Original Article

Analysis of copy number variations of the autosomal genome in 156 patients with azoospermia

Yongjie Liu[1], Dongmei Shi[1], Fan Zhang[1], Liang Dai[1], Wenzhi Ma[2]

[1]Department of Reproductive Center, Yinchuan Maternal and Child Health Care Hospital, Yinchuan, China; [2]Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, and Key Laboratory of Reproduction and Genetics of Ningxia Hui Autonomous Region, School of Basic Medical Science, Ningxia Medical University, Yinchuan, China

Contributions: (I) Conception and design: Y Liu, F Zhang; (II) Administrative support: L Dai; (III) Provision of study materials or patients: Y Liu; (IV) Collection and assembly of data: D Shi; (V) Data analysis and interpretation: F Zhang, L Dai, W Ma; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yongjie Liu. No. 56 Wenhua West Street, Yinchuan 750001, China. Email: lyj1722006@126.com.

Background: To analyze the relationship between copy number variations (CNVs) and azoospermia by analyzing the chromosome gene copy data of 156 patients with azoospermia.

Methods: A total of 156 azoospermia patients who were treated in our hospital from October 2018 to May 2021 were selected. Informed consent was signed, and semen analysis, testicular biopsy, and chromosome gene detection were carried out. CNVs were analyzed by next-generation sequencing (NGS) detection, and the obtained results were statistically analyzed.

Results: Among the 156 azoospermia patients, 81 cases had sperm in the testicular puncture and 75 cases had no sperm in the testicular puncture. There was a significant difference in CNV detection between the 2 groups ($P<0.05$). Detailed analysis of CNVs on chromosomes 2, 3, 5, 10, and 11 yielded the following results: 132 genes were found in autosomal chromosomes with CNVs and the percentage was >5%, including 8 deletions and 124 repetitions; CNVs on chromosome 2 found 11 genes, of which 3 were deleted and 8 were duplicated; 17 genes were found in CNVs on chromosome 3, including 3 deletions and 14 duplications; 12 genes were found in CNVs on chromosome 5, of which 2 were deleted and 10 were repeated; 72 genes were found in CNVs on chromosome 10, all of which were duplicates; CNVs on chromosome 11 found 20 genes, all of which were duplicates.

Conclusions: The chromosome changes caused by CNVs in structure or function may affect the component of spermatogenesis, interfere with mitosis and/or meiosis in the process of spermatogenesis, and lead to azoospermia.

Keywords: Azoospermia; copy number variation (CNV); next-generation sequencing (NGS)

Submitted Mar 28, 2022. Accepted for publication May 13, 2022.
doi: 10.21037/tau-22-301
View this article at: https://dx.doi.org/10.21037/tau-22-301

Introduction

Infertility affects about 10–15% of couples of gestational age. It has attracted attention as a common and frequently occurring condition, half of which is caused by male factors (1). Male factors mainly manifest as sexual dysfunction, oligoasthenospermia, azoospermia, abnormal seminal plasma, and immune infertility, among others. Azoospermia has a serious impact due to its complex etiology and was one of the most important factors of infertility. At present, the causes of spermatogenesis disorder are mainly involved the endocrine system, reproductive system ultrasound, chromosome abnormalities, and gene mutation (2). However, the etiology of most azoospermia cases is unknown, and the exact mechanism of spermatogenesis block has not yet been determined.

Therefore, in the present study, we screened 156 azoospermia patients with normal chromosomes and measured their genomic copy number variations (CNVs) by next-generation sequencing (NGS) technology, in order to determine the relationship between CNVs and azoospermia and lay a foundation for clarifying the possible mechanism of spermatogenic block caused by CNVs. We present the following article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-301/rc).

Methods

General information

A total of 156 azoospermia patients treated in our hospital from October 2018 to May 2021 were selected. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Yinchuan Maternal and Child Health Hospital (No. 2018-079) and the patients provided informed consent.

Inclusion criteria

B-ultrasound was performed to indicate that there was no abnormality in the bilateral testis, epididymis, and spermatic cord. Other inclusion criteria were as follows: genetic testing shown 46, XY, and no Y chromosome microdeletion was detected; no history of systemic diseases such as hepatitis, tuberculosis, and mumps; no history of exposure to radiation and chemicals; no urogenital infection, malformation, and varicocele. The ages ranged from 24 to 43 years, with an average of 30.79±6.43 years.

