GENETICS

Partial-AZFc deletions in Chilean men with primary spermatogenic impairment: gene dosage and Y-chromosome haplogroups

María Cecilia Lardone[1] · Victoria Ortega[1] · Eliana Ortiz[1] · Martha Flórez[1] · Antonio Piottante[2] ·
Mauricio Ebensperger[1,3] · Sandra Flores[4] · Patricio Pezo[4] · Michael Orellana[4] · Mauricio Moraga[4] · Andrea Castro[1]

Abstract

Purpose To investigate the association of partial-AZFc deletions in Chilean men with primary spermatogenic failure and their testicular histopathological phenotypes, analyzing the contribution of *DAZ* dosage, *CDY1* copies, and Y-chromosome haplogroups.

Subjects and methods We studied 479 Chilean men: 334 infertile patients with histological examination (233 cases with spermatogenic defects and 101 normal spermatogenesis, obstructive controls, OC), and 145 normozoospermic controls (NC). AZFc subdeletions were detected by single-tagged sequences and single nucleotide variants analysis. *DAZ*-copy number was quantified by real-time qPCR. Y-chromosome haplogroups (Y-hg) were hierarchically genotyped through 16 biallelic-markers.

Results The prevalence of AZFc-partial deletions was increased in cases (6%) compared with NC (1.4%) ($P = 0.035$). There was no difference between 143 Sertoli-cell only syndrome, 35 maturation arrest, or 35 mix atrophy patients and controls. However, gr/gr deletions were more frequent in 16 subjects with hypospermatogenesis compared with NC ($P = 0.003$) and OC ($P = 0.013$). Y-hg R was the most prevalent (~50%), but decreased among gr/gr deletions (21%, $P = 0.03$). The prevalence of Y-hg M increased in cases versus controls, both in total and non-deleted men (3.9 and 3.7% versus 0.4%, $P = 0.009$ and $P = 0.016$, respectively). Among gr/gr deletions, Y-hg H increased compared with non-deleted men (14.3% versus 0.4%, $P = 0.0047$).

Conclusion Partial-AZFc deletions in a Chilean admixed population are associated with secretory azo/oligozoospermia and might have a role in the development of hypospermatogenesis. Low represented haplogroups, Y-hg M and Y-hg H, show an association with the occurrence of spermatogenic failure and gr/gr deletions respectively; however, additional studies are required.

Keywords AZFc subdeletions · *DAZ* duplications · Y-chromosome haplogroups · Male infertility · Spermatogenic failure

---

María Cecilia Lardone and Victoria Ortega should be considered as joint first authors

✉ Andrea Castro
acastro@med.uchile.cl

[1] Institute of Maternal and Child Research, School of Medicine, University of Chile, Santa Rosa 1234, 8360160 Santiago, Chile

[2] Pathologic Anatomy Service, Clínica Las Condes, 7591046 Santiago, Chile

[3] Urology Department, Hospital Clínico San Borja Arriarán, Servicio Salud Metropolitano Central, 8360160 Santiago, Chile

[4] Human Genetic Program, ICBM, School of Medicine, University of Chile, 8380453 Santiago, Chile

Introduction

The classic deletions on the long arm of the Y chromosome (Yq-microdeletions), comprising one or more regions of the azoospermia factors (AZFa, AZFb, and AZFc), have a prevalence of 5–10% in secretory azo/oligozoospermic men. These deletions represent the main cause of primary spermatogenic failure among infertile men who exhibit a normal karyotype [1–5]. Among them, those that lead to the removal of AZFc are the most prevalent [3]. The complete loss of AZFc region leads to the absence of twelve genes, including three coding protein multicopy gene families (*DAZ*, *CDY1*, and *BPY2*) [3, 6]. In addition to these complete AZFc deletions, three main partial-AZFc deletions have been defined by a different organization of their amplicons, and have been named as gr/gr, b2/b3, and b1/b3 [6–8]. In contrast to the complete or classic deletions, the AZFc subdeletions are not only found among secretory azo/

🖄 Springer

oligozoospermic infertile men, but they are also observed in obstructive, fertile, and/or healthy normozoospermic men. Several meta-analysis of selected case-control studies have suggested that the most prevalent gr/gr and b2/b3 deletions represent true risks for infertility, depending on the ethnicity and geographic location of the subjects, and call for performing larger additional studies [9–11].

Regarding South American populations, association studies of partial-AZFc deletions with spermatogenic failure and the connection with certain Y-chromosome lineages are scarce. To our knowledge, three studies performed in South America have shown a similar prevalence gr/gr deletions between cases and controls [12–14], with an apparently lower prevalence compared with Caucasians and European populations [9, 10]. This evidence suggests that these discrepancies can be caused by the different genetic composition of admixed populations and particular Y-chromosome haplogroups, which may have a genetic predisposition to rearrangements, and a different prevalence among human populations. A previous study showed that the haplogroup Q1a3a (Q-M3), characteristic of the Y chromosome in South Amerindians, was more frequent among Chilean patients with complete AZFb deletions, although no difference was observed in patients with complete or subdeletions of AZFc [15].

Greater complexity in the analysis of the effects of AZFc gene variations and subdeletions, and the impact of different lineages of the Y chromosome are related to the occurrence of duplications that involve this region, either in men without deletions or as a subsequent event in men partially deleted. The analysis of the Y chromosome structure and gene dosage in AZFc has revealed the presence of duplications on complete [16] or on AZFc partially deleted Y chromosomes [17, 18], with no certainty regarding their spermatogenic effects. Recently, a meta-analysis showed that AZFc duplications, including gr/gr duplication-only or more than 4 DAZ gene copies, seem to increase the infertility risk by two- to threefold in Asian, although not in European men [19].

The aim of this study was to investigate the association of partial-AZFc deletions with histologically diagnosed primary spermatogenic failure by comparing their prevalence between Chilean secretory infertile and control men. For this purpose, we classified the different types of AZFc subdeletions (gr/gr, b2/b3, and b1/b3) by the presence or absence of single-tagged sequence (STS) markers, the type of *DAZ*, and *CDY1* copy retained by sequence nucleotide variants (SNVs) analysis and *DAZ* dosage by qPCR. Additionally, we investigated the contribution of Y-chromosome haplogroups.

Material and methods

Subjects

We studied 334 consecutive Chilean men who consulted for infertility and required testicular sperm extraction (TESE)

between March 2003 and March 2017 at two University infertility clinics in Santiago, Chile. Among them, the first 248 subjects had been previously defined for the Q1a3a (YCC2008) haplogroup by the Y chromosome single-nucleotide polymorphism M3 (Y-SNP M3) [15]. All patients underwent a complete evaluation that included a physical examination, hormonal determinations, analysis of Y chromosome microdeletions, and karyotype. Patients were excluded if they had Y chromosome microdeletions, karyotype abnormalities, cryptorchidism, varicocele grades II or III, chronic diseases, morbid obesity, hypogonadism hypogonadotropic, hyperprolactinemia, recent or concomitant hormonal treatment, chronic diseases, retractile testis, male accessory gland infections, genital trauma, occupational exposure to pesticides, and excessive drugs consumption.

Histological analysis was performed as previously described [15], showing 233 patients with spermatogenic failure (cases) and 101 with normal spermatogenesis (obstructive controls). Among the 233 cases biopsy-documented, we observed 143 Sertoli cell-only syndrome (SCOS, 111 complete and 32 focal), 35 maturation arrest (MA), 30 mixed atrophy (MixA), 16 hypospermatogenesis (HSP), and 9 severe atrophy (SA) (Supplementary Table S1).

