Analysis of the correlation between gene copy deletion in the AZFc region and male infertility in Japanese men

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Abstract

Deletion of the azoospermia factor c (AZFc), located on the long arm of the Y chromosome, is a cause of male infertility. The structure of the Y chromosome is diversified by the copy number of various genes, such as deleted in azoospermia (*DAZ*), basic protein Y2, chromodomain Y1, testis-specific transcript Y-linked 4, and Golgi autoantigen golgin subfamily a2 like Y, located in the AZF region. In this study, we investigated the deletion of each gene copy and analyzed its relationship with Japanese male infertility. Deletions of single nucleotide variants of each gene copy in 721 proven fertile men as controls, 139 patients with non-obstructive azoospermia (NOA), and 56 patients with oligozoospermia (OS) were analyzed via polymerase chain reaction-restriction fragment length polymorphism analysis. Their association with infertility was analyzed using logistic regression analysis adjusted for the Y-chromosome haplogroup, D1a2a. Deletions of DAZ/II in the r1 region and DAZ/V in the r1 and r2 regions showed significant associations with NOA (odds ratio [OR] = 4.15, 95% confidence interval [CI] = 1.18–14.6, $P = 0.026$; OR = 4.19, 95% CI = 1.19–14.7, $P = 0.025$, respectively). They did not show any association with OS. Partial deletion of the AZFc region affects spermatogenesis in Japanese male.

Keywords: Y chromosome; AZF; non-obstructive azoospermia; infertility; Japanese men

1. Introduction

Infertility is defined as not leading to pregnancy despite sexual intercourse for more than 12 months without regular contraception [1]. Infertility is observed in approximately 15% of all couples, with 45–50% attributed to the male side [2]. Many of male infertility cases are caused by spermatogenic failure, which is classified into non-obstructive azoospermia (NOA), oligozoospermia (OS), and asthenozoospermia. The World Health Organization defines azoospermia as the absence of sperm in the ejaculated semen, OS as the presence of less than 16 million sperms per mL of semen, and asthenozoospermia as sperm motility of less than 42% [3]. Klinefelter's syndrome and microdeletion of the Y chromosome are known genetic causes of spermatogenic dysfunction that remain undiagnosed in approximately 60% of male infertility cases [4-5].

The long arm of the Y chromosome contains three azoospermia factor (AZF) regions (AZFa, b, and c) [6]. In the AZF regions, there are multiple repetitive sequences called amplicons, which were classified using a color code by Kuroda Kawaguchi et al. [7] (Fig. 1). Deletion due to homologous recombination between repetitive sequences occurs in the AZF region, and deletion of the entire AZFc region is called complete deletion, while partial deletion is called subdeletion of the AZFc region. In gr/gr deletion

(1.6 Mb), g1/r1 to g2/r3 regions are deleted; in b2/b4 deletion, b2 to b4 regions are deleted, part of the AZF region is inverted; and in b2/b3 deletion (1.8 Mb), the b3 region is deleted from the b2 region [7-9]. Patients with a deletion of the AZFa or AZFb region are completely azoospermic, and the sperm cannot be recovered by microscopic intratesticular sperm extraction (micro-TESE). Deletion of the AZFc region causes azoospermia or severe OS, but the sperm can be recovered by micro-TESE [10].

In the AZFc region, there are eight multi-copy gene families: deleted in azoospermia (*DAZ*), basic protein Y2 (*BPY2*), chromodomain Y1 (*CDY1*), golgi autoantigen golgin subfamily a2 like Y (*GOLGA2LY*; GOLY), chondroitin sulfate proteoglycan 4 like Y (*CSPG4LY*), testis-specific transcript Y-linked (*TTTY*)-3, *TTTY4*, and *TTY17* [11]. These genes are suggested to play an important role in spermatogenesis, as they are exclusively or predominantly expressed in the testes [12]. *DAZ* has a polymorphic structure, with a total of four copies in the r1, r2, r3, and r4 regions. In particular, the exon 7 length is likely to change as the number of copies changes [13]. *DAZ* deletion accounts for spermatogenic dysfunction in 10% of all men, and infertile men lacking a copy of *DAZ* are more likely to develop azoospermia or severe OS [14-16]. Severe OS is believed to be caused by the deletion of *DAZ* clusters containing *DAZ1* and *DAZ2* [16, 17]. *CDY* contains two identical genes (*CDY1A* and *CDY1B*) in the AZFc