Semen analysis

According to the fifth edition of the WHO standard (1) and the Guidelines on Male Infertility of the European Society of Urology, patients were required to abstain for 3 to 5 days then masturbate and collect semen into a sterile cup. The sample was placed in a 36 ℃ incubator for 30 to 60 minutes to liquefy, and any patient who had no sperm after microscopic examination and centrifugal precipitation was determined as infertile.

Testicular biopsy

After routine disinfection and laid the drapes, 2% lidocaine spermatic cord block was performed. The left ring finger and middle finger on the opposite side of the biopsy were fixed to make the scrotal skin on the biopsy side in a tense state. The left thumb and index finger were twisted tightly in the middle of the testis to counter the biopsy pressure, then the vas deferens separation forceps were held in the right hand to slowly separate the skin and subcutaneous tissue at the needle eye during spermatic cord block to reach the testicular white membrane. The white membrane continued to be blunt friction to make it thinner. When there was a sense of breakthrough, the vas deferens separation forceps entered the testis. Some testicular tissue was clamped and sent to the laboratory for microscopic examination. After the procedure, gentle pressure was applied for a moment to stop bleeding.

Chromosome detection

The fasting venous blood of the patient's upper limb was collected for chromosome detection which was performed by G-banding karyotype analysis technology, and at least 20 cells were analyzed.

Detection of CNVs by NGS

The venous blood of the upper limbs was collected to extract genomic DNA, and 10 ng DNA was used to construct the library using "rapid PCR free library construction technology", cut the DNA, repaired the enzyme digestion gap and double stranded ends, phosphorylate and modify them and connect them with primers, measure the sequence with the NextSeqCN500 gene sequencer, and analyze the data with the data analysis software from Hangzhou Berry Genomics Diagnosis Technology Company. The results were determined by comprehensively referring to the latest published data of public databases such as GRCh37 (hg19): ENSEMBL release_59/61/64/68/69/75, Database of Genomic Variants (DGV), human chromosome imbalance and phenotype database (DECIPHER), Online Mendelian Inheritance in Man (OMIM) database, University of California Santa Cruz (UCSC) database, and PubMed. Analyses were completed by Beijing Berry Genomics Biotechnology Co., Beijing, China.

Statistical analysis

The statistical analyses were performed using the Statistical

Table 1 Comparative analysis of testicular puncture results and CNVs

Operation	Result	CNV test results, n (%)		χ^2	P
		Normal	Abnormal		
Testicular puncture	Sperm	49 (60.49)	32 (39.51)	4.958	0.037
	Azoospermia	32 (42.67)	43 (57.33)		

CNV, copy number variation.

Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL, USA). Non-normally distributed metric variables were analyzed by the Kruskal-Wallis test and Mann-Whitney U-test. The mean surface areas of the endometriotic implants between the same group (before and after the medical treatment) were analyzed by Wilcoxon's signed-rank test. $P \leq 0.05$ was considered statistically significant. Values were expressed as mean ± standard deviation, unless stated otherwise.

Results

Comparison between testicular puncture results and CNVs

Among the 156 patients, 81 had sperm in the testicular puncture and 75 had no sperm in the testicular puncture. There was a significant difference in CNV detection between the 2 groups ($P<0.05$), as shown in *Table 1*.

Analysis of CNVs on each chromosome

Among the 156 patients, 75 patients had CNVs, with an incidence of 48.08%, including 59 patients with 1 CNV, 9 patients with 2 CNVs, 5 patients with 3 CNVs, 1 patient with 4 CNVs, and 1 patient with 5 CNVs. The chromosomes with CNVs which accounted for more than 5% were 2, 3, 5, 10, 11, and Y, as shown in *Table 2*.

