Features of constitutive gr/gr deletion in a Japanese population

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BACKGROUND: The relationship between male infertility and gr/gr deletions that remove multiple genes of the Y chromosome varies among countries and populations. The aim of this study was to investigate the association between gr/gr deletions and spermatogenic phenotype in fertile and infertile Japanese men.

METHODS: The subjects were screened by sequence-tagged site (STS) analysis to detect gr/gr deletions, and haplogroups were assigned using eight highly informative markers. In total, 395 infertile men and 377 fertile men (controls) participated in our study. Of the 772 subjects, 260 individuals carried confirmed gr/gr deletions and were used in further analysis of deletion subtype and gene copy number, specifically loss and gain of CDY1 and DAZ copies. These 260 subjects were divided into a control group (n = 131) all with normozoospermia, and an infertile group (n = 129) with 89 infertile subjects exhibiting azoospermia (absence of sperm) and 40 exhibiting oligozoospermia (reduced sperm concentration).

RESULTS: There were gr/gr deletions in 33.7% (260/772) of all subjects and the deletions were widespread in haplogroup D (86.2%). There were no significant differences in the frequency of gr/gr deletions between the infertile and control groups. The gr/gr deletion subtypes were not distributed randomly among haplogroups; the CDY1a + DAZ1/2 genes were deleted in 96.9% (217/224) of haplogroup D individuals, whereas the O lineage had a variety of gr/gr deletion types. The loss of CDY1a + DAZ1/2 was not associated with spermatogenic impairment in haplogroup D (P = 0.33).

CONCLUSIONS: Taken together, gr/gr deletions in haplogroup D occur constitutively, are associated with the loss of CDY1a + DAZ1/2 and are phenotypically neutral. Further studies are needed to establish whether Y-linked compensatory factors outside the AZFc region can counteract the pathogenic effect of a gr/gr deletion in the D lineage.

Key words: Y microdeletion / Y haplogroup / homologous recombination / AZF / male infertility

Introduction

The male-specific region of the Y chromosome (MSY) escapes recombination with its meiotic partner, the X chromosome, and is rich in transcription units and functional genes related to spermatogenesis. The MSY region contains long, tandem duplicated sequences that facilitate genomic deletions via non-allelic homologous recombination due to sequence similarities of internal amplicons (Sun et al., 2000; Kuroda-Kawaguchi et al., 2001; Repping et al., 2002; Koh et al., 2005; Choi et al., 2008). The most well-characterized deletions of the MSY region are in the azospermia factor regions (AZF) a, b and c, and these result in spermatogenic failure and severely reduced sperm concentrations (Vogt, 1998; Foresta et al., 2001; Simoni et al., 2004; Krausz and Degl’Innocenti, 2006).

The complete deletion of AZFc due to homologous recombination between the b2 and b4 amplicons results in azoospermia (Kuroda-Kawaguchi et al., 2001). However, it is unclear whether partial deletions in the AZFc region that result in significant gene loss (i.e. b1 / b3, b2/b3 and gr/gr deletions) contribute to infertility (Hucklenbroich et al., 2005). The phenotypic association between gr/gr deletion and spermatogenic impairment is variable and apparently depends on the population and country (Repping et al., 2003; de Carvalho et al., 2006; Zhang et al., 2006; Visser et al., 2009). The boundaries of gr/gr deletion encompass CDY1, the DAZ family and several genes that are divided into different amplicons. There are four different gr/gr deletion subtypes that are based on the genes lost: CDY1a + DAZ1/2; CDY1a + DAZ3/4; CDY1b + DAZ1/2; and CDY1b + DAZ3/4 (Krausz et al., 2009; Fig. 1).
The CDY1 transcript encodes a chromodomain and histone acetylase transferase, exclusively found in mature spermatid and spermatozoa and possibly required in late stage spermatogenesis (Kleiman et al., 2003). The DAZ family members are expressed in the testis at different stages of spermatogenesis (Habermann et al., 1998; Reijo et al., 2000; Huang et al., 2008). The biological functions of CDY and DAZ families are not confirmed, but the expression levels and patterns suggest that they are important to spermatogenesis. On the basis of expression data, much research has focused on deletion frequency and the types of loss of CDY and DAZ family members and their relationship to male infertility (Giachini et al., 2005).

