Frequent polymorphism of the mitochondrial DNA polymerase gamma gene (POLG) in patients with normal spermiograms and unexplained subfertility

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BACKGROUND: Male fertility largely depends on the quality of sperm production, which may be affected by environmental and genetic factors. In this study, we explored a possible role of the polymerase gamma (POLG) gene polymorphism, recently reported to be associated with male infertility in some populations.

METHODS: The polymorphic CAG repeat (usually 10 codons long) in the POLG gene was studied in 1298 male subjects: 429 patients with infertility/subfertility, and 869 controls (495 men from the general population with unknown fertility and 374 recent fathers). In all subjects, the POLG polymorphism was assessed in relation to their semen quality, and—in the fertile controls—with biological fecundity measured as waiting time-to-pregnancy (TTP) for the couples. In the patients lacking the common POLG allele, the outcome of the assisted reproductive techniques (ART) for the couples was evaluated.

RESULTS: The absence of one (¹10/¹10) or both common POLG alleles (¹10/¹10) was more frequent among the subfertile patients than among fertile controls (P = 0.021 and P = 0.04 respectively). The estimated predictive value for infertility in a man homozygous for the POLG polymorphism was 15.5% (95% CI: 4.8–51%). There was a positive association with sperm concentration: 14.3% of the normospermic subfertile patients were homozygous for the absence of the common POLG allele (¹10/¹10), in comparison with 2.3% of unselected controls (P = 0.001) and 0.9% of the fertile men (P = 0.0001). No association with sperm motility, morphology and TTP was found. Spermatozoa of the three ¹10/¹10 patients treated with IVF retained the ability to penetrate the egg, but the fertilization rate was low. Nine homozygous ¹10/¹10 patients were treated with ICSI, resulting in pregnancy in seven couples.

CONCLUSIONS: The POLG gene polymorphism should be considered as a possible contributing factor in patients with unexplained subfertility and normal spermiograms. The oocyte penetration ability of sperm may be partially impaired in the ¹10/¹10 patients but most of them can be successfully treated with ICSI.

Key words: gene polymorphism/male infertility/mitochondrial DNA/sperm quality/POLG gene

Introduction

Infertility in humans affects 10–15% of couples; ~20% of the cases are attributable solely to the male partner, in a further 25% a subfertile male contributes to the problem, and in ~15% no factor can be defined in either partner (WHO, 1987; Skakkebæk et al., 1994; Huynh et al., 2002).

Clinical assessment of male infertility/subfertility is primarily based on the analysis of semen quality. The most informative parameters are sperm concentration and total sperm count, followed by sperm motility and morphology (WHO, 1999). Sperm concentration exceeding 20 × 10⁶/ml is usually considered normal, although values up to 40 × 10⁶/ml have been associated with subfertility (Bonde et al., 1998). There is also ongoing debate concerning the predictive value of other sperm parameters, such as motility and morphology, for the assessment of fertility potential (Comhaire and Vermeulen, 1995; Zinaman et al., 2000; Guzick et al., 2001; Menkveld et al., 2001).

Studies of sperm function, especially motility, turned the attention of researchers to the possible role of sperm mitochondria which produce large quantities of energy (Folgero et al., 1993). Mitochondria have their own genome
(mtDNA), which codes for proteins involved in the respiratory chain and oxidative phosphorylation system. Numerous studies indicated an association between different polymorphisms, mutations or deletions in the mitochondrial genome and sperm dysfunction (Lestienne et al., 1997; Ruiz-Pesini et al., 2000; Holyoake et al., 2001; St John et al., 2001; Spriopoulos et al., 2002). One of these studies (Ruiz-Pesini et al., 2000) was particularly informative as it identified specific mtDNA haplogroups that are associated with asthenozoospermia. On the other hand, in one study no association of mtDNA with sperm dysfunction was found (Cummins et al., 1998), and some recent reports showed an increased mtDNA content in infertile men (Diez-Sanchez et al., 2003; May-Panloup et al., 2003).