Detailed analysis of CNVs on chromosomes 2, 3, 5, 10, and 11

A total of 132 genes were found on autosomes with CNVs and the percentage of CNVs was more than 5%, including 8 deletions and 124 repetitions. CNVs on chromosome 2 found 11 genes, of which 3 were deleted and 8 were duplicated; 17 genes were found in CNVs on chromosome 3, including 3 deletions and 14 duplications; 12 genes were found in CNVs on chromosome 5, of which 2 were deleted and 10 were repeated; 72 genes were found in CNVs on chromosome 10, all of which were duplicates; CNVs on chromosome 11 found 20 genes, all of which were duplicates. The specific location, size, and genes of CNVs on chromosomes 2, 3, 5, 10, and 11 are shown in *Tables 3*-7, respectively.

Discussion

Spermatogenesis is a programmed, multigene, and complex process (3-7). Any abnormality in any link may lead to the stagnation of spermatogenesis, which may then lead to severe oligozoospermia or azoospermia (8-10). The deletion of azoospermia factor (AZF) confirmed by a large number of studies is a typical example. In fact, there are thousands of genes involved in spermatogenesis, and most of the mechanisms are not clear (3,11,12). It is particularly important to find and clarify the mechanisms of genes related to spermatogenesis, which can aid in many aspects, such as species evolution, spermatogenic arrest, *in vitro* culture, and clinical intervention (13-15).

In this study, we examined the CNVs of 156 azoospermia patients and found that each chromosome had more or less CNVs. We further screened for chromosomes with CNVs which accounted for more than 5% which yielded chromosomes 2, 3, 5, 10, 11, and Y, indicating that there may be important gene fragments affecting spermatogenesis. A large number of genes on the Y chromosome have been confirmed to cause spermatogenesis arrest, while autosomal gene abnormalities have rarely been reported (16-18). In this experiment, 132 genomic CNVs were found in the 5 autosomal chromosomes 2, 3, 5, 10, and 11, including 8 deletions and 124 repeats. A large number of studies have been carried out on gene deletions and mutations, which could be verified by animal experiments through gene knockout, but gene duplication has always been a research blind spot until the emergence of NGS (19-23). This technique provides a strong technical guarantee for the detection of gene duplication, but how to confirm the impact of gene duplication on spermatogenesis in the next step will be challenging.

The size of CNV fragments detected in this study was 0.1–4.64 Mb, and gene free fragments accounted for 25.58% (11/43), suggested that even if there were no known genes in CNVs, the changes in chromosome structure caused by CNVs are of great importance and may also affect spermatogenesis, which is worthy of attention and in-depth research.

NGS technology analyzes Y chromosome aberrations by detecting genomic CNVs over 100 kb. Zhu *et al.* (24)

Table 2 CNV frequency of each chromosome

Chromosome	Defect		Repeat		Total	
	Amount	Proportion (%)	Amount	Proportion (%)	Amount	Proportion (%)
1	0	0.00	3	4.23	3	2.97
2	3	10.00	8	11.27	11	10.89
3	2	6.67	5	7.04	7	6.93
4	0	0.00	2	2.82	2	1.98
5	4	13.33	6	8.45	10	9.90
6	3	10.00	2	2.82	5	4.95
7	2	6.67	2	2.82	4	3.96
8	0	0.00	4	5.63	4	3.96
9	2	6.67	0	0.00	2	1.98
10	0	0.00	7	9.86	7	6.93
11	1	3.33	7	9.86	8	7.92
12	1	3.33	2	2.82	3	2.97
13	1	3.33	0	0.00	1	0.99
14	3	10.00	2	2.82	5	4.95
15	0	0.00	3	4.23	3	2.97
16	2	6.67	2	2.82	4	3.96
17	0	0.00	2	2.82	2	1.98
18	0	0.00	1	1.41	1	0.99
19	0	0.00	1	1.41	1	0.99
20	0	0.00	2	2.82	2	1.98
21	1	3.33	0	0.00	1	0.99
22	0	0.00	0	0.00	0	0.00
X	1	3.33	4	5.63	5	4.95
Y	4	13.33	6	8.45	10	9.90
Total	30	100	71	100	101	100

CNV, copy number variation.

showed that the size of CNVs correlated with male infertility. This study found that duplication (DUP) of Y chromosome CNVs detected by NGS is currently undetectable by sequence tagged site (STS)-polymerase chain reaction (PCR). Therefore, we speculated that gene duplication also has the potential to cause azoospermia. In terms of detection of gene deletions, there are no reports on the concordance between Y-chromosome AZF microdeletions detected by STS-PCR and Y-chromosome CNVs deletions (del) detected by NGS. It has been documented that CNVs in the AZFc region are associated with an increased risk of male spermatogenic failure, but the significance with respect to the detection of deletions in both genes is not clear. Since the current tests are for CNVs over 100kb, there may be some cases of missed STS loci, especially novel STS such as sY1192 or small deletions. If future technologies allow detection of smaller segments of CNVs, it will be of great value to explore Y chromosome gene level contributions to male infertility (2).