Phenotypic diagnosis of gr/gr deletion is inconsistent and variable across study populations. In fact, the association between infertility, the frequency of gene loss and the frequency of gr/gr deletion differs between Y haplogroups. For instance, the gr/gr region is uniformly deleted in haplogroup Q1 individuals, DAZ3/4 gene is frequently eliminated in haplogroup N, and neither haplogroup is associated with spermatogenic impairment (Fernandes et al., 2004; Lu et al., 2009). In contrast, gr/gr deletion frequency was significantly higher (P < 0.001) in men with impaired sperm production when compared with normozoospermic controls in a large Italian study population [OR = 7.9 (95% CI, 1.8–33.8)] (Giachini et al., 2008). The gr/gr deletions are nearly fixed in haplogroup D individuals, and there is no evidence for significant levels of associated infertility (Kuroki et al., 1999; de Carvalho et al., 2006).

The Japanese population is composed of two major Y haplogroups; D and O (Hammer et al., 2006). The haplogroup D lineage has a high frequency of gr/gr deletions and is widely distributed in the Japanese population (Shinka et al., 1999). Thus, the Japanese population is a powerful model system in which to study the effects and phenotypic variations associated with gr/gr deletions. In this investigation, we focused on three factors in subjects carrying a gr/gr deletion: the type of gene loss, haplogroups and sperm parameters. We used these three parameters to investigate the relationship between gr/gr deletions and male infertility in the Japanese population.

**Materials and Methods**

The Ethics Committee of Kanazawa University Hospital approved the study, and informed consent was obtained from all participants (No. 172, H20.8.8).

**Study population**

In total, 395 infertile men and 377 fertile men were enrolled in this study. General clinical data and blood samples were obtained from Kiba Clinic, Kyono ART clinic and the Department of Urology of Kanazawa University.

![Figure 1](https://www.oxfordjournals.org/ourournals/humrep/article-figures/2397644/Figure1.png)

**Figure 1** (A) Structural features of boundaries of the gr/gr region. Schematic illustration of the Y chromosome: three azoospermic regions are depicted as bold lines (AZFa, AZFb and AZFc). (B) Enlargement of the AZFc region, each arrow indicates amplicons and individual palindromes are indicated above the arrows with direction. Transcription units and genes are depicted as colored boxes located in identical colored amplicons. (C) Each gr/gr deletion subtype is identified as loss of CDY1a + DAZ1/2 or CDY1a + DAZ3/4. (D) A b2/b4 duplication that occurred after gr/gr deletion; this chromosome recovered copies of the CDY1a and DAZ genes and contained the same copy number of a non-gr/gr-deleted Y chromosome. (E) Gr/gr deletion after b1/b3 inversion. CDY1b + DAZ1/2 and CDY1b + DAZ 3/4 are eliminated.
Hospital. Semen analysis was performed according to conventional methods (WHO, 1999). Subjects were divided into two groups; the infertile group and the control group. Subjects in the infertile group were diagnosed with azoospermia (no sperm in the ejaculate sample) or oligozoospermia (< 20 x 10^6/ml sperm in the ejaculate sample). All 377 fertile subjects in the control group produced one or more children, and they were confirmed as being normozoospermic, with normal sperm motility and morphology. Sperm concentration and total sperm counts were determined three times for each subject, and we used the mean value, excluding the lowest measurement (mean sperm concentration, MSC; mean total sperm counts, MTSC). Patients with karyotype abnormalities, confirmed obstructive azoospermia, other partial or complete deletions of the AZF region, or insufficient patient information were excluded from the study.

### STS Analysis
Basic genetic analysis was performed using the conventional Y chromosome sequence-tagged site (STS) test (AZFa: sY82, sY84 and sY86; AZFb: sY1264, sY1235, sY1227, sY1228, sY117, sY280, sY127, sY134, sY135, sY258, sY142 and sY143; AZFc: sY1161, sY1191, sY1197, sY1291, sY1125, sY1054, sY1206, sY1201, sY255 and sY254) (Simoni et al., 2004; Fukushima et al., 2006). The presence of a gr/gr deletion was assessed using eight STS markers; the absence of sY1291 in combination with the presence of sY142, sY116, sY1191, sY1197, sY1291, sY1125, sY1054, sY1206, sY1201, sY1201 and sY1201 indicated a gr/gr deletion (Giachini et al., 2005). All collected samples were subjected to STS testing using these eight markers (www.FamilyTreeDNA.com).

