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Analysis of the AZF Region in Slovak Men with Azoospermia

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***Abstract*—**Y chromosome microdeletions are the most common genetic cause of male infertility and screening for these microdeletions in azoospermic or severely oligospermic men is now standard practice. Analysis of the Y chromosome in men with azoospermia or severe oligozoospermia has resulted in the identification of three regions in the euchromatic part of the long arm of the human Y chromosome (Yq11) that are frequently deleted in men with otherwise unexplained spermatogenic failure. PCR analysis of microdeletions in the AZFa, AZFb and AZFc regions of the human Y chromosome is an important screening tool. The aim of this study was to analyse the type of microdeletions in men with fertility disorders in Slovakia. We evaluated 227 patients with azoospermia and with normal karyotype. All patient samples were analyzed cytogenetically. For PCR amplification of sequence-tagged sites (STS) of the AZFa, AZFb and AZFc regions of the Y chromosome was used Devyser AZF set. Fluorescently labeled primers for all markers in one multiplex PCR reaction were used and for automated visualization and identification of the STS markers we used genetic analyzer ABi 3500xl (Life Technologies). We reported 13 cases of deletions in the AZF region 5,73%. Particular types of deletions were recorded in each region AZFa,b,c .The presence of microdeletions in the AZFc region was the most frequent. The study confirmed that percentage of microdeletions in the AZF region is low in Slovak azoospermic patients, but important from a prognostic view.

***Keywords*—**AZF, male infertility, microdeletions, Y chromosome.

I. Introduction

INFERTILITY affects 15% of couples worldwide, and in roughly half of these cases, the defect can be traced to the male factors [1], [2]. Several factors have been implicated in male infertility such as hormonal abnormalities, erectile dysfunction, infections, antisperm antibodies, exposure to chemical agents and radiations, testicular cancer, varicose, genetic factors and others. The main genetic cause of male infertility is chromosomal abnormalities, which account for almost 5% of infertile males, and the prevalence increases to 15% in the azoospermic males [3]. Men with non-obstructive azoospermia have high prevalence of aneuploidy, particularly in their sex chromosomes [4]. The second most common genetic cause of male infertility is microdeletion in the azoospermia factor (AZF) region of the Y chromosome [5]. The azoospermia factor (AZF) region contains essential genes for spermatogenesis. Microdeletions in this region cause defect in spermatogenesis that leads to development of azoospermia and oligozoospermia [6]. Three major loci have been identified in the AZF and named AZFa, AZFb and AZFc regions. These three loci contain 16 coding genes that play a role in the process of spermatogenesis such as regulation of gene expression, RNA processing and trafficking [7].

The origin of human X and Y chromosomes dates back to about 300 million years ago, i.e. long before the development of mammals. They evolved from a pair of identical chromosomes of reptiles. The Y chromosome is always in a haploid state and therefore transmitted in an intact state through the paternal line. The human Y chromosome is one of the smallest chromosomes. It contains 57 million base pairs and a relatively low number of functional genes. The most important genes are those necessary for proper development of testis and spermatogenesis [8]. The SRY gene, located in the terminal region of the short arm of Y chromosome with its lenght of 1kb [9], plays the key role in the development of testis [10].

Azoospermia Factor Region (AZF) was defined on the long arm of Y chromosome. Genes and gene families belonging to this region are responsible for the faultless course of spermatogenesis [11]. Deletions of AZF locus are genomic deletions of the euchromatic part of the long arm of Y chromosome (Yq11) related to azoospermia or severe oligospermia. It is generally accepted that such deletions determine the dysfunction of Y chromosome genes responsible for spermatogenesis [12]. The final number of genes in the above mentioned subregions of AZF locus was determined after defining the complete sequence of the Yq11 region in 2003 [13]. It has been found out that the AZF region contains locations of 31 Y- specific genes, which are expressed in human testes and are located in some of the further described subregions. Fourteen of them code proteins and 17 represent non-protein-coding transcripts. AZFc subregion is evolutionally young and AZF genes were found also in mouse Y chromosome [14]. Additionally, it was shown thet genes of AZFb and AZFc subregions play a significant role as a control mechanism during the pre-meiotic X and Y chromosome-pairing process [15], [16].

