) was detected.

## Y chromosome haplogroups

In infertile (azoospermic and oligozoospermic) males and in normozoospermic controls, similar Y haplogroup distribution was detected with the highest frequency of haplogroup P (>40%). The gr/gr deleted patients' Y chromosome haplogroups are the following: P ($n$ = 9, 53%), E ($n$ = 3, 17.65%), J ($n$ = 3, 17.65%), and K ($n$ = 2, 11.76%). The gr/gr deletion with P haplogroup was more frequent in the oligozoospermic

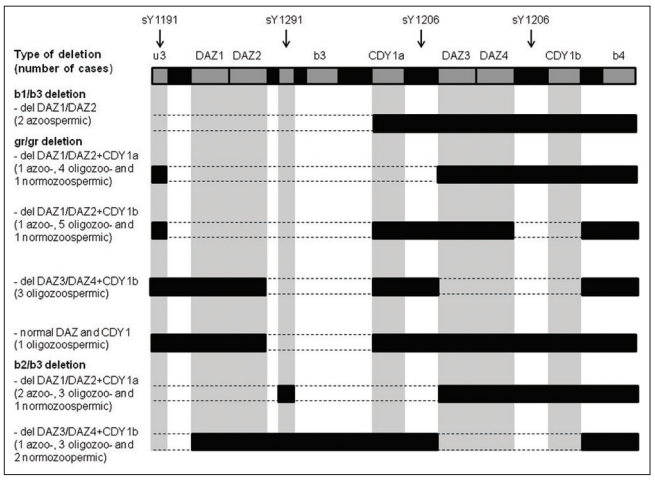


**Figure 2:** Schematic representation of the seven deletion patterns (based on the type of *DAZ* and *CDY* copies deleted) found in the AZFc deleted patients. Distribution of deletion (number of cases) in azoospermic, oligozoospermic, and normozoospermic patients is shown in parentheses. Filled boxes indicate the presence of a given marker or gene

group than in the normozoospermic males, but the difference was not significant. The b2/b3 deleted patients' Y chromosome haplogroup distribution was different as follows: E ($n$ = 5, 41.7%), P ($n$ = 4, 33.3%), K ($n$ = 2, 16.7%), and J ($n$ = 1, 8.3%). The b2/b3 deletion with E haplogroup was the most frequent, found only in the normozoospermic group. The b1/b3 deletions were associated with *P* haplotype in our azoospermic patients.

## Discussion

The phenotypic incidence of AZFa deletion ranges from Sertoli cell-only syndrome (SCOS) to azoospermia.[22-26] In that cases, it is impossible to retrieve testicular spermatozoa for intracytoplasmic sperm injection. Complete deletions of AZFb and AZFbc (P5/proximal P1, P5/distal P1, and P4/distal P1) cause SCOS or spermatogenetic failure, resulting in azoospermia. Several reports have confirmed that similar to the deletions of AZFa region, no spermatozoa are found during testicular sperm extraction (TESE) in these patients.[23,25,27] Deletions of the AZFc region (b2/b4) are associated with various clinical phenotypes.[28-30] In men with AZFc deletion causing azoospermia, there is 50% chance to find spermatozoa from TESE.[2,3,30-32]

According to the screening of several thousand patients, clinically relevant Y chromosomal microdeletions were diagnosed only in azoospermic or severe oligozoospermic men with low sperm concentrations (<2 × $10^6$/mL). Some authors found deletions causing sperm concentration between 2 and 5 × $10^6$/mL,[3,33] but not in normozoospermic and proven fertile males. Similar frequency was observed in our study population: AZFbc and AZFc deletions were verified only in azoospermic and severe oligozoospermic men.

Patients with azoospermia before TESE should be recommended deletion screening because TESE is unnecessary in cases with complete deletion of the AZFa. TESE in azoospermic carriers of deletions of the AZFb or AZFbc is suggested. However, retrieving spermatozoa from these patients has very low chance. Consequently, Y microdeletion screening has prognostic value and can affect the clinical therapy.[7]

Unlike for the complete AZFc deletion, genotype–phenotype correlations for the partial AZFc deletion are more confused. Gr/gr and b2/b3 deletion types were verified in the normospermic males, indicating that these deletions are not specific for spermatogenic impairment. Gr/gr deletion carriers have seven times higher chance for oligozoospermia than males without deletions.[7] The frequency of gr/gr deletions was significantly higher in our oligozoospermic group than in control males, suggesting that this polymorphism on spermatogenic output can have a harmful effect. However, the sperm concentration, motility, and morphology were not significantly different in the oligozoospermic group between men with gr/gr deletions and those without. Several studies have found relationship between sperm concentration and gr/gr deletion frequencies;[6,19,34-36] others found no such relationship.[13,15,37,38] The similar frequency of the b2/b3 deletion in patients and normozoospermia does not prove the pathogenic role for this deletion; however, it has been demonstrated that it occurs in higher frequency in the general population of northern part of Europe with haplogroup N.[14,15] In contrast, the b2/b3 deletion with E haplogroup was the most frequent in our study population, found only in the normozoospermic group.