region and two genes closely related to palindrome P5 (*CDY2A* and *CDY2B*). Simultaneous deletion of both *DAZ* and *CDY1* copies [16, 18] and *CDY1b* copy is associated with OS or azoospermia [19]. However, the fact that some men are fertile or have normal sperm even with a *CDY1* deletion suggests that these genes are not essential for spermatogenesis [16, 17]. *BPY2* consists of three approximately identical copies of *BPY2A*, *BPY2B*, and *BPY2C* on the Y chromosome, two of which are located at the boundaries of the gr/gr deletion in the *DAZ* gene group. Mutations in *BPY2* are associated with the Sertoli cell-only syndrome [20]. *GOLGA2LY* contains two copies of *GOLGA2P2Y* and *GOLGA2P3Y* in the AZFc locus, which are arranged in the opposite direction to that of palindrome P1. These two copies are suggested to play different roles in spermatogenesis [21]. There are three copies of *TTTY4*, namely *TTTY4A*, *TTTY4B*, and *TTTY4C*. This gene has not yet been studied in detail and is suggested to be a non-coding RNA that does not encode a protein [22, 23].

The Y chromosome is not recombined and is inherited from the father to the son in its original genome sequence. Therefore, men with the same variant on the Y chromosome are considered to have the same ancestry, from which a phylogenetic tree on the Y chromosome can be created [24, 25]. A group with the same variant on the Y chromosome is called the Y-chromosome haplogroup. Haplogroups are broadly classified

into 20 groups, from A to T [24]. The Y chromosome of Japanese men is mainly classified into three haplogroups: C (10.8%), D (32.1%), and O (54.1%) [26]. Previously, we reported that in Japan, males belonging to the haplogroup, D1a2a (M55), have a gr/gr deletion, and this lineage is associated with NOA [27] and sperm motility [28]. However, studies on the association between subdivided structural variations in the AZFc region and spermatogenesis are limited. In this study, we determined the deletion of single nucleotide variants (SNVs) in each gene copy of the AZFc region in fertile men and patients with NOA and OS and analyzed the association between the deletion of each gene copy and NOA or OS in Japanese men.

2. Material and methods

2.1. Subjects

First, 721 fertile men (aged 31.2 ± 4.8 years; mean ± standard deviation [SD]) who were the partners of pregnant women who attended obstetric clinics in four cities in Japan were recruited for this study (Sapporo, Kanazawa, Osaka and Fukuoka). These fertile subjects have been previously described [29]. Fertile Japanese men were also included in the control group. A total of 195 infertile patients were recruited from the Department of Urology, St. Mariana University Hospital, Kanagawa Prefecture; Center for Infertility and IVF International University of Health and Welfare Hospital, Tochigi Prefecture; Urology Department, Tsukuba Gakuen Hospital, Ibaraki Prefecture; and Department of Male Infertility Reproduction Center, Sanno Hospital, Tokyo, Japan. Some of the subjects and their diagnostic criteria have been described in previous reports [27]. Of these patients, 139 (aged 33.9 ± 6.0 years; mean ± SD) were diagnosed as having NOA and 56 (aged 35.2 ± 6.4 years; mean ± SD) were diagnosed as having OS. NOA was diagnosed based on semen analysis (absence of sperm in the ejaculate), serum hormone levels, and results of physical examinations. OS was defined as a sperm concentration $< 20 \times 10^6$/mL. We excluded patients with any known cause of infertility, such as obstructive azoospermia, varicocele, cryptorchidism, hypogonadotropic hypogonadism, karyotype abnormalities,

or complete deletion of AZFa, b, or c. The reason for excluding obstructive azoospermia from our study is that while this condition is diagnosed as azoospermia because sperm are not ejected due to the closure of the sperm transport ducts, sperm are still produced in the testes. Deletions in AZFa, b, and c were analyzed according to the European Academy of Andrology and European Molecular Genetics Quality Network best practice guidelines [30]. This study was approved (approval number: R2-35)by the ethics committees of Tokushima University, St. Marianna Medical University, International University of Health and Welfare Hospital, Tsukuba Gakuen Hospital, and Sanno Hospital. All participants provided written informed consent for their involvement in this study.