II. Material and Methods

Unrelated 227 infertile men with azoospermia were evaluated in the study over the period from 2011 to 2014. All patients were Caucasians from different regions of Slovakia.

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The mean age of subjects was 32,3 ± 7 years (range 24-42) years. DNA from the patient´s peripheral blood (native or frozen, in solution of 0.5 ml 0.5. M EDTA) was extracted by standard extraction procedures using ReliaPrep™ gDNA Tissue Miniprep System (Promega). All patient samples were analyzed cytogenetically. Chromosomal analysis was performed on all patients on cultured lymphocytes from peripheral blood using standard methods. The chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature standards. We examined 50 women and 50 healthy men as negative controls. For PCR amplification of sequence-tagged sites (STS) of the AZFa, AZFb and AZFc regions of the Y chromosome was used Devyser AZF set. All STS markers and control sequences, recommended by the European Academy of Andrology (EAA) and the European Quality Monitoring Network Group (EMQN) for basic Y-chromosomal microdeletion analysis were included in the kit. Fluorescently labeled primers for all markers in one multiplex PCR reaction were used and for automated visualization and identification of the STS markers we used genetic analyzer ABi 3500xl (Life Technologies). Our study was approved by the local ethics committee of our faculty.

The European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) published the laboratory guidelines for molecular analysis of Y chromosome microdeletions. Therefore we decided to alter previously used sequences and proceed in accordance with European guidelines, in which all three subregions are represented by Y sequences: sY84, sY86, sY625, sY127, sY131, sY134, sY157, sY254 and sY255.

III. Results

In this study, we examined microdeletions in AZF region of Y chromosome in 227 male with azoospermia. We reported 13 cases of deletions in the AZF region (5,73%). Particular types of deletions were recorded in each region AZFa,b,c. A total of 227 men with AZF deletions included two patients with AZFa deletion (SY 86, SY 84) three with AZFb deletion (SY 127, SY 134) and eight with AZFc deletion (SY 254, SY 255, SY 157). All men with deletions in AZF region were azoospermic. The detailed deletion specifications in AZFa and AZFb regions are shown in Table I and in AZFc region in Table II. Our results are consistent with most of the literature that indicate that the prevalence of microdeletions in AZFc is high compared to AZFb and AZFa regions. The study confirmed that percentage of microdeletions in the AZF region is low in Slovak azoospermic patients, but important from a prognostic view.

TABLE I
THE DELETION SPECIFICATION IN AZFA AND AZFB REGION OF THE Y CHROMOSOME

	Region AZFa			Region AZFb		
	SY 86	SY 625	SY 84	SY 127	SY 131	SY 134
Patient 1						
Patient 2						
Patient 3			Del			
Patient 4				Del		
Patient 5						
Patient 6						
Patient 7						
Patient 8						
Patient 9						
Patient 10				Del		Del
Patient 11						Del
Patient 12	Del		Del			
Patient 13						

TABLE II
THE DELETION SPECIFICATION IN AZFC REGION OF THE Y CHROMOSOME

	Region AZFc		
	SY 254	SY 255	SY 157
Patient 1		Del	
Patient 2			Del
Patient 3			
Patient 4			
Patient 5	Del		
Patient 6		Del	
Patient 7	Del	Del	
Patient 8	Del		
Patient 9	Del	Del	Del
Patient 10			
Patient 11			
Patient 12			
Patient 13	Del	Del	

TABLE III
THE FREQUENCY OF AZF MICRODELETIONS AMONG AZOOSPERMIC MALES IN SELECTED POPULATIONS

Population	Frequency of AZF microdeletions (%)	Reference
Slovakian	5.7	This study
Slovakian	6.9	[15]
Chinese	8.6	[9]
Algerian	2.0	[16]
Japanese	11.7	[13]
Turkish	1.3	[17]
Tunisian	11.8	[14]
Netherlander	8.1	[11]
USA	10.4	[12]
Indian	7.6	[10]