Y haplogroups represent very specific patterns of geographical distribution, allowing the determination of male populations based on haplogroup composition.[39,40] Gr/gr deletions have been demonstrated in the fixed haplogroups D2 and Q1 and b2/b3 deletions in haplogroup N.[6,13,14,16,41,42] In contrast, in our study population, the most frequent haplogroup with gr/gr deletion was *P* haplogroup. Since several populations show the significance of Y origin, deletions are considered a deleterious event on fertility, but it can neutralize some specific compensatory factors. The demonstration of an association between partial AZFc deletions and spermatogenic defects in a given population depends on its haplogroup composition. Some haplogroups seem to be able to compensate the degree of spermatogenic disruption in connection with partial deletions. Among gr/gr deleted Chinese men with spermatogenic failure, there are more represented haplogroups C and DE than in equally deleted normozoospermic males.[43] No b2/b3 deletions associated with this effect were detected, and no replication occurred in a different regional context. No evidence for distinct haplogroup distribution was found analyzing gr/gr deleted men from European centers, who had different sperm parameters.[44] Partial deletions affecting the proximal AZFc domain are particularly likely to occur in haplogroups C and G.[42] An analogous effect was observed in the North Italian male population, where deleted men showed an overrepresentation of haplogroup E.[45] In contrast, it has been suggested that the haplogroups O3, J, and R can offer some protection.[42,43,45]

There are too few publications about the genotype–phenotype correlation of partial AZFc duplications. It is generally accepted that neither partial nor complete AZFc duplications pose any risk for spermatogenic failure since the homeostatic mechanisms

of spermatogenesis can offer some compensation, and gene dosage increases are not balanced.[19,46,47] Men with AZFc duplications did not show qualitative and quantitative increased spermatogenic production.[19,44]

It was demonstrated that certain deletion motifs may be more pathogenic than others, namely a significantly higher frequency of *CDY1a* copy deletion and *DAZ1*/*DAZ2* deletion, which were observed in the oligozoospermic patients than in the normozoospermic group.[21] In our study, besides the *DAZ1*/*DAZ2*+*CDY1a* deletion, the *DAZ1*/*DAZ2*+*CDY1b* was more frequent in oligozoospermic group than in other groups. A similar distribution of gr/gr deletions removing the *DAZ1*/*DAZ2* copies was published in infertile men of Caucasian origin.[19,35,48]

In the case of partial AZFa or AZFb and AZFc deletion, the genetic counseling (with Y chromosome deletion screening) makes sense also for other males in the family. According to the literature, this type of deletion is transmittable.[49-51] Turner's syndrome is one of the most potential risks in the offspring as well as other genetic disorders of sex chromosome mosaicism such as ambiguous genitalia phenotype. There is a suggestion that in men with Y microdeletions[52,53] and in patients carrying a mosaic 46, XY/45, X karyotype with sexual ambiguity or Turner stigmata,[54] some AZF deletions are connected with an overall instable Y chromosome, which can create 45, X cell lines.

## Conclusions

The Y chromosome harbors a number of genes essential for testis development and function. Genetic tests include karyotype analysis on blood lymphocytes and assessment of microdeletions in the long arm of chromosome Y (Yq), also called AZF deletions. We recommend assessment of Yq microdeletions of infertile men with a sperm concentration $\leq 5 \times 10^6$/mL, which will be inherited to male offspring. Testing for this deletion has both diagnostic and prognostic values for testicular sperm retrieval in azoospermic men.