2.2. Genotyping SNVs, gr/gr deletion, and haplogroup D1a2a

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). SNVs of DAZ/I, DAZ/II, DAZ/III, DAZ/IV, DAZ/V, GOLY/I, BPY2/I, TTTY4/I, and CDY1 were typed via polymerase chain reaction restriction fragment length polymorphism analysis (PCR-RFLP). The primer sets, PCR amplification conditions, restriction enzymes, and SNV alleles are listed in Supplementary Table S1. The Y chromosome haplogroup, D1a2a, was typed by PCR using M55 allele-specific primers (forward: 5′-GTAGGCGTTTGACAGCAGTT-3′,

reverse: 5′-ACTGGATGACTGATGAAAAGGT-3′), according to the method described by Kumagai et al. [31].

2.3. Statistical analysis

The associations between infertility and SNVs or haplotypes of SNVs were assessed using Fisher's exact test and odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. When adjusted for Y chromosome haplogroup D1a2a, logistic regression analysis was used. Statistical analyses were performed using R version 4.0.3 (The R Project for Statistical Computing; http://www.r-project.org), and statistical significance was set at $P < 0.05$.

3. Results

The SNV typing results for DAZ/I, DAZ/II, DAZ/III DAZ/IV, DAZ/V GOLY/I, BPY2/I, TTTY4/I, and CDY1 for fertile men, patients with NOA, and patients with OA are shown in Table 1. DAZ SNV-IIIa (deleted DAZ SNV-IIIb) and DAZ SNV-IVa (deleted DAZ SNV-IVa) were not detected in any of the groups. DAZ SNV-IIa (deleted DAZ SNV-IIb), DAZ SNV-V B (deleted DAZ SNV-Vb), and TTTY4/I A (deleted TTTY4/Ib) were less frequently detected.

We analyzed the relationship between NOA or OS and each SNV allele and SNV A + B. The results showed that NOA was correlated with DAZ SNV-IIA and B (deleted DAZ SNV-IIa or b) (OR = 1.73, 95% CI = 1.16–2.57, $P = 0.0051$), DAZ SNV-IIB (deleted DAZ SNV-IIa) (OR = 1.77, 95% CI = 1.19–2.65, $P = 0.0036$), DAZ SNV-III B (deleted DAZ SNV-IIIa) (OR = 1.66, 95% CI = 1.11–2.47, $P = 0.010$), DAZ SNV-V A (deleted DAZ SNV-Vb) (OR = 1.79, 95% CI = 1.20–2.67, $P = 0.0035$), DAZ SNV-V A and B (deleted DAZ SNV-Va or b) (OR = 1.77, 95% CI = 1.19–2.65, $P = 0.0036$), GOLY/I B (deleted GOLY/Ia) (OR = 1.60, 95% CI = 1.05–2.45, $P = 0.024$), BPY2/I A and B (deleted BPY2/Ia or b) (OR = 1.75, 95% CI = 1.17–2.61, $P = 0.0044$), and BPY2/I B (deleted BPY2/Ia) (OR = 1.73, 95% CI = 1.14–2.62, $P = 0.0073$) (Table 2); however, no association was found in patients with OS.