IV. Discussion

Over the past few years, screening tests for detecting microdeletions on the long arm of the Y chromosome have established the distribution and characteristics of the deletions among different groups of infertile male patients Data from PCR studies have demonstrated that the prevalence of Y chromosome microdeletions among men with nonobstructive

idiopathic azoospermia or severe oligozoospermia ranges from 3 to 55.5%, the higher frequencies being detected in cases of severely impaired spermatogenesis [17]. A number of factors have been implicated in the wide variation of Y deletion frequencies reported such as patient selection criteria, experimental designs, environmental influences and ethnic variations [18].

The study has confirmed that the detection of microdeletions in the AZF region is significant from a diagnostic viewpoint. It is also useful to obtain reliable genetic information from infertile men to determine the etiology of the deletions, and to avoid unnecessary treatments and vertical transmission of genetic defects [19]. The frequency of AZF microdeletions in Slovak azoospermic infertile males is comparable to that observed in other populations (1%-15%). The frequency of AZF microdeletion observed in this study was about 5.7% among azoospermic males. This frequency is lower than that detected in patients from China (8.6%) [20], India (7.6%) [21], Netherland (8.1%) [22], USA (10.4%) [23], Japan (11.7%) [24], Tunisia (11.8%) [25] and Slovakia (6.9%) [26]. However, very low frequency of AZF microdeletions was reported in studies from Algeria (2%) [27] and Turkey (1.3%) [28], (Table III). The variation in the detected frequencies of AZF microdeletions among azoospermic infertile males could be due to method of detection of deletions, inclusion criteria and sample sizes. Our results are consistent with most of the literature that indicate that the prevalence of microdeletions in AZFc is high compared to AZFb and AZFa regions. AZFc region contains the DAZ family genes that encode proteins with RNA-binding motive and involved in the regulation of RNA metabolism during testicular development [29]. Deletions in DAZ gene cluster have been shown to be associated with a variety of spermatogenic alterations [30]. The reasons behind high frequency of microdeletions in AZFc could be due to the presence of repetitive sequences of the genes in this region that predispose it to intrachromosomal recombination. The results also suggest the importance of AZF microdeletion analysis for genetic counseling prior to providing assisted reproduction technique [31]. Deletions in the AZF region of the Y chromosome are specific within diagnoses with spermatogenesis disorders,no deletions were recorded in the group of normospermic men. From this point of view it is more appropriate to indicate that microdeletions of the Y chromosomal AZF region are more responsible for oligo or azoospermia than for the cause of infertility. Apart from possible oligospermia or azoospermia there is a potential risk of developing ananomaly 45,X0 Turner syndrome, or other forms of sex chromosome mosaicism, including hermaphroditism. Yq deletions are associated with Y chromosome instability. We can say that identification of Y chromosomal microdeletions has significant diagnostic and prognostic value and provides useful information for genetic counseling in these patients [32].