The key nuclear enzyme involved in the elongation and repair of mtDNA strands is DNA polymerase gamma (POLG), believed to be the only polymerase acting in the mitochondria (Bolden et al., 1977). The catalytic subunit of POLG is encoded by the POLG gene, which was mapped to chromosome 15q24 and includes a CAG repeat region (Ropp and Copeland, 1996). A recent investigation of the frequency of different CAG repeat lengths in different European populations showed a high frequency (88%) of 10 codons (Rovio et al., 2001), indicating that this common allele is maintained by selection. According to that study (Rovio et al., 2001), the absence of the common allele on both chromosomes (x/x or 10/10 according to our nomenclature) seems to be associated with male infertility, since it was observed in 3.5–9% of infertile males with impaired sperm quality (after exclusion of azoosperma and severe oligozoosperma), while none of the fertile control individuals was homozygous for the lack of the common allele. These intriguing findings prompted us to examine the polymorphism of the POLG gene among Danish men, whose average sperm counts appear to be low (Andersen et al., 2000; Jørgensen et al., 2001). In particular, we were interested in exploring possible associations between the POLG polymorphism and semen quality and fecundity. We report here the results of our analysis of a large series of well characterized patients and controls.

**Materials and methods**

**Studied subjects**

Our study series included 1298 males divided into three groups. Infertile (azoospermic) and subfertile (with retained spermatogenesis) patients, nearly all Danish, included 429 males from infertile couples (unable to conceive for at least 1 year), referred to our clinic for an andrological work-up before fertility treatment. All patients were karyotyped and tested for microdeletions of the Y chromosome, and those with genetic abnormalities were excluded.

The fertile control group of 374 Danish subjects was invited to participate in research projects via their female partners. Their fatherhood was not genetically verified. However, these men were recruited to the study by their pregnant spouses, who knew that they would be subjected to genetic studies. Moreover, we have complete hormone and semen quality data on these individuals, and they are consistent with their good reproductive function. These subjects included: (A) 306 men recruited from the antenatal care unit for a study of reproductive health of Danish men (Jørgensen et al., 2001). Only couples that achieved the pregnancy without any fertility treatment were invited to participate. (B) Sixty-eight young men, selected from couples recruited for a prospective study of the association of time-to-pregnancy (TTP) with reproductive variables (Bonde et al., 1998; Jensen et al., 2000; 2001). Only men who achieved fatherhood during the study period (12 months) were included in the current investigation.

The unselected control group of 495 young Danish men from the general population with unknown fertility was recruited for prospective studies of reproductive health in Europe (Jørgensen et al., 2001; 2002) while attending a compulsory medical examination before being considered for military service.

**Clinical analysis**

All subjects underwent a thorough andrological examination, including a comprehensive analysis of reproductive hormones in serum (testosterone, inhibin B, LH, FSH, oestradiol and sex hormone-binding globulin) and semen analyses. Control subjects donated only one semen sample. The semen analysis was performed as previously described (Bonde et al., 1998; Andersen et al., 2000; Jørgensen et al., 2001; 2002). Three parameters of sperm quality, concentration, motility and morphology, were used for the statistical analysis of an association with the POLG polymorphism. Sperm concentration was divided into clinical categories defined as: azoosperma and severe oligozoosperma, <5 × 10^6/ml; moderate oligozoosperma, 5–20 × 10^6/ml; and normozoosperma, >20 × 10^6/ml. Motility was assessed according to the WHO guidelines (WHO, 1999) with small modifications (Jørgensen et al., 2001; 2002), and a cut-off value of 50% of motile sperm was used to define normal motility. Sperm morphology was assessed either according to the WHO (Rowe et al., 1993; WHO, 1999), or using the so-called strict criteria (Menkveld et al., 1990; 2001). In order to compare the data, we chose different ‘normal’ cut-off values, according to previous studies of the impact of sperm morphology on fecundity. For the subjects analysed according to the WHO, 21% of normal forms was the lower cut-off value (Menkveld et al., 2001), 39% as the upper threshold (Slama et al., 2002), and 22–38% was considered the uncertain zone. It was difficult to select the cut-off value for the strict criteria, because of some disagreement in the literature. We selected 8% of normal spermatozoa as the lower cut-off value (Guzick et al., 2001), 19% as the upper threshold (Slama et al., 2002), and 9–18% as the uncertain zone. In addition, we calculated the median values of spermatozoa with defects of mid-piece (neck) and tail in all studied subjects, which were 8 and 3% respectively, and assessed the distribution of subjects below and above these values.