### Typing of gr/gr deletions
Sequence family variant (SFV) analysis was performed to screen for deletions in CDY1 and DAZ. CDY1-7750 was amplified from the subjects’ genomic DNA, and the PCR products were treated with the restriction enzyme PvuII, which enabled recognition of CDY1a and/or CDY1b deletions. The STS sY587 was used to discriminate DAZ1/2 deletions from DAZ3/4 deletions. We performed PCR amplification using sY587 and digested the products using Dral. Digested fragments were separated on a 2.0% agarose gel to sort four different types of gr/gr deletions as follows: loss of CDY1a + DAZ1/2, CDY1a + DAZ3/4, CDY1b + DAZ1/2 and CDY1b + DAZ3/4.

### Gene dosage analysis
CDY (primers: oMY953a/o1023) and DAZ (primers: oMY953a/o1313) were amplified, and the PCR products were 5′-end labeled with cyanine 5.5 and used as probes to evaluate the copy number of each gene using the CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Brea, CA, USA). The primer pair oMY953a/o1023 amplified across a 3-bp in/del gap of CDY. We coamplified the DAZ family and DAZL using the oMY953a/o1313 primer set that amplified across a 3-bp in/del gap between DAZa and DAZL. Two copies of CDY2 and DAZL reside in palindrome 3 of the Y chromosome and in chromosome 3, respectively, allowing for the use of an internal standard for quantification of CDY1a and the DAZ family in the gr/gr region (Krausz et al., 2009).

### Y chromosome haplogrouping
On the basis of eight markers—YAP, RPS4Y711, M213, M174, M9, M116, M231 and M175—subjects were partitioned into eight haplogroups (YChromosomeConsortium, 2002). The PCR products from each marker were compared with an absence or a presence of locus, then sequenced, and digested with the designated restriction enzymes to identify haplogroup-specific polymorphisms on the Y chromosome. From this study, we listed eight haplogroups: D*(excluding D2; xD2), D2, E, C, F*(excluding K; xK), K* (excluding O; xO), N and O clade (Fig. 2).

### Statistical analysis
An analysis of the distribution of the Y chromosome haplogroups in the 772 subjects was performed to determine population differentiation using Arlequin ver 3.11 (Excoffier et al., 2005). Median values of sperm concentration were compared between the infertile (oligozoospermia) and control groups using the Mann–Whitney U-test and Student’s t-test. The frequency of azoospermia and oligozoospermia compared with normozoospermia was analyzed using the χ² test. All analyses were performed using the Statistical Package for Social Sciences, version 11.0 (SPSS, Chicago, IL, USA), and P values were corrected using Fisher’s exact test.

### Results
The Japanese population is said to have a high frequency of gr/gr deletions. We first investigated the frequency of Y chromosomal haplogroups in the Japanese population. We then carefully observed the distribution of gr/gr deletions and verified the frequency of gr/gr deletion subtypes within infertile or fertile subject groups. Subsequently, we evaluated whether the constitutive gr/gr deletion adversely affects fertility or sperm production.

### Distribution and frequency of gr/gr deletion in Japanese population
In total, 772 subjects were analyzed; 395 subjects were infertile men and 377 subjects had at least one child and were normozoospermic.
Gr/gr deletions in a Japanese population

Types of gr/gr deletion

The gr/gr deletions resulted in several types of gene loss owing to different combinations of ampiclons, and these differences in gene loss potentially had an effect on spermatogenic failure. In this study, we defined gr/gr deletion subtypes according to the loss and gain of gene copies (CDY1a + DAZ1/2; CDY1a + DAZ3/4; CDY1b + DAZ1/2; and CDY1b + DAZ3/4) (Fig. 1). The frequency of gr/gr deletions differed across the Y chromosomal haplogroups; 84.5% (224/265) of the gr/gr deletions occurred constitutively in haplogroup D. On the basis of this observation, we divided subjects with gr/gr deletions into haplogroup D and non-haplogroup D.