Additionally, we recruited healthy volunteers for seminal analysis and selected 145 men as normozoospermic controls. Eighty-eight of them had been selected previously [15]. The volunteers were students recruited from the Faculty of Medicine, University of Chile, and fertile patients or public employees from one of the university hospitals where the infertile patients came from. They were interviewed regarding lifestyle, andrological history, chronic diseases, and fertility history. Subjects with chronic diseases, morbid obesity, and drug consumption were excluded. Similarly to infertile men, blood samples were obtained for hormonal assessment and weight and height were determined for BMI calculation. .

All subjects were genetically unrelated and originated from different geographic regions of Chile [20].

Hormonal measurements

Blood samples were collected between 8 and 10 a.m. for determinations of LH and FSH by immunoradiometric assay (Siemens Medical Solutions Diagnostics, LA, CA, USA), and total testosterone by radioimmunoassay (Diagnostic System Laboratories, Webster, TX, USA).

Semen analysis

Semen quality was assessed according to the criteria of World Health Organization (WHO) for the examination of human semen [21]. The Kruger's strict criteria was used for the evaluation of sperm morphology [22], and the cut-off ≥ 4 of normal forms was used to describe a morphologically normal

sample according to WHO 2010 [23]. The seminal pattern of the subjects was defined by at least two separate seminal analyses.

Screening of complete and partial-AZFc deletions

Peripheral blood was obtained for DNA isolation using the Wizard® genomic DNA purification kit (Promega, WI, USA). Azo/oligozoospermic patients were evaluated for Yq-microdeletions with a standard set of 21 Y-specific STS primers covering the AZFa (sY83, sY85, *DFFRY*, *DBY*, sY90), AZFb (*CDY2*, *XKRY*, *EIF1AY*, sY142, sY143, *RBMY*), and AZFc (BPY2a, sY221, sY255, sY153, sY283, sY158) regions, inter-region markers (sY98), Yp (*TSPY*), the centromere (sY78), and heterochromatic region (sY160). Reaction was performed in a multiplex end-point PCR as previously described [24]. Yq-microdeletions were detected in 6.8% of all azo/oligozoospermic infertile men and in 13% of secretory infertile men. Partial-AZFc deletions were evaluated according to a three-step screening of STS described by Repping et al. [6] and Lardone et al. [15], with some modifications. Briefly, PCR assays included the amplification of STS in single reactions (sY1258, sY1197, sY1161, sY1191, sY1291, sY1206, sY1201), and also in multiplex reactions (mix-a: sY1291 and sY1191 in a ratio 2:1; mix-b: sY1201, sY1206, and sY1161 in a ratio 1:1:1; mix-c: sY1258 and sY14 in a ratio 2:1). Indicative of the different subtypes of AZFc subdeletions were the exclusive absence of sY1291 (gr/gr) or sY1191 (b2/b3) and the loss of sY1191, sY1291, sY1161, and sY1197 (b1/b3). At least three independent PCR assays were performed for confirmation of the negative PCR reactions. As a positive control, we used a pair of primers that amplify one specific STS in the DAZ gene (sY254), and another for the centromere of the Y chromosome (sY78).

In order to discriminate among *DAZ* copies, we performed SNVs analysis by the amplification of the target sequence and the following digestion with restriction enzymes (PCR-RFLP) for *DAZ1*, *DAZ2*, *and DAZ4*, and *DAZ3* by PCR of the STS Y-DAZ-3, as previously described [12, 15]. In addition, to differentiate between *CDY1* copies (*CDY1a* and *CDY1b*) in partial-AZFc-deleted subjects, we analyzed the C/A SNV situated 7750 bp upstream of the *CDY1* translation start codon (CDY1–7750: o1025/o1026 pair of primers) by PCR followed by digestion of *CDY1b* with the PvuII restriction enzyme as previously described [25]. Further characterization of the deleted subjects with partial deletions of AZFc included the detection of additional STS to determine the breakpoints to Yq palindromes [26–30].

Quantification of the number of DAZ copies

DAZ gene copy number was quantified by real-time PCR through the amplification of STS SHGC-35663 (GenBank accession number G29902) mapped one time in each DAZ genes (exon 10). As reference gene, a sequence of the single-copy DAZL gene was amplified using a pair of primer designed with Primer3 software (DAZL-sense: 5′-GAATGCTGAATTTT TACTCTTGAAG-3′ and DAZL-antisense: 5′-CTCT ATACGTGGCTAGAGTTC-3′). PCR was performed in triplicate and in separate reactions for DAZ and DAZL gene copy quantification. Each reaction consisted in 10 µl of Fast-Plus EvaGreen® qPCR Master Mix (Biotium, Fremont, CA), 350 nM of each primer and 100 ng of genomic DNA in a final volume of 20 µl. PCR conditions for both amplification were an initial step of 2 min at 95 °C, 40 cycles of 5 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, and a final step of 15 s at 95 °C. The efficiency of PCR was calculated using the slope of a standard curve generated through the amplification of serial dilutions of known concentrations of an identical fragment (1×10^6 to 1×10^2 fragment copies/µl) to that amplified in the sample and inserted in a plasmid. PCR efficiencies for the amplification of STS 60325 and DAZL were 96% and 101%, respectively. The number of copies of the DAZ gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The quantification was expressed relatively to a calibrator group ($n = 10$) selected among those control samples with a ΔCt value between $Ct_{reference}$—$Ct_{target} \approx 1$. The same calibrator group was used in every PCR run. Additionally, 2 DNA samples with AZFc partial deletion, identified by STS markers as described above, were included in every experiment as control of 2 *DAZ* gene copies as well as 2 DNA samples with AZFc duplication detected in previous experiments. *Rq* or fold-change equal to 1 means the double of *DAZ* gene copies compared to the reference gene (i.e 4 *DAZ* copies). *Rq* equal to 0.5, 1.5, and 2 stands for 2, 6, and 8 *DAZ* gene copies, respectively. Cut-off values for *Rq* were determined as the mean ± 2 SD in a first set of subjects that included 209 samples with $Rq \approx 1$ (Rq 0.97 ± 0.12), 8 subjects with partial deletions (Rq 0.52 ± 0.03), 4 samples with $Rq \approx 1.5$ (Rq 1.46 ± 0.9) and 3 subjects with $Rq \approx 2$ (1.77 ± 0.06).

Genotyping of the Y-chromosome haplogroups

We genotyped all 479 male DNA samples (100 ng/µl) for Y-chromosome haplogroups with a set of 16 biallelic markers (SNPs) following a hierarchical approach: first DE-YAP and F-M89, then we determined the derived linages of DE-YAP (D-M174, E-M40), F-M89 (G-P257, H-L901, I-M258J-M304, J2-M172, K-M9), K-M9 (L-M20, M-Page93, P-M45), and P-M45 (Q-M242, Q1a3a1-M3 and R-M207), according to Karafet et al. [31]. The primers for G-P257, H-L901, M-Page93, and R-M207 were designed using the online software Primer-BLAST [32], while the other 14 come from literature (Supplementary Table S2). Since there is knowledge about the proportion of paternal lineages in admixed Chilean population [33], the haplogroups Q1a3a1-M3 and R-M207 were examined first on those individuals belonging to P-M45 haplogroup, as they are the most frequent in the Amerindian and European populations,

respectively. The Q-M3 haplogroup was also independently re-analyzed as previously described [15, 34].

The determination of the allelic state of SNPs was done by PCR-RFLP. The temperatures of annealing and restriction enzymes used are available on Supplementary Material (Supplementary Table S2). Due to the mutation defining haplogroup DE (Alu insertion), only a PCR was carried out, enabling the direct observation of the base pair differences through an agarose gel (2%). The results of the applied techniques were directly analyzed by electrophoresis in an agarose gel (2%).