After examining the combinations of SNV deletions, it was dived into 47 haplotypes (Supplementary Table S2). We performed an association analysis of 47 haplotypes with NOA and OS. Only haplotype 47 (DAZ SNV-I B, DAZ SNV-II B, DAZ SNV-III B, DAZ SNV-IV B, DAZ SNV-A, GOLY/I B, BPY2/I B, TTTY4/I B, and CDY1 B) was associated with NOA (OR = 1.65, 95% CI = 1.10–2.46, $P = 0.011$) (Table 2).

Previously, we showed that the Y chromosome haplogroup, D1a2a (M55 lineage), is associated with NOA [27]. Therefore, we performed an additional association analysis of NOA with the Y-chromosome haplogroup, D1a2a, as a covariate. Only DAZ SNV-II B (deleted DAZ SNV-IIa in the r1 region) (OR = 4.15, 95% CI = 1.18–14.6, $P = 0.026$) and DAZ SNV-V A (deleted DAZ SNV-Vb in the r1 and r2 regions) (OR = 4.19, 95% CI = 1.19–14.7, $P = 0.025$) were found to be associated with NOA (Table 3).

4. Discussion

In this study, we analyzed the association between the deletion of each gene copy in the AZFc region and NOA or OS in Japanese men. We found an association between NOA and the copy deletions of *DAZ*, *GOLY*, and *BPY2*. We also found an association with haplotype 47, which consisted of a copy deletion pattern for each gene. Infertile men lacking a copy of *DAZ* are more likely to develop azoospermia and OS [14-16]. A copy of *GOLY* is suggested to play an important role in spermatogenesis [21]. Mutations in *BPY2* are associated with the Sertoli-only syndrome [20]. Therefore, the results of this study support those of previous reports. Haplotype 47, consisting of a copy deletion pattern in each gene, matched the Y chromosome haplogroup, D1a2a. Previously, we showed that men with the Y chromosome haplogroup, D1a2a, always have a gr/gr deletion. Various studies have been conducted to assess the relationship between gr/gr deletions and spermatogenesis [28, 32-36]. In the Estonian male population, gr/gr deletion is significantly higher in patients with idiopathic male infertility than in control males, but there are differences in semen parameters, with and without gr/gr deletion, in control males [36]. In Japanese men, gr/gr deletion is associated with decreased sperm motility [28]. In addition, haplogroup D1a2a is associated with azoospermia [27]. We then analyzed the SNVs, which were significantly associated with azoospermia, using the Y

haplogroup, D1a2a, as a covariate. Only DAZ SNV-II B (deleted DAZ SNV-IIa) and DAZ SNV-V A (deleted DAZ SNV-Vb) were associated with azoospermia. Therefore, deletion of DAZ SNV-II in the r1 region and DAZ SNV-V in the r1 and r2 regions is associated with azoospermia, regardless of the Y haplogroup, D1a2a (gr/gr deletion). Fernandes et al. concluded that the deletion of *DAZ1/DAZ2* was responsible for the decrease in sperm count in oligozoospermic patients [17]. Similarly, Kumari et al. reported that *DAZ1/DAZ2* deletion was associated with spermatogenic disorders, whereas *DAZ3/DAZ4* deletion had only a small effect on them [37]. On the other hand, Hallast et al. reported that deletion of the *DAZ1/DAZ2* and *DAZ3/DAZ4* gene pairs did not directly cause sperm dysfunction in the Estonian male population, while the deletion of *DAZ1/DAZ3* and *DAZ2/DAZ4* gene pairs causes sperm dysfunction [36]. There are three types of gr/gr deletions caused by homologous recombination among g1/g2, r1/r3, and r2/r4 amplicons in the palindrome of P1 and P2 of AZFc, accompanied by structural changes, such as inversion [36]. Therefore, the differences between these results may be due to the difference in the contribution of each subtype to spermatogenesis.