Overall, gr/gr deletions occurred in 33.7% of the Japanese population, and there were no significant differences between the frequency of gr/gr deletions in subjects with azoospermia (35.0%, $P = 0.52$) or those with oligozoospermia (28.4%, $P = 0.54$) when compared with subjects in the control group. This observation did not vary between haplogroups; the frequency of gr/gr deletions did not differ in the azoospermia and oligozoospermia groups and control groups in each haplogroup ($P > 0.05$) (Table I). Furthermore, we did not observe any significant differences in the frequency of gr/gr deletions between azoospermic (9/126) and control (11/206) subjects in haplogroup O. In the case of minor haplogroups such as E, K*, and N, sample sizes were too small to assess significance. Even when we considered the frequency of gr/gr deletions in 100% of the haplogroup D* samples and 82.6% of D2 samples, whereas they occurred in only 5.1% of the O haplogroup samples, indicating that gr/gr deletions occurred constitutively in haplogroup D. On the basis of this observation, we divided subjects with gr/gr deletions into haplogroup D and non-haplogroup D.

To investigate gr/gr deletion subtypes, we divided subjects into haplogroup D and non-haplogroup D. We defined gr/gr deletion subtypes according to the loss and gain of gr deletions. The groups shared a low frequency of gr/gr deletions in the azoospermia groups (50%, 9/18 gr/gr deletions), oligozoospermia (39%, 11/28 gr/gr deletions), normozoospermia (26%, 4/15 gr/gr deletions), and control groups (45%, 11/24 gr/gr deletions). The non-haplogroup D (E*, C, K*, N, and O) also carried the CDY1a + DAZ1/2 deletion in the azoospermia (77%, 10/13 gr/gr deletions), oligozoospermia (20%, 1/5 gr/gr deletions) and normozoospermia groups (50%, 9/18 gr/gr deletions). The groups shared a low frequency of CDY1a + DAZ1/2, CDY1b + DAZ1/2, and CDY1b + DAZ3/4 gr/gr deletion subtypes. Although the sample sizes were not sufficient to calculate statistical significance in these groups, the O lineage showed a greater distribution of gr/gr deletion subtypes [loss of CDY1a + DAZ1/2 (52.4%,

Haplogroup D

The haplogroup D lineage possessed the CDY1a + DAZ1/2 deletion with extremely high frequency among the infertile and control groups. The distribution of gr/gr subtype CDY1a + DAZ1/2 in the infertile and control groups was similar: azoospermia, 97.4% (74/76); oligozoospermia, 94.3% (33/35); normozoospermia, 96.5% (109/113). Thus, the constitutive gr/gr deletion removes CDY1a + DAZ1/2, and removal of these copies apparently does not confer a genetic risk for impaired sperm production in association with haplogroup D. Loss of CDY1a + DAZ3/4 was not observed, although CDY1b + DAZ1/2 and CDY1b + DAZ3/4 occurred with low frequency (CDY1b + DAZ1/2: azoospermia, 1.3% (1/74); oligozoospermia, 1.3% (1/74); normozoospermia, 2.2% (2/13); and CDY1b + DAZ3/4: azoospermia, 1.3% (1/74); normozoospermia, 1.2% (1/74)).

Subsequently, we examined b2/b4 duplication and gene amplification for compensatory mechanisms for the high frequency of loss of CDY1a + DAZ1/2 in haplogroup D (Table II). The most abundant genomic rearrangement type was simple gr/gr deletion without any other gene compensation (203/220), and b2/b4 duplication after gr/gr deletion arose in ~10% of individuals among the groups, whereas single gene amplification was scarcely observed. Gr/gr deletions followed by b2/b4 duplications (restoring the initial AZFc gene dosage) are not a prevalent feature of haplogroup D, and thus do not explain the lack of pathogenic effects. Haplogroup D was not associated with duplication or amplification of individual genes as a compensatory mechanism.

Non-haplogroup D

Unlike in haplogroup D, the distribution of gr/gr deletion subtypes varied in non-haplogroup D groups. The non-haplogroup D (E*, C, K*, N, and O) also carried the CDY1a + DAZ1/2 deletion in the azoospermia (77%, 10/13 gr/gr deletions), oligozoospermia (20%, 1/5 gr/gr deletions) and normozoospermia groups (50%, 9/18 gr/gr deletions). The groups shared a low frequency of CDY1a + DAZ1/2, CDY1b + DAZ1/2 and CDY1b + DAZ3/4 gr/gr deletion subtypes. Although the sample sizes were not sufficient to calculate statistical significance in these groups, the O lineage showed a greater distribution of gr/gr deletion subtypes [loss of CDY1a + DAZ1/2 (52.4%,

Table I The distribution and frequency of gr/gr deletions within haplogroups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Y chromosome haplogroup</th>
<th>Total I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haplogroup D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D*</td>
<td>D2</td>
</tr>
<tr>
<td>Infertility</td>
<td>AZ*</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>E*</td>
<td>2/3</td>
</tr>
<tr>
<td>Control</td>
<td>OZ*</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>NZ*</td>
<td>13/13</td>
</tr>
<tr>
<td>Total 2</td>
<td></td>
<td>30/30</td>
</tr>
</tbody>
</table>

AZ, azoospermia; OZ, oligozoospermia; NZ, normozoospermia; Total 1, sum of each group; Total 2, sum of each haplogroup.