Statistical analysis

We used the SPSS software version 21 (IBM Corp, Armonk, NY, USA) for statistical comparisons. Differences in proportions between cases and controls were tested by Pearson's χ^2 with Bonferroni correction in contingency tables greater than 2×2, and by Fisher's exact test in contingence tables of 2×2. The strength of the association between dichotomous variables between two groups was calculated by the odds ratio (OR) and was used to estimate the risk among different subsets of cases and/or controls. Differences in continuous variables among groups were compared by the Kruskal–Wallis test and the Mann-Whitney U test. P values less than 0.05 (2-sided) were considered as statistically significant.

Results

Subjects

Clinical and hormonal characterization of the infertile and normozoospermic subjects is shown in Supplementary Material (Table 1). All infertile patients ($N = 334$) required sperm testicular extraction and most had azoospermia (85%) or severe oligozoospermia (11%) (0.8 ± 1.6; range: 0.1–5.2 millions/ml) followed by aspermia or cryptozoospermia (4%). The proportion of azoospermia and oligozoospermia between cases and obstructive controls was similar, and there was a greater proportion of aspermia in obstructive controls (8% versus 1%, $P = 0.001$). As expected, infertile patients with spermatogenic impairment showed increased gonadotropin concentrations, reduced testosterone levels, and a high proportion of subjects had reduced testicular volume compared with controls. Normozoospermic controls were younger, had lower BMI, higher testosterone, and lower FSH than obstructive controls.

Proportion of partial-AZFc deletions and characterization of DAZ/CDY1 gene copies

The screening for partial deletions of AZFc in 233 secretory azo/oligozoospermic men and 246 controls (101 obstructive and 145 normozoospermic) showed a total of 21 subjects (4.4%) with partial-AZFc deletions (Table 2). No statistical differences were observed in the proportion of partial-AZFc deletions between total cases and total or obstructive controls ($P = 0.118$ and $P = 0.802$, respectively), nor between the control groups ($P = 0.127$). Nevertheless, we observed a greater prevalence of partial-AZFc deletions in total cases compared with normozoospermic controls (6% versus 1.4%, $P = 0.035$). Furthermore, we detected statistical differences when we grouped the cases by testicular phenotypes of spermatogenic failure and compared the prevalence of partial-AZFc deletions with both groups of controls ($P = 0.024$), normozoospermic ($P = 0.011$), or all controls together ($P = 0.026$) (Supplementary Table S3).

In this sense, only cases with the testicular phenotype of HSP showed a statistical significant higher proportion of partial-AZFc deletion compared with total controls (18.8% versus 2.8%, $P = 0.017$), with an OR of 7.88 (95% CI = 1.82–34.03), and with normozoospermic controls (18.8% versus 1.4%, $P = 0.007$) with an OR of 16.5 (95% CI = 2.52–107.82) (Table 2). Likewise, the proportion of gr/gr subtype was significantly increased in HSP compared with the total and normozoospermic controls ($P = 0.013$ and $P = 0.003$, respectively), reaching an OR of 9.2 (95% CI = 2.07–41.12) and 33.2 (95% CI = 3.22–342.67), respectively. Among men with severe atrophy, the b1/b3 deletion showed a higher proportion compared with total controls (11.1% versus 0%, $P = 0.036$) (Table 2). Additional comparisons performed for the types and subtypes of partial deletions in more than two groups of subjects are shown in Supplementary Table S3.

Supplementary data on Table S4 and S5 shows the clinical, histological, genetic, and seminal characterization of men with partial-AZFc deletions.

Concerning which of the DAZ gene copies were removed in partial-AZFc deletions, we observed that the loss of the gene pair DAZ1/2 (11/14, 78.6%) was more frequent than the loss of DAZ3/4 in gr/gr deletions compared with b2/b3 deletions where the loss of DAZ3/4 was more common (5/6, 83.3%) ($P = 0.018$). The same analysis in cases showed that gr/gr deletions involved the loss of DAZ1/2, and that b2/b3 deletions involved exclusively the loss of DAZ3/4 ($P = 0.07$), and no differences were observed in controls. Moreover, no significant differences were observed when we separated by types of spermatogenic failure or control groups (Supplementary Table S3). Concerning b1/b3 deletions, the only patient with this deletion showed the loss of DAZ1/2 gene copies.

As regards the absent *CDY1* copy in partial deletions, the same proportion of loss of *CDY1a* and *CDY1b* was observed in subjects with gr/gr (7/14, 50%) or b2/b3 deletions (3/6, 50%). In addition, there was no difference in the proportion of *CDY1* copy types deleted between cases and controls either with gr/gr or b2/b3 deletions (Supplementary Table S3).

Table 1 Clinical and hormonal characterization in cases and controls

	Cases	All controls	OC	NC
Age (years)	34 (30–38)[a, b]	32.0 (25–38)[b]	35.0 (32–39)[a, c]	27.0 (23–35)[a, c]
BMI (Kg/m^2)	27.1 (25–30)[a]	26.8 (24–29)	26.9 (25–29)[c]	23.7 (21–27)[a, c]
FSH (mIU/ml)	13.8 (9–24)[a, b]	2.9 (2–5)[b]	3.1 (2–6)[a, c]	2.8 (2–4)[a, c]
LH (mIU/ml)	5.1 (3–8)[a, b]	2.4 (2–4)[b]	2.2 (2–3)[a, c]	2.5 (2–4)[a, c]
Testosterone (nmol/l)	11.1 (8–14)[a, b]	13.9 (10–19)[b]	11.8 (9–15)[c]	15.9 (12–23)[a, c]
Estradiol (pmol/l)	122.4 (97–169)	124.8 (94–159)	118.8 (81–164)	128.5 (107–158)
SHBG (nmol/l)	27.1 (21–39)	31.2 (19–48)	27.0 (19–45)[c]	47.9 (35–67)[c]
Free testosterone (pmol/l)	218.1 (174–305)	241.8 (184–297)	241.7 (184–295)	245.0 (183–389)
Sperm count	0.0 (0.0–0.0)	–	0.0 (0.0–0.0)	89.3 (54–149)
Reduced testicular volume	54	6	7	0
Number of subjects	233	246	101	145

Values show the median (25th–75th percentile) or percentage of patients with reduced testicular volume (average of both testicles less than 15 ml measured with the Prader orchidometer). [a] $P < 0.05$ between Cases and obstructive controls (OC) or normozoospermic controls (NC) by Mann-Whitney test. [b] $P < 0.05$ between Cases and All controls by Mann-Whitney test. [c] $P < 0.05$ between OC and NC by Mann-Whitney test. Reference ranges (commercial kit): FSH 1.0–8.0 mIU/ml; LH 1.0–8.0 mIU/ml; Testosterone (T) 2.0–8.0 ng/ml; Estradiol < 50 pg/ml; SHBG 10–80 nmol/l. Free testosterone was calculated from T and SHBG as previously described [1]

1. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84(10):3666–72. https://doi.org/10.1210/jcem.84.10.6079

In patients with HSP, the constitution gr/gr-del DAZ1/2 was significantly more frequent compared with total controls (18.8% vs 1.2%, $P = 0.004$), obstructive controls (18.8% vs 2.0%, $P = 0.019$), or normozoospermic controls (18.8% vs 0.7%, $P = 0.003$); and the association with the loss of CDY1a (gr/gr-del DAZ1/2-CDY1a) was observed exclusively in five cases (2.2% vs 0% of controls, $P = 0.026$) including two patients with HSP (13.3% versus 0% of controls; $P = 0.003$). Furthermore, when we compared gr/gr deletions without DAZ duplication, DAZ1/2(DAZx2), and gr/gr-DAZ1/2(DAZx2)-CDY1a, significant higher proportions were observed in cases with HSP compared with normozoospermic (20% vs 0.7%; $P = 0.003$ and 14.3% vs 0%; $P = 0.08$, respectively), or obstructive controls (20% vs 2.2%; $P = 0.020$ and 14.3% vs 0%; $P = 0.018$, respectively). As expected, the subject with the b1/b3 deletion showed two copies of CDY1 (CDY1a and CDY1b).