### Financial support and sponsorship

This research was supported by the Hungarian Academy of Sciences Postdoctoral Scholarship.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE, *et al.* Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: Microdeletions are specific for spermatogenic failure. J Clin Endocrinol Metab 2001;86:2638-42.
2. Simoni M, Tüttelmann F, Gromoll J, Nieschlag E. Clinical consequences of microdeletions of the Y chromosome: The extended Münster experience. Reprod Biomed Online 2008;16:289-303.
3. Lo Giacco D, Chianese C, Sánchez-Curbelo J, Bassas L, Ruiz P, Rajmil O, *et al.* Clinical relevance of Y-linked CNV screening in male infertility: New insights based on the 8-year experience of a diagnostic genetic laboratory. Eur J Hum Genet 2014;22:754-61.
4. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, *et al.* The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001;29:279-86.
5. Yen P. The fragility of fertility. Nat Genet 2001;29:243-4.
6. Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, *et al.* Polymorphism for a 1.6-mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 2003;35:247-51.
7. Krausz C, Hoefsloot L, Simoni M, Tüttelmann F; European Academy of Andrology, European Molecular Genetics Quality Network, *et al.* EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: State-of-the-art 2013. Andrology 2014;2:5-19.
8. Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, *et al.* Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 2002;71:906-22.
9. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, *et al.* Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995;10:383-93.
10. Yen PH. Putative biological functions of the DAZ family. Int J Androl 2004;27:125-9.
11. Lahn BT, Page DC. Retroposition of autosomal mRNA yielded testis-specific gene family on human Y chromosome. Nat Genet 1999;21:429-33.
12. Lahn BT, Tang ZL, Zhou J, Barndt RJ, Parvinen M, Allis CD, *et al.* Previously uncharacterized histone acetyltransferases implicated in mammalian spermatogenesis. Proc Natl Acad Sci U S A 2002;99:8707-12.
13. Zhang F, Lu C, Li Z, Xie P, Xia Y, Zhu X, *et al.* Partial deletions are associated with an increased risk of complete deletion in AZFc: A new insight into the role of partial AZFc deletions in male infertility. J Med Genet 2007;44:437-44.
14. Repping S, van Daalen SK, Korver CM, Brown LG, Marszalek JD, Gianotten J, *et al.* A family of human Y chromosomes has dispersed throughout Northern Eurasia despite a 1.8-mb deletion in the azoospermia factor c region. Genomics 2004;83:1046-52.
15. Machev N, Saut N, Longepied G, Terriou P, Navarro A, Levy N, *et al.* Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. J Med Genet 2004;41:814-25.
16. Fernandes S, Paracchini S, Meyer LH, Floridia G, Tyler-Smith C, Vogt PH, *et al.* A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men in Y haplogroup N. Am J Hum Genet 2004;74:180-7.
17. Vogt PH. Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. Mol Cell Endocrinol 2004;224:1-9.
18. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva,