For *BPY2* and *GOLY*, no association with infertility was found, considering the effects of haplogroup D1a2a. Approximately 32% of Japanese men belong to the Y haplogroup, D1a2a [26], and many copy deletions of *BPY2* and *GOLY* are included in the

haplogroup, D1a2a (SNV haplotype 47). Other *BPY2* and *GOLY* copy deletions were scattered across various haplotypes, so no association would have remained when Y haplogroup, D1a2a, was used as a covariate. Deletion of *DAZ3/4-CDY1* is associated with male infertility [38], while the deletion of both *DAZ* and *CDY1* copies in men results in azoospermia [18, 19]. Therefore, *CDY1* may affect spermatogenesis by undergoing simultaneous deletion with *DAZ*. There are various possibilities that the deletion alone does not affect spermatogenesis unless it is supplemented by other copies, or that the effect differs depending on the gene dose.

Gene copy deletions and SNV haplotypes were not found to be associated with OS. This may be due to the small sample size in this study. Therefore, further analysis using a larger sample size is necessary.

One of the limitations of our analysis of CNVs was that we did not use the Multiplex Ligand Probe-dependent Amplification (MLPA) method. MLPA is an excellent method for identifying deletions, duplications, and rearrangements. The purpose of our analysis was to examine the deletion of each gene copy within the AZFc region and analyze its relationship with male infertility. Deletion can be analyzed by PCR-RFLP method. Therefore, the MLPA method was not used in this study. However, the relationship between duplications and male infertility also needs to be examined, and this

is an issue to be addressed in the future.

In summary, several gene copy deletions and SNV haplotypes in the AZFc region were found to be associated with NOA in Japanese men. Previously, it was reported that the Y chromosome haplogroup, D1a2a, is associated with azoospermia in Japanese men. In this study, the association between the deletion of the DAZ SNV II, V copy and NOA remained significant after adjusting for the Y chromosome haplogroup, D1a2a (gr/gr deletion). Therefore, the gene copy number in the AZFc region, especially DAZ, seems to be important for spermatogenesis, regardless of gr/gr deletion in Japanese men. AZFc is one of the most genetically dynamic regions in the human genome. This property may serve to counteract genetic degeneracy associated with the lack of a meiotic partner. The specific deletions in this region could be linked to ethnicity. Therefore, the application of the results of this study is restricted to Japanese men. However, future studies on subjects of varying ethnicities may help to broaden the applicability of this work.

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Author contributions

YS conceived and designed the study; YN, AT, YS performed the experiments and data acquisition; YN, YS analyzed the data; KK, HT, MK, MU, KY, TI collected the samples; YN, YS wrote the paper. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

References

[1] World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva. 2018.

[2] World Health Organization WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge University Press, Cambridge, UK. 2000.

[3] WHO laboratory manual for the examination and processing of human semen, Sixth edition. 2021.

[4] Tüttelmann F, Werny F, Cooper TG, Kliesch S, Simoni M, Nieschlag E. Clinical experience with azoospermia: aetiology and chances for spermatozoa detection upon biopsy. Int J Androl 2011;34:291–8. doi:10.1111/j.1365-2605.2010.01087.

[5] Punab M, Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, Korrovits P, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod 2017;32:18–31. doi: 10.1093/humrep/dew284.

[6] 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. doi:10.1093/hmg/5.7.933.

[7] 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. doi: 10.1038/ng757.

[8] de Carvalho CMB, Zuccherato LW, Fujisawa M, Shirakawa T, Ribeiro-Dos-Santos AKC, Santos SEB, et al. Study of AZFc partial deletion gr/gr in fertile and infertile Japanese males. J Hum Genet 2006;51:794–9. doi:10.1007/s10038-006-0024-2.

[9] Yang B, Ma YY, Liu YQ, Li L, Yang D, Tu WL, et al. Common AZFc structure may possess the optimal spermatogenesis efficiency relative to the rearranged structures mediated by non-allele homologous recombination. Sci Rep 2015;5:10551. doi: 10.1038/srep10551.

[10] 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. doi: 10.1038/ejhg.2013.253.

[11] Vogt PH. Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. Mol Cell Endocrinol 2004;224:1–9. doi: 10.1016/j.mce.2004.06.008.

[12] Skaletsky, Kuroda-Kawaguchi, T, Minx PJ, Cordum HS, Hillier L, Brown LG, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003;423:825–37. doi: 10.1038/nature01722.