The complete characterization of the STS employed and the breakpoints defined for subjects with partial-AZFc deletions based on the structure of the non-inverted reference Y chromosome is shown in Fig. 1. The possible rearrangements and Y-chromosome structures from which these deletions might be occurred are shown in Supplementary Material (Fig. S1).

Dosage of DAZ gene copies in partial-AZFc deleted and non-deleted men

The quantification of DAZ copies was performed in 473/479 subjects. We observed that most men with AZFc-partial deletions showed 2 DAZ copies, except for 2 patients with complete SCOS and gr/gr-DAZ1/2-CDY1b deletion in whom 4 DAZ gene copies were detected (Table 3 and Supplementary Table S4). As expected, in most non-deleted men, the number of DAZ copies was 4, and only 25 of them showed a higher

Table 2 Frequency of partial-AZFc deletions in cases and controls

| | All cases | Histopathological phenotypes in cases | | | | | Type of controls | | |
		SCOS	MA	HSP	MixA	SA	All controls	OC	NC
Partial-AZFc deletions	14 (6)[a]	8 (5.6)	0	3 (19)[b, c]	2 (6.7)	1 (11)	7 (2.8)[b]	5 (5)	2 (1.4)[a, c]
gr/gr	8 (3.4)	3 (2.1)	0	3 (19)[d, e]	2 (6.7)	0	6 (2.4)[d]	5 (5)	1 (0.7)[e]
b2/b3	5 (2.1)	5 (3.5)	0	0	0	0	1 (0.4)	0	1 (0.7)
b1/b3	1 (0.4)	0	0	0	0	1 (11)[f]	0[f]	0	0
Total	233	143	35	16	30	9	246	101	145

Values are presented as number of subjects (percentage). SCOS, Sertoli cell only syndrome; MA, maturation arrest; HSP, hypospermatogenesis; MixA, mixed atrophy; SA, severe atrophy; OC, obstructive controls; NC, normozoospermic controls. Same superscript letter indicates statistical difference between a group of cases and a control group, by Fisher's exact test; [a] $P = 0.035$; [b] $P = 0.017$; [c] $P = 0.007$; [d] $P = 0.013$; [e] $P = 0.003$; [f] $P = 0.036$
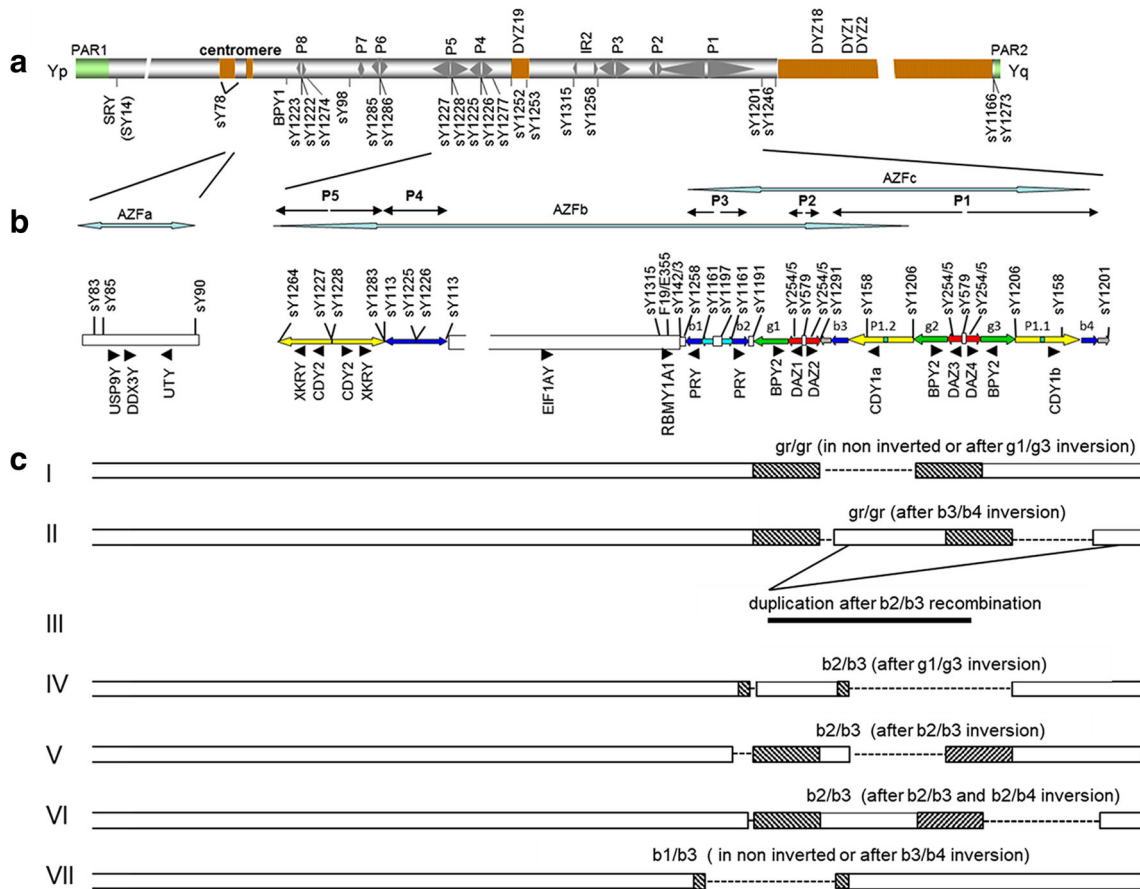
Fig. 1 Schematic representation of the Y chromosome in patients with partial-AZFc deletions. (A) Reference Y-chromosome structure (R1b haplogroup) showing palindromes P1 through P8 (gray arrowheads), pseudoautosomal regions PAR1 and PAR2 (green blocks) and hetero-chromatic (orange blocks). (B) Expanded and detailed view of AZF regions and palindromes, with STSs employed in the determination of partial-AZFc deletion and full characterization of the patients. AZF region residing genes are indicated with black arrowheads. (C) Schematic line-drawing showing different types of partial-AZFc deletions observed in this study with each possible structure of the Y chromosomes from which the rearrangements could have originated in parenthesis (see Fig. S1 for more detail). Solid white bars encompass STSs found to be present. Dotted lines indicate the chromosomal segments and/or STS deleted. Diagonally striped bars indicate breakpoint intervals involved in the recombination

number of *DAZ* copies (5/133 SCOS, 1/35 MA, 1/16 HSP, 5/28 MixA, 13/236 controls): with 6 (7 cases and 12 controls), 8 (4 cases and 1 control) or > 8 (1 case) DAZ gene copies (Table 3). Regardless of the presence of partial-AZFc deletions, the comparison of *DAZ* duplications between cases and controls, and among the different histological groups, showed no significant differences.

Haplogroups of the Y chromosome

The analysis of the Y-chromosome haplogroups (Y-hg) showed that Y-hg R was the most prevalent (51%), followed by the haplogroups J, E, Q-M3, I (16–8%) and F, M, G, H and Q-M242 (2.3–0.2%). No significant differences were observed between obstructive and normozoospermic controls ($P = 0.063$), hence why they were treated as one group for further comparisons. Moreover, a similar distribution of the Y-hg was observed when we compared cases and both control groups originating from different regions of Chile ($P = 0.889$). Among total subjects, 368 (77%) were living in the Metropolitan Region (MR),

Table 3 DAZ gene duplications in partial-AZFc deleted and non-deleted men

Group of Subjects	Cases	All controls	OC	NC
Non-deleted	216	236	94	142
DAZ duplicated	12 (5.6)	13 (5.5)	6 (6.4)	7 (4.9)
Partial-AZFc deleted	14	7	5	2
DAZ duplicated	2 (14.3)	0	0	0
gr/gr deleted	8	6	5	1
DAZ duplicated	2 (25.0)	0	0	0

For each group of subjects, the number of men is shown in the upper row and the number and percentage (in parenthesis) of subjects with duplications of *DAZ* gene copies is shown in the row below. No significant differences in the proportion of *DAZ* duplications were observed ($P > 0.05$, $\chi 2$ test after Bonferroni correction and Fisher's exact tests)

and the rest in different regions of Continental Chile (I–XII and XIV–XVI). While normozoospermic controls came mainly from MR (97%), and only 67% of cases and 71% obstructive controls came from this area, similar residence was observed among cases and subgroups of controls in MR ($P = 0.332$). In addition, a similar geographic distribution was observed between subjects with or without partial-AZFc deletion ($P = 0.980$) or among gr/gr, b2/b3, and b1/b3 subdeletions ($P = 0.941$).