The use of STS-PCR to detect Y-chromosomal AZF

Table 3 Correlation of CNVs on chromosome 2

Zone	Type	Specific location	Gene	Size (Mb)
q24.2	del	161320001_161420000	*RBMS1*	0.1
p15	dup	61660000_61820000	*USP34*, *XPO1*	0.16
p24.3	dup	13560001_14340000	There is no gene in this region	0.78
p23.3	dup	25620001_25980000	*ASXL2*, *DTNB*	0.36
p16.3p16.2	del	52860001_53040000	*MIR4431*	0.18
q12.3	dup	109160001_109400000	*RANBP2*, *LIMS1*	0.24
q22.2	dup	142900001_143500000	There is no gene in this region	0.60
p24.3	dup	13560000_14340000	There is no gene in this region	0.78
p16.3	del	50940000_51060000	*NRXN1*	0.12
q37.3	dup	237380001_237720000	*IQCA1*, *ACKR3*	0.34
q14.1	dup	116920001_117340000	There is no gene in this region	0.42

CNV, copy number variation; del, deletion; dup, duplication.

Table 4 Correlation of CNVs on chromosome 3

Zone	Type	Specific location	Gene	Size (Mb)
q11.2	del	94700001_94980000	*LINC00879*	0.28
q29	dup	197020001_197340000	*DLG1-AS1*, *BDH1*, *MIR4797*, *DLG1*	0.32
q26.1	del	165180001_165940000	*BCHE*, *LINC01322*	0.76
q25.32	dup	158060001_158600000	*MFSD1*, *GFM1*, *RARRES1*, *LXN*, *RSRC1*, *LOC100996447*, *MLF1*	0.54
q26.31q26.32	dup	175540001_175880000	There is no gene in this region	0.34
p26.3	dup	820000_1460000	*LINC01266*, *CNTN6*	0.64
p24.2	dup	25160000_25380000	*RARB*	0.22

CNV, copy number variation; del, deletion; dup, duplication.

Table 5 Correlation of CNVs on chromosome 5

Zone	Type	Specific location	Gene	Size (Mb)
q23.1	del	116740001_116920000	*LINC00992*	0.18
p15.31	del	6960000_7200000	There is no gene in this region	0.24
p14.1	del	25080001_25360000	There is no gene in this region	0.28
p15.32p15.31	dup	5720000_6500000	*UBE2QL1*, *MED10*, *LINC02145*	0.78
p12p11	dup	45980000_46400000	There is no gene in this region	0.42
p13.3	dup	32040000_32200000	*PDZD2*, *GOLPH3*	0.16
q23.1	del	117100001_117320000	*LOC102467224*	0.22
q15	dup	94020001_94180000	*SLF1*, *MCTP1*	0.16
q13.2	dup	72620001_72800000	*FOXD1*, *BTF3*, *LINC01386*	0.18
q34	dup	161640000_162360000	There is no gene in this region	0.72

CNV, copy number variation; del, deletion; dup, duplication.