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REFERENCES

[1] J. Poongothai, T. S.Gopenath, S. Manonayaki “Genetics of human male inferertility.” *Singapore Med.,*vol.50, pp.336-347,April 2009.
[2] K.L.O´Flynn,O´Brien,A.C.Varghese,A.Agarwali, “The genetic causes of male factor infertility : a review.”*Fertil Steril*,vol.93,no.1,pp.1-12,Jan 2010.
[3] B.R.Emery,D.T.Carrell,”The effect of epigenetic sperm abnormalities on early embryogenesis.” *Asian J,Androl.,*vol.8,no21,pp.131-142,March 2006.
[4] G.D.Palermo,L.T.Coôombero,J.J.Harriprashad,P.N.Schlegel,Z.Rosen-Waks,”Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI.” *Hum.Reprod,* vol.17, no.3,, pp.570-575,March 2002.
[5] R.Slezak,M.Sasiadek,”Chromosome Y microdeletions in the pathogenesis of male infertility.”*Pol Merkur Lekarski,*vol.13 ,no.75, pp.229-233, Sep 2002.
[6] C.Britom-Jones,C.J.Haines,”Microdeletions on the long arm of the Y chromosome and their association with male factor infertility.”*Hong Kong Med.J.,*vol.6,no.2,pp.184-189, June 2000.
[7] T.Shinka,Y.Nakahori,”The azoospermic factor on the &y chromosome.”*Acta Paediatr Jpn.,*vol.38,no.4,pp.399-404, Aug.1996.
[8] J.C.Venter,M.D.Adams,E.W.Myers, "The sequence of the human genome." *Science,* vol. 291, no.5507,pp.1304-1351, Feb 2001.
[9] C.Mitchell,V.R.Harley,"Biochemical defects in eight SRY missense mutations causing XY gonadal dysgenesis." *Mol Genet Metab*, vol.77,no.3,pp217-225, Nov 2002.
[10] A.H.Sinclair,P.Berta,M.Palmer,J.R.Hawkins,B.L.Griffiths,M.J.Smith,J. W.Foster,A.M.Frischauf,R.Lovell-Badge,P.N.Goodfellow, "A gene from the human sex-determining region encodes a protein with homology to conserved DNA-binding motif." *Nature*, vol.346,pp.240-246,July 1990.
[11] J.C.Achermann,G.Ozisik,M.Ito,U.A.Orun,K.Harmanci,B.Gurakan,J.L.Jameson, "Gonadal determination and adrenal development are regulated by the orphan nuclear receptor steroidogenic factor-1, in a dose-dependent manner." *J Clin.Endocrinol Metab*, vol.87,pp.1829-1833, Feb 2002.
[12] A.Fwrlin,E.Moro,A.Rossi,B.Dallapiccola,C.Foresta,"The huma Y chromosome ´s azoospermia factorb (AZFb) region: sequence, structure, and deletion analysis in infertile men*". J Med Genet*, vol.40,no.1,pp.18-24, Jan 2003.
[13] S.Simoni,E.Bakker,C.Krausz," EAA/EMQN best practice guidelines for molecular diagnosis of Y -chromosomal microdeletions. State of the art 2004. *Int J Androl*, vol.27,no.4, pp. 240-249, Aug 2004.
[14] C.Kamp,K.Huellen,S.Fernandes,M.Sousa,P.N.Schlegel,A.Mielnik,S.Kleiman,H.Yavetz,W.Krause,W.Kupker,R.Johannison,W.Schulze,W.Weidner,A.Barros,P.H.Vogt," High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome."*Molec Hum Reprod*, vol. 7,no.10,pp.987-994, July 2001.
[15] P.H.Vogt," AZF deletions and Y chromosomal haplogroups : history ad updates on sequence." Hum.Reprod., vol. 11,no.4, pp. 319-336, Aug 2005.
[16] P.H.Vogt, C.L.Falcao, R.Hanstein,J.Zimmer, "AZF Proteins. " *Int.J.Androl*., vol. 31, no.4,pp. 383-394, May 2008.
[17] C.Foresta , E.Moro A. Ferlin ,” Y chromosome microdeletions and alterations of spermatogenesis.” *Endocrine Reviews*, vol.22,no.2, pp. 226-23, July 2013.
[18] S.L. SãoPedro, R. Fraietta, D. Spaine, C.S. Porto, M. Srougi, A.P. Cedenho and M.C.W. Avellar ,“Prevalence of Y chromosome deletions in a Brazilian population of nonobstructive azoospermic and severely oligozoospermic men.” *Braz J Med Biol Res,* vol.36 ,no.6,pp.787-793, June 2003.
[19] M.Totonchi,A.Mohseni Meybody,P.Borijan,P.Boroujeni,M.Sedighi Gilani,N.Amadani,H.Gourabi,”Clinical data for 185 infertile Iranian men

with Y-chromosome microdeletion."*J.Assist.Reprod.Genet.,*vol.29, no.8,pp.847-853, Aug 2012.

[20] Y.S.Zhang,R.I.Dai,R.X.Wang,H.G.Zhang,S.Chen,R.Z.Liu,"Analysis of Y chromosome microdeletion in 1738 infertile men from northeastern China."*Urology,*vol.82,no.3,pp.584-588, Sep 2013.