**Molecular analysis of the POLG gene**

DNA was isolated from peripheral blood samples using a kit (Roche Diagnostics GmbH, Mannheim, Germany). Two primers (GTCCTCCTGACACAAACATGA and CTTGCGGGAGATTGTGC-TCGT), matching positions 267–286 and 535–555 respectively, of the POLG mRNA (accession number X98093) were used to amplify a 286 bp DNA fragment, using the Pfu DNA polymerase (Stratagene, San Diego, CA). PCR was performed in 30 μl of (final concentrations): 12 mmol/l Tris–HCl, pH 8.8; 10 mmol/l KCl; 10 mmol/l (NH₄)2SO₄, 2.0 mmol/l MgSO₄; 0.1% Triton X-100; 0.1 mg/ml BSA; 250 μmol/l dNTP; 30 pmol of each primer and 2 IU Pfu polymerase. PCR conditions were: 98°C for 5 min, 40 cycles of 98°C for 30 s, 63°C for 1 min, 72°C for 1 min and 45 s and one cycle of 72°C for 5 min. DNA fragments were purified from 1% agarose gels and analysed first by applying a Cy5-labelled sequencing primer (CY5-CTGGATGTCCAAATGGGTGTG, position 494–513) under standard PCR conditions, resulting in a DNA fragment, which was sequenced on an ALF-
Results

Distribution of the POLG alleles among patients and controls

CAG repeat lengths ranging from eight to 13 triplets were detected. In concert with the previous report (Rovio et al., 2001), homozygotes with 10 repeats in both alleles (10/10) were the most common and constituted 75% of all subjects. The following proportions of men in the studied groups had normal haplotypes: 71.8% (95% CI: 67.3–76%) of infertile/subfertile patients, 73.5% (95% CI: 69.4–77.4%) of healthy young men from the general Danish population (fertility unknown), and 80.7% (95% CI: 76.4–84.6%) of fertile controls (Figure 1). A highly significant trend (γ = 0.15; P = 0.004) towards increasing proportion of the common allele with the increasing fertility was immediately apparent, the OR of having 10/10 relative to the unselected controls was increasing from 0.9 (95% CI: 0.7–1.2) among the patients to 1.5 (95% CI: 1.1–2.1) among fertile controls. The absence of one common allele (heterozygous, 10/¹0) was marginally more frequent among the patients (25.4%, 95% CI: 21.4–29.8%) than among the unselected controls (24.8%, 95% CI: 21.1–28.9%, P = 0.88, not significant) but significantly more frequent than among the fertile controls (18.4%, 95% CI: 14.6–22.8%, P = 0.021). The frequency of the absence of the common POLG allele (¹0/¹0) was very low in all groups: 2.8% (95% CI: 1.5–4.8%) in the subfertile/infertile group, 0.8% (95% CI: 0.2–2.3%) in the fertile group, and 1.6% (95% CI: 0.7–3.2%) in our sample of the general population. The frequencies of POLG’s CAG ¹0/¹0 in the fertile control group (0.8%) and the subfertile patients (2.8%) were significantly different (3.6, 95% CI: 0.95–20, P = 0.04). Furthermore, the tendency toward a higher frequency of both ¹0/¹0 and ¹0/¹0 with declining fertility was also significant (γ = 0.12; P = 0.021, and γ = 0.37, P = 0.032 respectively).

Based on the frequencies of double ‘mutant’ haplotypes (CAG ¹0/¹0) among infertile patients (12/428) and fertile controls (3/374), we assessed the predictive value of homozygosity for the POLG gene polymorphism. Assuming that a prevalence of infertility is 5%, the probability that a man, randomly chosen from the general population, is infertile is 5%. If the man is known to be homozygous for the POLG polymorphism, the estimated probability that he is infertile increases to 15.5% (95% CI: 4.8–51%). With an assumed prevalence of infertility of, for example, 7%, the predictive value increases to 20.8% (95% CI: 6.7–60%).

Is there an association between the POLG gene polymorphism and spermatogenesis and semen quality?

The subjects were stratified according to sperm concentration and motility (Table I). Only one patient with POLG’s CAG ¹0/¹0 was found in the subgroup with azoospermia or severe oligozoospermia (his mean sperm concentration was 2 × 10⁶/ ml), and only three in the subgroup with moderate oligozoospermia. The proportion of heterozygotes was relatively higher among the infertile/subfertile patients with azoospermia or severe oligozoospermia in comparison with the controls. These low percentages among the controls may be due to chance because of the very small numbers of subjects with severe oligozoospermia—as is indicated by the very broad CIs.