Table 6 Correlation of CNVs on chromosome 10

Zone	Type	Specific location	Gene	Size (Mb)
p14p13	dup	11940001_12300000dup	*MIR548AK*, *UPF2*, *NUDT5*, *DHTKD1*, *SEC61A2*, *CDC123*	0.36
p11.21	dup	35700001_36000000dup	*CCNY*, *MIR4683*, *GJD4*, *FZD8*	0.3
q26.3	dup	135260000_135440000dup	*SPRNP1*, *FRG2B*, *SCART1*, *SYCE1*, *CYP2E1*	0.18
q11.22q11.23	dup	46960000_51600000dup	*ANTXRL*, *GDF10*, *BMS1P5*, *LRRC18*, *OGDHL*, *SYT15*, *GPRIN2*, *AGAP9*, *ZNF488*, *FRMPD2*, *C10orf128*, *C10* or *f71*, *DRGX*, *SLC18A3*, *MSMB*, *TIMM23*, *HNRNPA1P33*, *ANXA8*, *LOC107001062*, *LOC105378292*, *RBP3*, *FAM25C*, *FAM170B*, *PGBD3*, *PARG*, *LINC00842*, *MAPK8*, *ARHGAP22*, *WDFY4*, *FAM170B-S1*, *ERCC6*, *AGAP7P*, *ANXA8L1*, *ANTXRLP1*, *CTSLP2*, *PTPN20*, *CHAT*, *GDF2*, *VSTM4*, *C10* or *f53*	4.64
p12.1	dup	28000001_28760000dup	*ARMC4*, *MKX*, *MIR8086*, *MKX-AS1*, *MPP7*	0.76
p12.33	dup	17540001_17640000dup	*PTPLA*	0.10
q11.22	dup	46960000_47700000dup	*ANTXRL*, *SYT15*, *GPRIN2*, *FAM25BP*, *FAM35DP*, *LOC102724593*, *NPY4R*, *HNRNPA1P33*, *LINC00842*, *ANXA8L1*, *ANTXRLP1*	0.74

CNV, copy number variation; dup, duplication.

Table 7 Correlation of CNVs on chromosome 11

Zone	Type	Specific location	Gene	Size (Mb)
p15.1	dup	16820001_17060000	*PLEKHA7*	0.24
q22.3	dup	105860001_106620000	*AASDHPPT*, *MSANTD4*, *KBTBD3*, *LOC101928535*, *GUCY1A2*	0.76
p14.3	dup	22840001_23280000	*CCDC179*, *SVIP*	0.44
q22.1	del	97440001_97640000	There is no gene in this region	0.20
q22.3	dup	105860001_106620000	*AASDHPPT*, *MSANTD4*, *KBTBD3*, *LOC101928535*, *GUCY1A2*	0.76
q22.3	dup	106660000_107020000	*GUCY1A2*	0.36
p15.4	dup	3780001_4200000	*MIR4687*, *PGAP2*, *RRM1*, *RHOG*, *NUP98*, *STIM1*	0.42
q24.3	dup	129540001_129660000	There is no gene in this region	0.12

CNV, copy number variation; del, deletion; dup, duplication.

microdeletions is an important laboratory genetic test for azoospermia, but exactly how many STS loci are more appropriate to detect remains to be explored. The use of NGS to detect Y-chromosome CNVs has not been widely adopted, and we believe that the 2 assays complement each other and that, typically, NGS may be considered for screening in patients with azoospermia, whereas STS-PCR may be used for validation.

According to the overall analysis, among the 156 patients, 75 had CNVs, with an incidence of 48.08%. Especially in those without sperm in the testicular puncture, the incidence of CNVs was as high as 57.33% (43/75). This phenomenon itself deserves attention. Therefore, it could be inferred that the chromosomal changes caused by CNVs in structure or function may affect the component of spermatogenesis. In this way, it interferes with mitosis and/or meiosis in the process of spermatogenesis, making spermatogenesis stagnate in a certain spermatogenesis stage, resulting in partial or complete spermatogenesis block, clinically manifesting as severe oligozoospermia or azoospermia. Based on this, the next step is to interfere with the related CNVs *in vitro* or *in vivo*, open the obstacle of spermatogenic block, and then restore spermatogenesis, which will be the subject of our future studies.

Acknowledgments

Funding: This research was supported by the Ningxia

Natural Science Foundation (Item No. 2020AAC03495), and the Key Research and Development Program of Ningxia Hui Autonomous Region (2021BEG02029).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-22-301/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-22-301/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-301/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Yinchuan Maternal and Child Health Hospital (No. 2018-079) and written informed consent was obtained from all patients.