The analysis of different Y chromosome lineages showed different distribution between cases and controls ($P = 0.005$) (Fig. 2). We observed a higher proportion of Y-hg M in cases, both in all subjects or in those without partial-AZFc deletions ($P \leq 0.01$), with an OR of 9.8 and 9.0, respectively (Fig. 2).We did not observe any significant difference in the proportion of Y-hg M among all types of documented spermatogenic failure (MIxA, MA, SCOS, HSP, and SA, $P = 0.283$).

Although a similar Y-hg distribution was observed between cases and controls with AZFc-partial deletions ($P = 0.71$) or gr/gr deletions ($P = 0.277$), Y-hg H, one of the least represented haplogroups in our population, showed an increased proportion among subjects with partial-AZFc deletions ($P = 0.009$), or with the gr/gr deletion ($P = 0.004$) compared with non-deleted subjects, increasing the risk estimation by 24- and 38-fold, respectively (Fig. 2 and Supplementary Table S4). Moreover, we observed that Y-hg H is highly represented in gr/gr and gr/gr-DAZ1/2-CDY1a deleted cases ($P = 0.034$ and $P = 0.02$, respectively). In contrast, the analysis of the Y-hg R, the most prevalent Y-hg in our population, showed a lower proportion in AZFc partially deleted and in gr/gr-deleted patients, compared with those without partial-AZFc deletions ($P = 0.04$) (Fig. 2).

Meanwhile, Y-hg G was exclusively present in controls (4 obstructive and 5 normozoospermic), all of which showed absence of partial deletions or *DAZ* duplications.

On the other hand, the analysis of Y-hg distribution between subjects with or without *DAZ* duplications did not show any statistical difference, either in all subjects ($P = 0.969$), in cases ($P = 0.987$), in total controls ($P = 0.887$), in obstructive $P = 0.756$), or normozoospermic controls ($P = 0.825$). *DAZ* duplications were found in patients with R (14/241), Q-M3 (2/42), M (1/10), J (4/72), I (1/37), F (1/11), and E (4/46) Y-haplogroups.

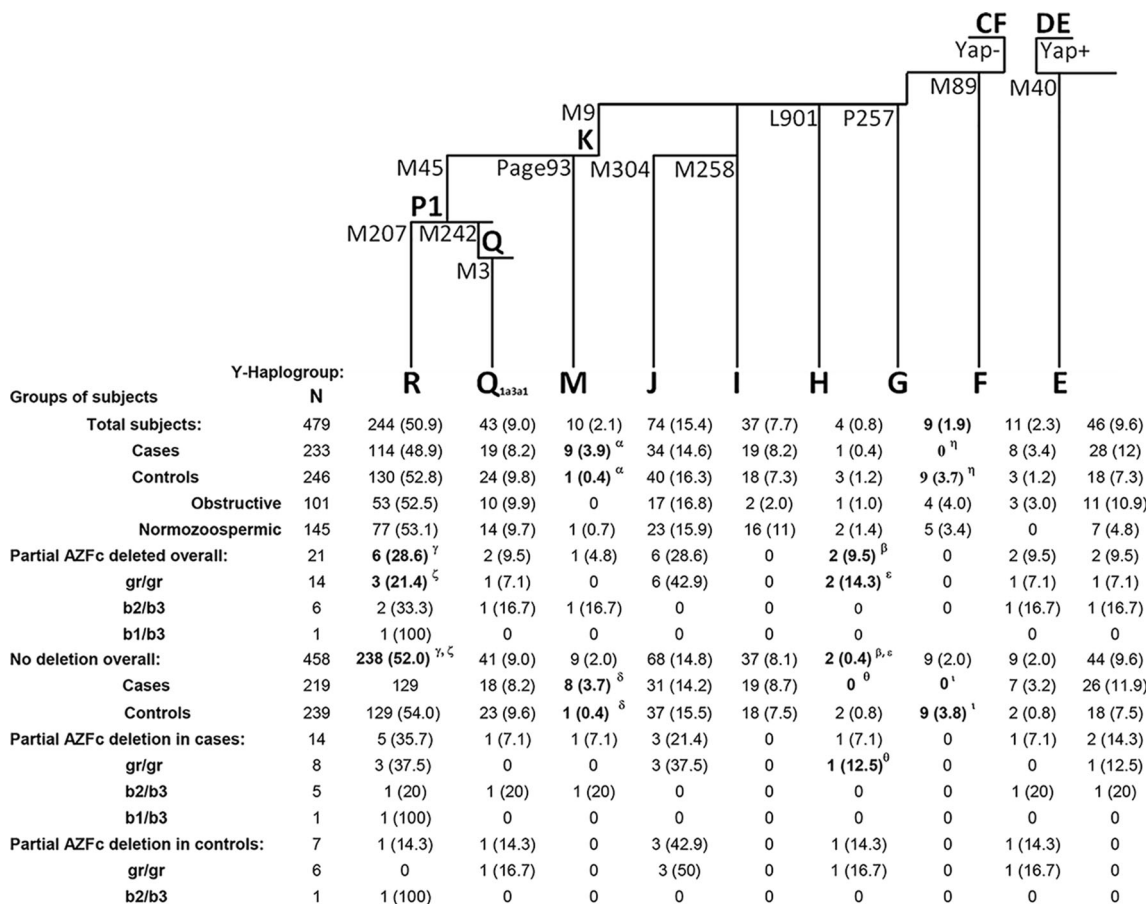| Groups of subjects	N	R	Q$_{1a3a1}$	M	J	I	H	G	F	E
Total subjects:	479	244 (50.9)	43 (9.0)	10 (2.1)	74 (15.4)	37 (7.7)	4 (0.8)	9 (1.9)	11 (2.3)	46 (9.6)
Cases	233	114 (48.9)	19 (8.2)	9 (3.9) $^\alpha$	34 (14.6)	19 (8.2)	1 (0.4)	0 $^\eta$	8 (3.4)	28 (12)
Controls	246	130 (52.8)	24 (9.8)	1 (0.4) $^\alpha$	40 (16.3)	18 (7.3)	3 (1.2)	9 (3.7) $^\eta$	3 (1.2)	18 (7.3)
Obstructive	101	53 (52.5)	10 (9.9)	0	17 (16.8)	2 (2.0)	1 (1.0)	4 (4.0)	3 (3.0)	11 (10.9)
Normozoospermic	145	77 (53.1)	14 (9.7)	1 (0.7)	23 (15.9)	16 (11)	2 (1.4)	5 (3.4)	0	7 (4.8)
Partial AZFc deleted overall:	21	6 (28.6) $^\gamma$	2 (9.5)	1 (4.8)	6 (28.6)	0	2 (9.5) $^\beta$	0	2 (9.5)	2 (9.5)
gr/gr	14	3 (21.4) $^\zeta$	1 (7.1)	0	6 (42.9)	0	2 (14.3) $^\varepsilon$	0	1 (7.1)	1 (7.1)
b2/b3	6	2 (33.3)	1 (16.7)	1 (16.7)	0	0	0	0	1 (16.7)	1 (16.7)
b1/b3	1	1 (100)	0	0	0	0	0	0	0	0
No deletion overall:	458	238 (52.0) $^{\gamma,\zeta}$	41 (9.0)	9 (2.0)	68 (14.8)	37 (8.1)	2 (0.4) $^{\beta,\varepsilon}$	9 (2.0)	9 (2.0)	44 (9.6)
Cases	219	129	18 (8.2)	8 (3.7) $^\delta$	31 (14.2)	19 (8.7)	0 $^\theta$	0 $^\iota$	7 (3.2)	26 (11.9)
Controls	239	129 (54.0)	23 (9.6)	1 (0.4) $^\delta$	37 (15.5)	18 (7.5)	2 (0.8)	9 (3.8) $^\iota$	2 (0.8)	18 (7.5)
Partial AZFc deletion in cases:	14	5 (35.7)	1 (7.1)	1 (7.1)	3 (21.4)	0	1 (7.1)	0	1 (7.1)	2 (14.3)
gr/gr	8	3 (37.5)	0	0	3 (37.5)	0	1 (12.5) $^\theta$	0	0	1 (12.5)
b2/b3	5	1 (20)	1 (20)	1 (20)	0	0	0	0	1 (20)	1 (20)
b1/b3	1	1 (100)	0	0	0	0	0	0	0	0
Partial AZFc deletion in controls:	7	1 (14.3)	1 (14.3)	0	3 (42.9)	0	1 (14.3)	0	1 (14.3)	0
gr/gr	6	0	1 (16.7)	0	3 (50)	0	1 (16.7)	0	1 (16.7)	0
b2/b3	1	1 (100)	0	0	0	0	0	0	0	0