[21] A.Mitra,R.Dada,R.Kumar,N.P.Gupta,K.Kucheria,S.K.Gupta,"Screening for Y-chromosome microdeletions in infertile Indian males : utility of simplified multiplex PCR." *Indian J Med.Res,*vol.127,no.2,pp.124-132, Feb 2008.

[22] G.R.Dohle,D.J.Halley,J.O.Van Hemel,A.M.Van Den Douwel,M.H. Pieters,R.F.Weber,L.C.Govaerts,"Genetic risk factors in infertile men with severe oligozoospermia and azoospermia." *Hum Reprod,*vol.17, no.1,pp.13-16, Jan 2002.

[23] P.J.Stahl,P.Masson,A.Mielnik,M.B.Marean,P.N.Schlegel,D.A.Paduch," A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia." *Fertil.Steril.,*vol.94 no.5,,pp.1753-1756, Oct 2010.

[24] M.Nakashima,E.Koh,M.Namiki,A.Yoshida,"Multiplex sequence-tagged site PCR for efficient screening of microdeletions in Y chromosome in infertile males with azoospermia or severe oligozoospermia." *Arcg Androl.,*vol.48,no.5,pp.351-358, Sep-Oct 2002.

[25] I.Rejeb,R.M´rad,F.Maazoul,M.Trabelsi,L.Ben Jemaa,M.Caabouni,F.Zhio ua,H.Chaabouni."Y chromosome microdeletions in Tunisian infertile males."*Pathol .Biol (Paris),*vol.56,no.3,pp.111-115, May 2008.

[26] R.Behulova,I.Varga,L.Strhakova,A.Bozikova,D.Gabrikova,I.Boronova,V .Repiska,"Incidence of microdeletions in the AZF region of the Y chromosome in Slovak patients with azoospermia."*Biomed.Pap.Med.Fac. Univ.Palacky Olomouc,Czech Repub.,*vol.155,no.1,pp.33-38, March 2011

[27] D.Chellat,M.L.Rezgoune,K.Mc.Elreavey,N.Kherouatou,S.Ben-Bouhadja, H.Douadi,B.Cherifa,N.Abadi,D.Satta," First study of microdeletions in the Y chromosome of Algerian infertile men with idiopathic oligo or azoospermia.*Urol .Int.,*vol.90,no.4,pp.455-459, March 2013.

[28] M.Balkan,S.Tekes,A.Gedik,"Cytogenetic and Y chromosome microdeletion screening studies in infertile males with Oligozoospermia and Azoospermia in Southeast Turkey."*J.Assist.Reprod.Genet.,* vol.25,no.11-12,pp.559-565,Nov 2008.

[29] A.Ferlin,E.Motto,A.Garolla,C.Foresta,"Human male infertility and Y chromosome deletions: role of the YZF candidate genes DAZ,RBM and DFFRY." *Hum Reprod,*vol.14,no.7,pp.1710-1716, July 1999.

[30] S.E.Kleiman,L.Yogev,R.Gamzu,R.Hauser,A.Botcha,G.Paz,J.B.Lessing,, Y.Yaron,H.Yavetz,"Three generations evaluation of Y-chromosome microdeletion."*J .Androl,*vol20,no.3,394-398, May-June 1999.

[31] O.F.Khabour,A.S.Fararjeh,A.A.Alfaduri,"Genetic screening for AZF Y microdeletions in Jordan azoospermic infertile men."*Intl J Mol Epidemiol Genet.,*vol.17,no.5,pp.47-50, Feb 2014.

[32] R. Behulova, I.Varga, L. Strhakova, A.Bozikova, D. Gabrikova,I. Boronova, V. Repiska**, "**Incidence of microdeletions in the AZF region of the Y chromosome I Slovak patients with azoospermia.." *Biomed Pap Med Fac Univ Palacky Olomouc,* Czech Repub.,vol. 155,no.1,pp.33–38, March 2011.