References

1. Lu JC, Huang YF, Lü NQ. WHO Laboratory Manual for the Examination and Processing of Human Semen: its applicability to andrology laboratories in China. Zhonghua Nan Ke Xue 2010;16:867-71.
2. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol 2018;15:369-84.
3. Agarwal A, Baskaran S, Parekh N, et al. Male infertility. Lancet 2021;397:319-33.
4. Selvaraju V, Baskaran S, Agarwal A, et al. Environmental contaminants and male infertility: Effects and mechanisms. Andrologia 2021;53:e13646.
5. Joshi M, Rajender S. Long non-coding RNAs (lncRNAs) in spermatogenesis and male infertility. Reprod Biol Endocrinol 2020;18:103.
6. Szymusik I, Warzecha D, Wielgoś M, et al. Infertility in Female and Male Solid Organ Recipients - From Diagnosis to Treatment: An Up-To-Date Review of the Literature. Ann Transplant 2020;25:e923592.
7. Liu Y, Zhang F, Dai L. Cyclooxygenase 1 (COX1) as an indicator of sperm quality in humans. Andrologia 2020;52:e13537.
8. Bertolla RP. Sperm biology and male reproductive health. Sci Rep 2020;10:21879.
9. Alhathal N, Maddirevula S, Coskun S, et al. A genomics approach to male infertility. Genet Med 2020;22:1967-75.
10. Schlegel PN, Sigman M, Collura B, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. Fertil Steril 2021;115:54-61.
11. Neto FT, Bach PV, Najari BB, et al. Spermatogenesis in humans and its affecting factors. Semin Cell Dev Biol 2016;59:10-26.
12. Liu Y, Wang G, Zhang F, et al. An NGS-based approach to identify Y-chromosome variation in non-obstructive azoospermia. Andrologia 2021;53:e14201.
13. Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. Reprod Biol Endocrinol 2018;16:14.
14. Griswold MD. 50 years of spermatogenesis: Sertoli cells and their interactions with germ cells. Biol Reprod 2018;99:87-100.
15. Ibtisham F, Honaramooz A. Spermatogonial Stem Cells for In Vitro Spermatogenesis and In Vivo Restoration of Fertility. Cells 2020;9:745.
16. Pacheco HA, Rezende FM, Peñagaricano F. Gene mapping and genomic prediction of bull fertility using sex chromosome markers. J Dairy Sci 2020;103:3304-11.
17. He S, Xi H, Chen J, et al. Prenatal genetic analysis and differential pregnancy outcomes of two de novo cases showing mosaic isodicentric Y chromosome. Mol Cytogenet 2020;13:7.
18. Ciccarelli M, Giassetti MI, Miao D, et al. Donor-derived spermatogenesis following stem cell transplantation in sterile NANOS2 knockout males. Proc Natl Acad Sci U S A 2020;117:24195-204.
19. Cannarella R, Precone V, Guerri G, et al. Clinical Evaluation of a Custom Gene Panel as a Tool for

Precision Male Infertility Diagnosis by Next-Generation Sequencing. Life (Basel) 2020;10:242.
20. Vara C, Paytuví-Gallart A, Cuartero Y, et al. Three-Dimensional Genomic Structure and Cohesin Occupancy Correlate with Transcriptional Activity during Spermatogenesis. Cell Rep 2019;28:352-367.e9.
21. Maezawa S, Sakashita A, Yukawa M, et al. Super-enhancer switching drives a burst in gene expression at the mitosis-to-meiosis transition. Nat Struct Mol Biol 2020;27:978-88.
22. Zitzmann M, Aksglaede L, Corona G, et al. European academy of andrology guidelines on Klinefelter Syndrome Endorsing Organization: European Society of Endocrinology. Andrology 2021;9:145-67.
23. Atshan M, Kakavand K, Hosseini SH, et al. Evaluation of sperm DNA fragmentation and chromatin structure in infertile men with immotile short-tail sperm defect. Andrologia 2020;52:e13445.
24. Zhu Y, Hu L, Cao D, et al. Chromosomal microarray analysis of infertile men with azoospermia factor microdeletions. Gene 2020;735:144389.

(English Language Editor: C. Betlazar-Maseh)

Cite this article as: Liu Y, Shi D, Zhang F, Dai L, Ma W. Analysis of copy number variations of the autosomal genome in 156 patients with azoospermia. Transl Androl Urol 2022;11(5):675-682. doi: 10.21037/tau-22-301