CH: World Health Organization; 2010.
19. Giachini C, Laface I, Guarducci E, Balercia G, Forti G, Krausz C, *et al.* Partial AZFc deletions and duplications: Clinical correlates in the Italian population. Hum Genet 2008;124:399-410.
20. Rosser ZH, Zerjal T, Hurles ME, Adojaan M, Alavantic D, Amorim A, *et al.* Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. Am J Hum Genet 2000;67:1526-43.
21. Giachini C, Guarducci E, Longepied G, Degl'Innocenti S, Becherini L, Forti G, *et al.* The gr/gr deletion(s): A new genetic test in male infertility? J Med Genet 2005;42:497-502.
22. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, *et al.* Human Y chromosome azoospermia factors (AZF) mapped to different subregions in yq11. Hum Mol Genet 1996;5:933-43.
23. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: What is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod 2000;15:1431-4.
24. Kamp C, Huellen K, Fernandes S, Sousa M, Schlegel PN, Mielnik A, *et al.* High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. Mol Hum Reprod 2001;7:987-94.
25. Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN, *et al.* Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 2003;18:1660-5.
26. Kleiman SE, Almog R, Yogev L, Hauser R, Lehavi O, Paz G, *et al.* Screening for partial AZFa microdeletions in the Y chromosome of infertile men: Is it of clinical relevance? Fertil Steril 2012;98:43-7.
27. Kleiman SE, Yogev L, Lehavi O, Hauser R, Botchan A, Paz G, *et al.* The likelihood of finding mature sperm cells in men with AZFb or AZFb-c deletions: Six new cases and a review of the literature (1994-2010). Fertil Steril 2011;95:2005-12, 2012.e1-4.
28. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996;347:1290-3.
29. Luetjens CM, Gromoll J, Engelhardt M, Von Eckardstein S, Bergmann M, Nieschlag E, *et al.* Manifestation of Y-chromosomal deletions in the human testis: A morphometrical and immunohistochemical evaluation. Hum Reprod 2002;17:2258-66.
30. Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod 2002;17:2813-24.
31. Peterlin B, Kunej T, Sinkovec J, Gligorievska N, Zorn B. Screening for Y chromosome microdeletions in 226 Slovenian subfertile men. Hum Reprod 2002;17:17-24.
32. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, *et al.* Molecular and clinical characterization of Y chromosome microdeletions in infertile men: A 10-year experience in Italy. J Clin Endocrinol Metab 2007;92:762-70.
33. Maurer B, Simoni M. Y chromosome microdeletion screening in infertile men. J Endocrinol Invest 2000;23:664-70.
34. de Llanos M, Ballescà JL, Gázquez C, Margarit E, Oliva R. High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. Hum Reprod 2005;20:216-20.
35. Ferlin A, Tessari A, Ganz F, Marchina E, Barlati S, Garolla A, *et al.* Association of partial AZFc region deletions with spermatogenic impairment and male infertility. J Med Genet 2005;42:209-13.
36. Navarro-Costa P, Pereira L, Alves C, Gusmão L, Proença C, Marques-Vidal P, *et al.* Characterizing partial AZFc deletions of the Y chromosome with amplicon-specific sequence markers. BMC Genomics 2007;8:342.
37. Hucklenbroich K, Gromoll J, Heinrich M, Hohoff C, Nieschlag E, Simoni M, *et al.* Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. Hum Reprod 2005;20:191-7.
38. Wu B, Lu NX, Xia YK, Gu AH, Lu CC, Wang W, *et al.* A frequent Y chromosome b2/b3 subdeletion shows strong association with male infertility in Han-Chinese population. Hum Reprod 2007;22:1107-13.
39. Seielstad MT, Minch E, Cavalli-Sforza LL. Genetic evidence for a higher female migration rate in humans. Nat Genet 1998;20:278-80.
40. Tyler-Smith C. An evolutionary perspective on Y-chromosomal variation and male infertility. Int J Androl 2008;31:376-82.
41. de Carvalho CM, Zuccherato LW, Fujisawa M, Shirakawa T, Ribeiro-dos-Santos AK, Santos SE, *et al.* Study of AZFc partial deletion gr/gr in fertile and infertile Japanese males. J Hum Genet 2006;51:794-9.
42. Balaresque P, Bowden GR, Parkin EJ, Omran GA, Heyer E, Quintana-Murci L, *et al.* Dynamic nature of the proximal AZFc region of the human Y chromosome: Multiple independent deletion and duplication events revealed by microsatellite analysis. Hum Mutat 2008;29:1171-80.
43. Yang Y, Ma M, Li L, Zhang W, Xiao C, Li S, *et al.* Evidence for the association of Y-chromosome haplogroups with susceptibility to spermatogenic failure in a Chinese Han population. J Med Genet 2008;45:210-5.
44. Krausz C, Giachini C, Xue Y, O'Bryan MK, Gromoll J, Rajpert-de Meyts E, *et al.* Phenotypic variation within European carriers of the Y-chromosomal gr/gr deletion is independent of Y-chromosomal background. J Med Genet 2009;46:21-31.
45. Arredi B, Ferlin A, Speltra E, Bedin C, Zuccarello D, Ganz F, *et al.* Y-chromosome haplogroups and susceptibility to azoospermia factor c microdeletion in an Italian population. J Med Genet 2007;44:205-8.
46. Bosch E, Jobling MA. Duplications of the AZFa region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. Hum Mol Genet 2003;12:341-7.
47. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, *et al.* Large-scale copy number polymorphism in the human genome. Science 2004;305:525-8.
48. Fernandes S, Huellen K, Goncalves J, Dukal H, Zeisler J, Rajpert De Meyts E, *et al.* High frequency of DAZ1/DAZ2 gene deletions in patients with severe oligozoospermia. Mol Hum Reprod 2002;8:286-98.
49. Krausz C, Degl'Innocenti S, Nuti F, Morelli A, Felici F, Sansone M, *et al.* Natural transmission of USP9Y gene mutations: A new perspective on the role of AZFa genes in male fertility. Hum Mol Genet 2006;15:2673-81.
50. Luddi A, Margollicci M, Gambera L, Serafini F, Cioni M, De Leo V, *et al.* Spermatogenesis in a man with complete deletion of USP9Y. N Engl J Med 2009;360:881-5.
51. Plotton I, Ducros C, Pugeat M, Morel Y, Lejeune H. Transmissible microdeletion of the Y-chromosome encompassing two DAZ copies, four RBMY1 copies, and both PRY copies. Fertil Steril 2010;94:2770.e11-6.
52. Siffroi JP, Le Bourhis C, Krausz C, Barbaux S,

Quintana-Murci L, Kanafani S, *et al.* Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. Hum Reprod 2000;15:2559-62.

53. Rajpert-De Meyts E, Ottesen AM, Garn ID, Aksglaede L, Juul A. Deletions of the Y chromosome are associated with sex chromosome aneuploidy but not with Klinefelter syndrome. Acta Paediatr 2011;100:900-2.
54. Patsalis PC, Sismani C, Quintana-Murci L, Taleb-Bekkouche F, Krausz C, McElreavey K, *et al.* Effects of transmission of Y chromosome AZFc deletions. Lancet 2002;360:1222-4.