Fig. 2 Phylogeny of the Y chromosome with the Y-haplogroups determined by binary markers. For each of the haplogroups of the Y chromosome, it is shown the number of subjects and in parentheses the percentage that they represent. Odds ratio (OR) were calculated for a 2 × 2 contingence table and P values were calculated by two-tailed Fisher's exact test.$^\alpha$OR = 9.84; 95% CI = 1.24–78.32; $P = 0.009$.$^\beta$ OR = 24; 95% CI = 3.21–179.66; $P = 0.010$.$^\gamma$ OR = 0.37; 95% CI = .14–0.97; $P = 0.044$.$^\delta$ OR = 9.0; 95% CI = 1.12–72.74; $P = 0.016$.$^\varepsilon$ OR = 38, 95% CI = .93–292.86; $P = 0.0047$. $^\zeta$ OR = 0.25, 95% CI = 0.07–0.92; $P = 0.03$.$^\eta$ $P = 0.037$. $^\theta$ $P = 0.0035$.$^\iota$ $P = 0.0039$. Symbol indicates the compared groups

Discussion

Most of the studies which associate partial-AZFc deletions with an increased risk of infertility have been performed in azo/oligozoospermic men and show that gr/gr deletions have an impact among Caucasian populations [9, 10]. Besides, the contribution of b2/b3 deletion is more controversial and has shown some significance in East Asia and North Africa populations [9–11]. Otherwise, there are very few studies conducted in South American patients, and even less in patients with known spermatogenic phenotypes [12–14, 35–37]. Moreover, until now, there were no studies that analyze the contribution of DAZ gene dosage, CDY1 copies, and haplogroups of the Y chromosome in Chilean men, which is characterized by the admixture of European and Native indigenous population (Amerindians and Polynesians from Easter Island) [38, 39].

Several studies have reported that partial-AZFc deletions occur in azoospermic, oligozoospermic, or normozoospermic men; however, they are more commonly associated with low sperm counts. Therefore, gr/gr deletions are considered predisposing factor for oligozoospermia in European and South Asia populations [9, 40, 41]. In agreement with this concept, we observed that patients with the histopathological phenotype of hypospermatogenesis showed a higher proportion of total partial-AZFc deletions and gr/gr subdeletions which, as expected, was the most common type. Moreover, the loss of DAZ1/2 pair observed among gr/gr deletions was significantly increased in patients with hypospermatogenesis, in concordance to previous findings of their association with severe oligozoospermia or spermatogenic impairment [7, 42, 43]. Even though we studied a limited number of 16 subjects with hypospermatogenesis, all our cases with primary spermatogenic failure were documented by a rigorous evaluation of testicular biopsy. On the other hand, most of the previous studies include patients classified as secretory testicular insufficiency after physical examination and anamnesis [9, 10, 16, 44], while others exclusively show the spermatogenic phenotypes of patients with partial deletions [35], likely under- or over-estimating the impact of partial deletions on each testicular phenotypes. It should be noted that SCOS was the main histological condition observed among our secretory patients and, similarly to the MA and MixA groups, had a comparable prevalence of partial AZFc deletions. Therefore, in Chilean secretory azo/oligozoospermic men, partial-AZFc deletions do not seem to represent a risk factor for all types of spermatogenic failure; and they are more likely associated with the hypospermatogenesis phenotype. However, a larger group of subjects with histological diagnosis of hypospermatogensis or, at least secretory oligozoospermia, would be necessary to support these findings.

In this work, we also included azoospermic obstructive infertile patients, as quantitative normal spermatogenesis controls since these subjects were recruited from the same infertile population and the same geographical area as cases, preventing selection biases for the subsequent Y chromosome haplogroup analysis. Even though obstructive controls present normal spermatogenesis in their biopsy analysis, this cannot predict normal seminal parameters other than sperm count, leaving sperm morphology and motility as non-evaluable parameters on biopsy. Therefore, the suggestive higher relative proportion of partial-AZFc deletions in obstructive than normozoospermic controls (not statistically significant, Table 2) could be attributed to the association of partial-AZFc deletions with sperm morphology and/or motility, as some authors have suggested [18, 37].

Among ethnic factors, the differential prevalence of certain lineages of the Y chromosome may affect the association between partial-AZFc deletions and spermatogenic failure in human populations. For example, after the exclusion of the N1 or N* haplogroups, which are fixed with b2/b3 deletion and distributed in North/Eastern Europe and Asia [6, 45, 46], the b2/b3 deletion has a lesser prevalence than gr/gr deletions [11]. In addition, the greater prevalence of the gr/gr deletion in Asian men may be due to the presence of the gr/gr-deleted Q1 [47] and D2 [6, 48] haplogroups, commonly seen in China and Japan, respectively.

The typing of Y-chromosome biallelic markers in the present study shows similar prevalences to those obtained by Toscanini et al. [38] in 978 unrelated Chilean males using Y-haplogrouping prediction. European Y chromosomes belonging to the Y-hg R are the most prevalent in Chilean population, with R1b reaching 97% within Y-hg R [38]. Moreover, a similar distribution of haplogroups was observed in different regions of Chile, indicating that our population is quite uniform throughout the country. In addition, the distribution of the residential areas of the cases and controls (in or outside the capital) was similar, and between the subjects with or without partial-AZFc deletions, suggesting that this was not a contributing factor for the prevalence of partial-AZFc deletions.

In our subjects, Y-hg H was present at a low frequency, similarly as observed by Toscanini et al. [38]. This haplogroup is prevalent in Dravidians population of the Indian subcontinent (South Asia) [49], and in Romani subjects from Europe [50], but is also present in a lower proportion in Chile [38] and the rest of the world. In accordance with our findings, a Brazilian study observed that gr/gr-deleted men had the haplogroups R, F*, K*, and E, which were common among men in this population [14]. However, we discriminated among haplogroups G, H, I included in F* (xJ,K), and showed that Y-hg H increases the odds of partial-AZFc deletions and of gr/gr deletions by 24- and 38-fold respectively. One additional study performed in South Americans, showed that Uruguayan men have a higher prevalence of the Y-hg F(xK) among subjects with abnormal sperm morphology compared with men from other Y-chromosome lineages [37]. However,

it should be noted that Y-hg H was observed only in 4/479 subjects and, therefore, it is premature to draw definitive conclusions. Interestingly, the subclade H1a1a (M82) of the Y-hg H is prevalent in India, and recently Rani et al. [51] showed a higher proportion of AZFc partial deletions in this population.