No significant difference between 1 and 2 versus 3 (P > 0.05).
Table II  The distribution of types of genomic rearrangements.

<table>
<thead>
<tr>
<th>Rearrangement type</th>
<th>Haplogroup D</th>
<th>Non-haplogroup D</th>
<th>Total 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AZ, n</td>
<td>OZ, n</td>
<td>NZ, n</td>
</tr>
<tr>
<td>Gr/gr del</td>
<td>68</td>
<td>30</td>
<td>105</td>
</tr>
<tr>
<td>Gr/gr del B2/b4 dupl</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Gr/gr del CDY1 ampl</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gr/gr del DAZ ampl</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gr/gr del CDY1/DAZ ampl</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Total 2</td>
<td>74</td>
<td>33</td>
<td>113</td>
</tr>
</tbody>
</table>

|                    | Infertile    | Control          |         |
|                    | AZ, n        | OZ, n            | NZ, n   |
| Gr/gr del          | 11           | 5                | 15      |
| Gr/gr del B2/b4 dupl | 3           | –                | 1       |
| Gr/gr del CDY1 ampl | –           | –                | 1       |
| Gr/gr del DAZ ampl | –           | –                | 2       |
| Gr/gr del CDY1/DAZ ampl | 1      | –                | 4       |
| Total 2            | 15           | 7                | 18      |

del, deletion; dupl, duplication; ampl, amplification; AZ, azoospermia; OZ, oligozoospermia; NZ, normozoospermia; Total 1, sum of each genomic rearrangement type; Total 2, sum of each group. Non-haplogroup D group contained E*, C, K*, N and O.

Figure 3 Frequency of gr/gr deletion subtypes in Y chromosome haplogroup. ‘Others’ comprise E*, C, K* and N.

Comparison of phenotypes between CDY1a and CDY1b deletion subjects in the control group

We assumed that loss of CDY1b or CDY1a plays a role in phenotypic variation in the Y chromosome haplogroups.

We compared MSC in the control groups (Table IV). The loss of CDY1a was very frequent in haplogroup D (110/113), and the MSC in this group was 159.4 ± 96.1 × 10^6/ml. In contrast, loss of CDY1b was very infrequent in haplogroup D (3/113), and all three subjects with CDY1b deletion had reduced MSC (P = 0.03). With regard to MTSC, CDY1b deletion subjects (114.1 ± 230.8 × 10^6) also showed reduced MTSC when compared with CDY1a deletion subjects (508.8 ± 265.0 × 10^6) in the D haplogroup (P = 0.044).

Furthermore, loss of CDY1a and CDY1b of the O lineage showed that the MSC was more than 50% lower in CDY1b deletion subjects (47.5 ± 17 × 10^6/ml) when compared with CDY1a deletion subjects (115.2 ± 50.7/ml) (P = 0.02). In addition, a comparison of MTSC between CDY1a (474.6 ± 225.0 × 10^6) and CDY1b (145.8 ± 62.8 × 10^6) confirmed a reduced MTSC (P = 0.03) in the latter. However, the values of MSC and MTSC between loss of CDY1b and loss of CDY1a in the minor haplogroups (E, C, K* and N) did not reach significant differences (P > 0.1).

In our study, haplogroup O had a variety of gr/gr deletion subtypes and a high proportion of CDY1b loss (8/21, 38.1%), and gr/gr deletion in haplogroup O was associated with lower sperm concentrations in normozoospermic men. This interesting observation requires further confirmation in an independent study.
Discussion

The haplogroup D lineage had a high frequency of gr/gr deletions in this study. Two major haplogroups, D and O, are widespread in the Japanese population. Because haplogroup D was present ~12,000 years ago, it now occurs in 34.7% of the Japanese population (Tajima et al., 2004). In contrast, the O lineage appeared only ~2300 years ago but has spread to account for 51.8% of Japanese Y haplogroups (Hammer et al., 2006).