[13] Vogt PH, Fernandes S. Polymorphic DAZ gene family in polymorphic structure of AZFc locus: Artwork or functional for human spermatogenesis? APMIS 2003;111:115–26; discussion 126–7. doi:10.1034/j.1600-0463.2003.11101161

[14] Shahid M, Dhillon VS, Khali HS, Sexana A, Husai SA. Associations of Y-chromosome subdeletion gr/gr with the prevalence of Y-chromosome haplogroups in infertile patients. Eur J Hum Genet 2011;19:23–9. doi:10.1038/ejhg.2010.151.

[15] Lu C, Jiang J, Zhang R, Wang Y, Xu M, Qin Y, et al. Gene copy number alterations in the azoospermia-associated AZFc region and their effect on spermatogenic impairment. Mol Hum Reprod 2014;20:836–43. doi: 10.1093/molehr/gau043.

[16] Sen S, Ambulkar P, Hinduja I, Zaveri K, Gokral J, Pal A, et al. Susceptibility of gr/gr rearrangements to azoospermia or oligozoospermia is dependent on DAZ and CDY1 gene copy deletions. J Assist Reprod Genet 2015;32:1333–41. doi: 10.1007/s10815-015-0520-4.

[17] 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. doi:10.1093/molehr/8.3.286.

[18] Ghorbel M, Baklouti-Gargouri S, Keskes R, Chakroun N, Sellami A, et al. gr/gr-DAZ2-DAZ4-CDY1b deletion is a high-risk factor for male infertility in Tunisian population. Gene 2016;592:29–35. doi:10.1016/j.gene.2016.07.050.

[19] Ghorbel M, Baklouti-Gargouri S, Keskes R, Chakroun N, Sellami A, Fakhfakh F, et al. Deletion of CDY1b copy of Y chromosome CDY1 gene is a risk factor of male infertility in Tunisian men. Gene 2014;548:251–5. doi:10.1016/j.gene.2014.07.042.

[20] Choi J, Ko E., Suzuki H, Maeda Y, Yoshida, Namiki M. Alu sequence variants of the BPY2 gene in proven fertile and infertile men with Sertoli cell-only phenotype. Int J Urol 2007;14:431–5. doi:10.1111/j.1442-2042.2007.01741

[21] Noordam MJ, Westerveld GH, Hovingh SE, van Daalen SK, Korver CM, van der Veen F, et al. Gene copy number reduction in the azoospermia factor c (AZFc) region and its effect on total motile sperm count. Hum Mol Genet 2011;20:2457–63. doi: 10.1093/hmg/ddr119.

[22] 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. doi:10.1086/342928.

[23] 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. doi:10.1016/j.ygeno.2003.12.018.

[24] Karafet TM, Mendez FL, Meilerman MB, Underhill PA, Zegura SL, Hammer MF. New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. Genome Res 2008;18:830–8. doi: 10.1101/gr.7172008.

[25] Y Chromosome Consortium. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. Genome Res 2002;12:339–48. doi:10.1101/gr.217602.

[26] Sato Y, Shinka T, Ashraf AE, Yamauchi A, Iwamoto T, Nakahori Y. Overview of genetic variation in the Y chromosome of modern Japanese males. Anthropol Sci 2014;122:131–6.

[27] Sato Y, Shinka T, Iwamoto T, Yamauchi A, Nakahori Y. Y chromosome haplogroup D2* lineage is associated with azoospermia in Japanese males. Biol Reprod 2013;88:107.

doi:10.1095/biolreprod.112.105718.

[28] Sato Y, Iwamoto T, Shinka T, Nozawa S, Yoshiike M, Koh E, et al. Y chromosome gr/gr subdeletion is associated with lower semen quality in young men from the general Japanese population but not in fertile Japanese men. Biol Reprod 2014;90:116. doi:10.1095/biolreprod.114.118810.