Similarly, the Y-hg M also has a restricted geographical distribution. It is found in Oceania, mainly in Melanesia and less frequently in West and Central Polynesia [52–54]. In this study, Y-hg M was present in 10/479 (2.1%) of subjects that could have been originated in Easter Island (Chilean island in the South-Eastern Pacific Ocean), in agreement with the proposal of its Polynesian ancestral origin [53, 54], and/or with the repatriation of Polynesian slaves that were not native to this island in the nineteenth century [55, 56]. Although, the low frequency of this haplogroup does not allow us to draw definite conclusions, it is interesting to note that Y-hg M is more represented in cases than in controls, both in all subjects or after removing AZFc partially deleted men, showing a tenfold higher risk.

In contrast to Y-hg M, Y-hg G is widely represented in western Eurasia and was detected exclusively among the controls without partial-AZFc deletions, *CDY1* loss, or *DAZ* duplications. Balaresque et al. [57] reported that, compared with Y-hg R, this haplogroup has a higher rate of rearrangements in the proximal part of AZFc. In agreement with our results, other authors have reported that Y-hg R could have a protective role, based on their lesser representation in partial-AZFc deletions [1, 57]. Further studies that include the characterization of greater number of subjects and more refined Y-chromosome haplogrouping will help to elucidate the significance of the associations observed in this study.

In reference to the number of *DAZ* copies, some studies suggest a compensation in gr/gr-deleted males [18], or even further spermatogenic deterioration in b2/b3-deleted men [17]. Interestingly, the study of Lu et al. [58] in secretory azoospermic men of the Han Chinese population suggested that a greater susceptibility to spermatogenic impairment given by the Y-hg K* was related to an increased dosage of *DAZ* [58]. The Y-hg K* has been observed more frequently among azo/oligozoospermic Han Chinese men compared with fertile controls [59]. We did not observe an increased proportion of *DAZ* duplications in the derivate haplogroups R, Q-M3, and M. However, after discriminating haplogroups R, Q-M3, and M, we observed that the Y-hg M would increase the risk of primary spermatogenic failure but not partial-AZFc deletions or *DAZ* duplications.

Conclusions

In the present study we observed that partial-AZFc deletions are associated with spermatogenic failure, and gr/gr deletions show a potential association with the histological phenotype of hypospermatogenesis in Chilean men. Moreover, two underrepresented Y-chromosome haplogroups in this admixed population have an association with the occurrence of spermatogenic failure (Y-haplogroup M) or gr/gr deletions (Y-haplogropup H); however, larger studies are required

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent and ethical statement The Institutional Review Boards of the University of Chile, School of Medicine, and Hospital Clínico San Borja Arriarán (Santiago, Chile) approved the study in accordance with the Helsinki Declaration, and all subjects gave their informed consent.

References

1. Huang X, Wang F, Wang K. Paracetamol versus ibuprofen for the treatment of patent ductus arteriosus in preterm neonates: a meta-analysis of randomized controlled trials. J Matern Fetal Neonatal Med. 2017;31:1–7. https://doi.org/10.1080/14767058.2017.1338263.

2. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev. 2001;22(2):226–39.

3. Krausz C, Hoefsloot L, Simoni M, Tuttelmann F, European Academy of A, European Molecular Genetics Quality N. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology. 2014;2(1):5–19. https://doi.org/10.1111/j.2047-2927.2013.00173.x.

4. Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. J Clin Endocrinol Metab. 2005;90(1):152–6. https://doi.org/10.1210/jc.2004-1469.

5. Punab M, Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod. 2017;32(1):18–31. https://doi.org/10.1093/humrep/dew284.

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(3):247–51.

7. 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(3):286–98.

8. 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(6):1046–52.

9. 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. https://doi.org/10.1038/srep19798.

10. 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(2):197–209.

11. Bansal SK, Gupta G, Rajender S. Y chromosome b2/b3 deletions and male infertility: a comprehensive meta-analysis, trial sequential analysis and systematic review. Mutat Res Rev Mutat Res. 2016;768:78–90. https://doi.org/10.1016/j.mrrev.2016.04.007.

12. Lardone MC, Parodi DA, Ebensperger M, Penaloza P, Cornejo V, Valdevenito R, et al. AZFc partial deletions in Chilean men with severe spermatogenic failure. Fertil Steril. 2007;88(5):1318–26.

13. Lardone MC, Piottante A, Valdevenito R, Ebensperger M, Castro A. Histological and hormonal testicular function in oligo/azoospermic infertile men. Andrologia. 2013;45(6):379–85. https://doi.org/10.1111/and.12026.

14. Carvalho CM, Zuccherato LW, Bastos-Rodrigues L, Santos FR, Pena SD. No association found between gr/gr deletions and infertility in Brazilian males. Mol Hum Reprod. 2006;12(4):269–73. https://doi.org/10.1093/molehr/gal029.

15. Lardone MC, Marengo A, Parada-Bustamante A, Cifuentes L, Piottante A, Ebensperger M, et al. Greater prevalence of Y chromosome Q1a3a haplogroup in Y-microdeleted Chilean men: a case-control study. J Assist Reprod Genet. 2013;30(4):531–8. https://doi.org/10.1007/s10815-013-9950-z.

16. Giachini C, Laface I, Guarducci E, Balercia G, Forti G, Krausz C. Partial AZFc deletions and duplications: clinical correlates in the Italian population. Hum Genet. 2008;124(4):399–410. https://doi.org/10.1007/s00439-008-0561-1.

17. Lu C, Zhang F, Yang H, Xu M, Du G, Wu W, et al. Additional genomic duplications in AZFc underlie the b2/b3 deletion-associated risk of spermatogenic impairment in Han Chinese population. Hum Mol Genet. 2011;20(22):4411–21. https://doi.org/10.1093/hmg/ddr369.

18. 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(12):2457–63. https://doi.org/10.1093/hmg/ddr119.

19. Xie S, Zhang Y, Yang Y. Is the primary AZFc duplication a potential risk for male infertility?: a systematic review and meta-analysis. Andrology. 2020;8:996–1004. https://doi.org/10.1111/andr.12800.

20. Available from: https://www.bcn.cl/siit/nuestropais/nuestropais/div_pol-adm.htm. Accessed September, 24 2018.

21. WHO. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge; 1999.

22. Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, Acosta AA. A quick, reliable staining technique for human sperm morphology. Arch Androl. 1987;18(3):275–7.

23. WHO. WHO laboratory manual for the Examination and processing of human semen. 5th ed. Geneva; 2010.

24. Castro A, Zambrano N, Kaune H, Madariaga M, Lopez P, Mericq V. YqTER deletion causes arrest of spermatogenesis in early puberty. J Pediatr Endocrinol Metab. 2004;17(12):1675–8.

25. 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.

26. Castro A, Rodriguez F, Florez M, Lopez P, Curotto B, Martinez D, et al. Pseudoautosomal abnormalities in terminal AZFb+c deletions are associated with isochromosomes Yp and may lead to abnormal growth and neuropsychiatric function. Hum Reprod. 2017;32(2):465–75. https://doi.org/10.1093/humrep/dew333.

27. Lange J, Skaletsky H, Bell GW, Page DC. MSY breakpoint mapper, a database of sequence-tagged sites useful in defining naturally occurring deletions in the human Y chromosome. Nucleic Acids Res. 2008;36(Database issue):D809–14. https://doi.org/10.1093/nar/gkm849.