Generally, haplogroup D is rare in northeast Asia, but it was dispersed among the African, Tibetan and Japanese populations (Hammer and Horai, 1995; Wen et al., 2004). On the basis of this unique aspect of the Japanese population, our study size was appropriate for comparing the frequency of gr/gr deletions between these two major haplogroups (D and O). As has already been noted, the frequency of gr/gr deletions was high in both the infertile group (azoosperma 35.0% and oligozoosperma 28.4%) and the control group (34.7%). In addition, a majority of the gr/gr deletions in Japanese subjects occurred in the D lineage. All subjects with the D* carried a gr/gr deletion, and the gr/gr frequency in the D2 clade approached 82.6%(224/260), thus suggesting that gr/gr deletions are constitutive in these haplogroups.

Similarly, a study on a Chinese population reported that haplogroups N1 and Q1 have fixed b2/b3 and gr/gr deletions, respectively (Lu et al., 2009). According to the report by Lu et al., gr/gr deletions occurred in up to 10% of the Chinese population and were not strongly associated with infertility in the O lineage. The frequency of gr/gr deletions was not different among the infertile and control groups of haplogroup O, and the deletion frequency had no relationship with spermatogenic impairment in our study. The minor haplogroups (E*, C, K* and N) also showed a relatively low frequency of gr/gr deletions when compared with haplogroup D. If this trend is evident in additional studies in Japanese men, it can be concluded that gr/gr deletions have been fixed in the haplogroup D lineage during Japanese paternal inheritance. Studies of gr/gr deletions thus demonstrate different phenomena in different countries and study populations in a haplogroup-dependent manner.

We attempted to discover which gr/gr deletion subtypes had the most potent impact on infertility. In the case of haplogroup N, elimination of DAZ3/4 is common, but this does not lead to a reduction in MSC, whereas the absence of DAZ1/2 has been associated with reduced sperm numbers (Fernandes et al., 2002, 2004). In contrast, loss of CDY1a + DAZ3/4, which is involved in infertility, and other deletion subtypes has been regarded as neutral variants in a French study (Machev et al., 2004). According to a broad screening of European populations, gr/gr deletion subtypes are not related to phenotypic abnormalities ranging from azospermia to normozoosperma (Krausz et al., 2009).

In this survey, we found that loss of CDY1a + DAZ1/2 did not induce phenotypic abnormalities in the haplogroup D lineage. The comparison of MSCs between the gr/gr deletion and non-deletion subjects derived from haplogroup D showed no significant difference. In fact, no phenotypic variations were detected in our study. However, frequency of loss of CDY1b was low, and loss of CDY1b was associated with phenotypic variations in normozoospermic men (Tables III and IV). Of 133 normozoospermic subjects from the control group, 12 individuals with a CDY1b deletion were confirmed to have lower sperm concentrations although they are still within the normal range.
The biological function of CDY1 and the DAZ family is not fully understood, but their expression patterns indicate that they play a role in spermatogenesis. In particular, CDY1 transcripts have been detected in mature spermatids and spermatozoa (Kleiman et al., 2003). Taken together with previously reported data, the results of the present study suggest that the loss of CDY1a + DAZ1/2 is insufficient to disturb spermatogenesis in association with haplogroup D* and D2.

Conversely, haplogroup O showed various patterns of gr/gr deletion that included a high frequency of loss of CDY1b. When compared with control subjects lacking deletions, the O lineage showed decrease in both sperm concentration and total sperm counts though still within the normal range. Although the incidence was extremely low (1.5%, 5/337), a larger study population is necessary to confirm the association with sperm counts.

The frequency of gr/gr deletion subtypes differs with Y chromosome haplogroup, and it is thus essential to consider the distribution of gr/gr deletions among the haplotypes. In this study, we demonstrated that any phenotypic variation in gr/gr deletion carriers from haplogroup D is independent from the type of CDY1 and DAZ copy loss, since in all cases a homogenous pattern (loss of CDY1a + DAZ1/2) was observed. Further studies are needed to establish whether Y-linked compensatory mechanism outside the AZFc region, enable the haplogroup D individuals to counteract the pathogenic effect of a gr/gr deletion.

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