[29] Iwamoto T, Nozawa S, Yoshiike , Namiki M, Koh E, Kanaya J, et al. Semen quality of fertile Japanese men: a cross-sectional population-based study of 792 men. BMJ Open 2013;3:e002223. doi:10.1136/bmjopen-2012-002223.

[30] Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. Int J Androl 2004;27:240–9. doi: 10.1111/j.1365-2605.2004.00495.x.

[31] Kumagai R, Sasaki Y, Tokuta T, Biwasaka H, Matsusue A, Aoki Y, et al. Distinct breakpoints in two cases with deletion in the Yp11.2 region in Japanese population. Hum Genet 2010;127:537–43. doi:10.1007/s00439-010-0794-7.

[32] 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. doi:10.1038/ng1250.

[33] Hucklenbroich K, Gromoll J, Heinrich M, Hohoff C, Nieschlag E, Simoni M. 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. doi: 10.1093/humrep/deh558.

[34] Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, Sankhwar SN, et al. gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses. Sci Rep 2016;6:19798. doi: 10.1038/srep19798.

[35] Stouffs K, Lissens W, Tournaye H, Haentjens P. What about gr/gr deletions and male infertility? Systematic review and meta-analysis. Hum Reprod Update 2011;17:197–209. doi: 10.1093/humupd/dmq046.

[36] Hallast P, Kibena L, Punab M, Arciero E, Rootsi S, Grigorova M, et al. A common 1.6 mb Y-chromosomal inversion predisposes to subsequent deletions and severe spermatogenic failure in humans. Elife 2021;10:e65420. doi:10.7554/eLife.65420.

[37] Kumari A, Yadav SK, Misro MM, Ahmad J, Ali S. Copy number variation and microdeletions of the Y chromosome linked genes and loci across different categories of Indian infertile males. Sci Rep 2015;5:17780. doi:10.1038/srep17780.

[38] 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. doi:10.1136/jmg.2004.022111.

Figure Legends

Figure 1. Schematic diagram of the azoospermia factor c (AZFc) region. The AZFc region has a palindrome structure, which can be sub-divided into units of repetitive

sequences. Same color arrows indicate amplicons which exhibit > 99.9% sequence identify. Since there are multiple gene copies of very similar sequences, a and b are used to distinguish the single nucleotide variants (SNVs).

Figure 1

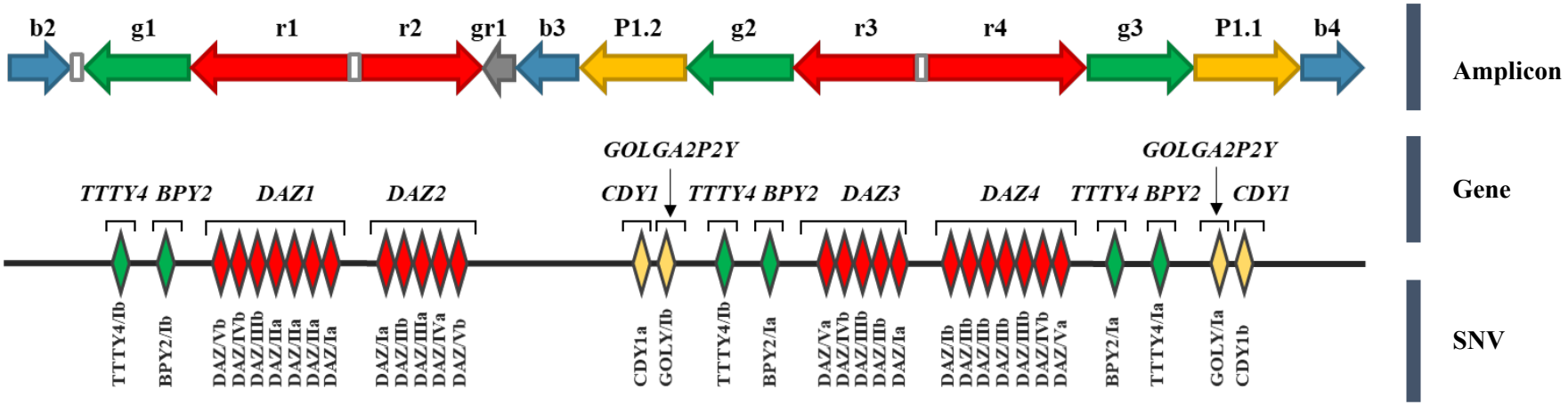
b2
g1
r1
r2
gr1
b3
P1.2
g2
r3
r4
g3
P1.1
b4
Amplicon
TTTY4
BPY2
DAZ1
DAZ2
GOLGA2P2Y
CDY1
TTTY4
BPY2
DAZ3
DAZ4
TTTY4
BPY2
GOLGA2P2Y
CDY1
Gene
TTTY4/Ib
BPY2/Ib
DAZ/Vb
DAZ/IVb
DAZ/IIIb
DAZ/IIa
DAZ/IIa
DAZ/Ia
DAZ/Ia
DAZ/IIb
DAZ/IIIa
DAZ/IVa
DAZ/Vb
CDY1a
GOLY/Ib
TTTY4/Ib
BPY2/Ia
DAZ/Va
DAZ/IVb
DAZ/IIIb
DAZ/IIb
DAZ/Ia
DAZ/Ib
DAZ/IIb
DAZ/IIb
DAZ/IIIb
DAZ/IVb
DAZ/Va
BPY2/Ia
TTTY4/Ia
GOLY/Ia
CDY1b
SNV