28. Lange J, Skaletsky H, van Daalen SK, Embry SL, Korver CM, Brown LG, et al. Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. Cell. 2009;138(5):855–69. https://doi.org/10.1016/j.cell.2009.07.042.

29. 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(4):906–22.

30. Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS, et al. The human Y chromosome: a 43-interval map based on naturally occurring deletions. Science. 1992;258(5079):52–9.

31. 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(5):830–8.

32. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 2012;13:134. https://doi.org/10.1186/1471-2105-13-134.

33. Moraga Vergara M, Pezo Valderrama P, de Saint Pierre Barrera M. El genoma de herencia uniparental en el estudio de las poblaciones fundadoras. In: Berrios del Solar S, editor. El ADN de los chilenos y sus orígenes genéticos. Santiago: Editorial Universitaria; 2016.

34. Underhill PA, Jin L, Zemans R, Oefner PJ, Cavalli-Sforza LL. A pre-Columbian Y chromosome-specific transition and its implications for human evolutionary history. Proc Natl Acad Sci U S A. 1996;93(1):196–200.

35. Li Q, Song NH, Cao WZ, Shao Q, Xie JJ, Liu C, et al. Relationship between AZFc deletions and testicular histology in infertile South Chinese men with azoospermia and severe oligospermia. SpringerPlus. 2016;5(1):1805. https://doi.org/10.1186/s40064-016-3512-7.

36. Ferrás C, Fernandes S, Marques CJ, Carvalho F, Alves C, Silva J, et al. AZF and DAZ gene copy-specific deletion analysis in maturation arrest and Sertoli cell-only syndrome. Mol Hum Reprod. 2004;10(10):755–61.

37. Skowronek MF, Velazquez T, Mut P, Figueiro G, Sans M, Bertoni B, et al. Associations between male infertility and ancestry in South

Americans: a case control study. BMC Med Genet. 2017;18(1):78. https://doi.org/10.1186/s12881-017-0438-z.

38. Toscanini U, Brisighelli F, Moreno F, Pantoja-Astudillo JA, Morales EA, Bustos P, et al. Analysis of Y-chromosome STRs in Chile confirms an extensive introgression of European male lineages in urban populations. Forensic Sci Int Genet. 2016;21:76–80. https://doi.org/10.1016/j.fsigen.2015.12.005.

39. Cifuentes L, Morales R, Sepulveda D, Jorquera H, Acuna M. DYS19 and DYS199 loci in a Chilean population of mixed ancestry. Am J Phys Anthropol. 2004;125(1):85–9.

40. Visser L, Westerveld GH, Korver CM, van Daalen SK, Hovingh SE, Rozen S, et al. Y chromosome gr/gr deletions are a risk factor for low semen quality. Hum Reprod. 2009;24(10):2667–73. https://doi.org/10.1093/humrep/dep243.

41. de Llanos M, Ballesca JL, Gazquez C, Margarit E, Oliva R. High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. Hum Reprod. 2005;20(1):216–20. https://doi.org/10.1093/humrep/deh582.

42. Li Q, Qiao D, Song NH, Ding Y, Wang ZJ, Yang J, et al. Association of DAZ1/DAZ2 deletion with spermatogenic impairment and male infertility in the south Chinese population. World J Urol. 2013;31(6):1403–9. https://doi.org/10.1007/s00345-013-1058-7.

43. Wang YM, Li Q, Song LB, Zhang JY, Yang J, Song NH. Association of the deleted DAZ gene copy related to gr/gr and b2/b3 deletions with spermatogenic impairment. Zhonghua Nan Ke Xue. 2016;22(1):17–21.

44. 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(9):794–9. https://doi.org/10.1007/s10038-006-0024-2.

45. Fernandes S, Paracchini S, Meyer LH, Floridia G, HTyler-Smith C, Vogt P. A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men in Y haplogroup N. Am J Hum Genet. 2004;74: 180–7.

46. Rootsi S, Zhivotovsky LA, Baldovic M, Kayser M, Kutuev IA, Khusainova R, et al. A counter-clockwise northern route of the Y-chromosome haplogroup N from Southeast Asia towards Europe. Eur J Hum Genet. 2007;15(2):204–11. https://doi.org/10.1038/sj.ejhg.5201748.

47. Yang Y, Ma M, Li L, Su D, Chen P, Ma Y, et al. Differential effect of specific gr/gr deletion subtypes on spermatogenesis in the Chinese Han population. Int J Androl. 2010;33(5):745–54. https://doi.org/10.1111/j.1365-2605.2009.01015.x.

48. Sin HS, Koh E, Shigehara K, Sugimoto K, Maeda Y, Yoshida A, et al. Features of constitutive gr/gr deletion in a Japanese population. Hum Reprod. 2010;25(9):2396–403. https://doi.org/10.1093/humrep/deq191.

49. Sengupta S, Zhivotovsky LA, King R, Mehdi SQ, Edmonds CA, Chow CE, et al. Polarity and temporality of high-resolution y-chromosome distributions in India identify both indigenous and

exogenous expansions and reveal minor genetic influence of Central Asian pastoralists. Am J Hum Genet. 2006;78(2):202–21. https://doi.org/10.1086/499411.

50. Rai N, Chaubey G, Tamang R, Pathak AK, Singh VK, Karmin M, et al. The phylogeography of Y-chromosome haplogroup h1a1a-m82 reveals the likely Indian origin of the European Romani populations. PLoS One. 2012;7(11):e48477. https://doi.org/10.1371/journal.pone.0048477.

51. Rani DS, Rajender S, Pavani K, Chaubey G, Rasalkar AA, Gupta NJ, et al. High frequencies of non allelic homologous recombination (NAHR) events at the AZF loci and male infertility risk in Indian men. Sci Rep. 2019;9(1):6276. https://doi.org/10.1038/s41598-019-42690-0.

52. Thorsby E. The Polynesian gene pool: an early contribution by Amerindians to Easter Island. Philos Trans R Soc Lond Ser B Biol Sci. 2012;367(1590):812–9. https://doi.org/10.1098/rstb.2011.0319.

53. Kayser M, Brauer S, Cordaux R, Casto A, Lao O, Zhivotovsky LA, et al. Melanesian and Asian origins of Polynesians: mtDNA and Y chromosome gradients across the Pacific. Mol Biol Evol. 2006;23(11):2234–44. https://doi.org/10.1093/molbev/msl093.

54. Kayser M. The human genetic history of Oceania: near and remote views of dispersal. Curr Biol. 2010;20(4):R194–201. https://doi.org/10.1016/j.cub.2009.12.004.

55. Hurles ME, Maund E, Nicholson J, Bosch E, Renfrew C, Sykes BC, et al. Native American Y chromosomes in Polynesia: the genetic impact of the Polynesian slave trade. Am J Hum Genet. 2003;72(5):1282–7. https://doi.org/10.1086/374827.

56. Fehren-Schmitz L, Jarman CL, Harkins KM, Kayser M, Popp BN, Skoglund P. Genetic ancestry of Rapanui before and after European contact. Curr Biol. 2017;27(20):3209–15 e6. https://doi.org/10.1016/j.cub.2017.09.029.

57. 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(10):1171–80. https://doi.org/10.1002/humu.20757.

58. Lu C, Wang Y, Zhang F, Lu F, Xu M, Qin Y, et al. DAZ duplications confer the predisposition of Y chromosome haplogroup K* to non-obstructive azoospermia in Han Chinese populations. Hum Reprod. 2013;28(9):2440–9. https://doi.org/10.1093/humrep/det234.

59. Lu C, Zhang F, Xia Y, Wu B, Gu A, Lu N, et al. The association of Y chromosome haplogroups with spermatogenic failure in the Han Chinese. J Hum Genet. 2007;52(8):659–63. https://doi.org/10.1007/s10038-007-0160-3.