Table 1. Frequency of each SNV in fertile men, NOA patients, and OS patients.

SNV	Fertile (n)			NOA (n)			OS (n)		
	A	B	A+B	A	B	A+B	A	B	A+B
DAZ SNV-I	399	248	74	67	63	9	31	18	7
DAZ SNV-II	6	250	465	0	66	73	1	18	37
DAZ SNV-III	0	274	447	0	69	70	0	22	34
DAZ SNV-IV	0	380	341	0	82	57	0	35	21
DAZ SNV-V	250	2	469	67	0	72	19	0	37
GOLY/I	108	256	357	12	66	61	12	19	25
BPY2/I	36	251	434	11	63	65	2	19	35
TTTY4/I	1	350	370	0	75	64	0	28	28
CDY1	106	388	227	12	80	47	12	26	18

Data are shown the number of SNV type.

A indicates deleted SNVb, B indicates deleted SNVa, and A+B indicates deleted SNVa or b.

NOA, non-obstructive azoospermia; OS, oligozoospermia.

Table 2. SNV markers and haplotype related to nonobstructive azoospermia

SNV marker or haplotype	SNV allele vs. A+B	OR (95%CI)	*P*-value
DAZ SNV-II	A and B	1.73 (1.16-2.57)	0.0051
DAZ SNV-II	B	1.77 (1.19-2.65)	0.0036
DAZ SNV-III	B	1.66 (1.11-2.47)	0.010
DAZ SNV-V	A	1.79 (1.20-2.67)	0.0035
DAZ SNV-V	A and B	1.77 (1.19-2.65)	0.0036
GOLY/I	B	1.60 (1.05-2.45)	0.024
BPY2/I	A and B	1.75 (1.17-2.61)	0.0044
BPY2/I	B	1.73 (1.14-2.62)	0.0073
Haplotype 47		1.65 (1.10-2.46)	0.011

Data are shown as odds ratios (ORs), 95% confidence intervals (CIs), and *P*-values. For SNV makers, OR indicates versus SNV A+B. For haplotype 47, OR was indicated versus other haplotypes.

Table 3. SNV markers related to nonobstructive azoospermia with adjustments for haplogroup D1a2a

SNV marker	SNV allele vs. A+B	OR (95%CI)	*P*-value
DAZ SNV-II	B	4.15 (1.18-14.6)	0.026
DAZ SNV-V	A	4.19 (1.19-14.7)	0.025

Data are shown as odds ratios (ORs), 95% confidence intervals (CIs), and *P*-values with adjustments for haplogroup D1a2a in SNV allele vs. A+B.