Interestingly, we found that 14.3% of those with normozoospermia and unexplained subfertility were homozygous ¹0/¹0, compared with only 2.3% in the unselected control group (P = 0.001) and 0.9% in the fertile group (P = 0.0001). Thus, the OR of having ¹0/¹0 and being normozoospermic and infertile was 18.1 (95% CI: 3.3–184) in comparison to fertile men.

We did not observe an association of the POLG polymorphism with decreased sperm motility; the majority of subjects either heterozygous or homozygous for the absence of the common allele had reasonably good motility (Table I). Sperm morphology had to be analysed separately, because two different methods of assessment were used, therefore the data are not included in Table I. We had to analyse the data using different thresholds for different morphological forms.
Regardless of how we defined the morphology thresholds, we found no association between the POLG polymorphism and sperm morphology.

The analysis of the reproductive hormone profile was consistent with the status of spermatogenesis and normal testicular–pituitary axis function in all patients and controls. There was no association between the hormone profile and the POLG polymorphism (data not shown).

**Analysis of association between the POLG gene polymorphism and fecundity**

We then addressed the question whether or not the POLG polymorphism has any association with fertility or fecundity. Most of our fertile controls were young men recruited when they awaited their first child; therefore, it was not possible to associate the distribution of subjects with the POLG polymorphism with the number of children. For all subjects in our fertile control group B we had recorded the waiting TTP during a previous prospective study of factors affecting couple fecundity (Bonde, 1998). In addition, a large proportion of men in the control group A provided retrospective information on TTP in a questionnaire. We used those data to analyse a possible association of TTP with the POLG polymorphism, especially the heterozygotes, since we found only three homozygotes among the fertile controls. The three fertile #10/#10 subjects had normal sperm parameters and reproductive hormones. In the fertile control group A, the male partners with #10/#10 reported an average TTP of 3.9 ± 0.4 (SE) months, whereas the #10/#10 men reported a mean TTP of 6.3 ± 1.9 months (P = 0.21, not significant). In the fertile control group B, those with #10/#10 had a mean TTP of 3.4 ± 0.27 months, those with #10/#10, 2.5 ± 0.42 months (P = 0.13, not significant). For both fertile subgroups combined, the mean TTP values were 3.8 ± 0.4 months (median 2 months) for the #10/#10 subjects versus 5.6 ± 1.6 months (median 2 months) for the #10/#10 subjects (P = 0.26, not significant). Thus, no significant association between the POLG gene polymorphism and TTP was found.

Eleven of the 12 patients homozygous for the POLG polymorphism were treated by assisted reproductive techniques (ART). In four cases a contributing female factor was present, with one exception (case 10, partial tubal occlusion) the problems were negligible. The outcome of the treatment is listed in Table II. Three cases underwent standard IVF. In one couple, three of four oocytes cleaved to an embryo, whereas only one of nine and one of five cleaved in the two other couples. One patient conceived but miscarried, two remaining patients conceived after ICSI, but one aborted. Nine of the 11 ART-treated #10/#10 patients underwent ICSI, either as the first treatment or after failed IVF. Apart from a single case, where none of 16 oocytes were fertilized after ICSI (case 4, Table II), all others had fertilization and cleavage. Seven partners conceived and delivered eight healthy children; one couple (case 1) subsequently underwent a second ICSI procedure which resulted in another child.

**Discussion**

In this large study of infertile/subfertile patients and well characterized controls, we found a high frequency of the absence of the common allele with 10 CAG repeats in the POLG gene in patients with fertility problems, but noteworthy, mainly among those with a truly idiopathic condition, in whom conventional parameters of semen quality were within the normal range. Male subfertility is a heterogeneous disorder and a symptom rather than a disease, because it may be due to a number of underlying systemic and local diseases, both inherited and acquired. In most cases, the pathogenesis involves impairment in sperm production, maturation or function. But in many subfertile patients no problem can be detected by semen analysis. Some of these patients may

### Table I. Proportions of subjects with the POLG gene polymorphism in subgroups divided according to semen quality parameters (sperm concentration and motility)

<table>
<thead>
<tr>
<th>Subgroups with a given parameter</th>
<th>Homozygous for the polymorphism (CAG #10/#10)</th>
<th>Heterozygous for the polymorphism (CAG 10/#10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subfertile/infertile</td>
<td>Unselected controls</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 × 10⁶/ml</td>
<td>0.7% (1/144)</td>
<td>0% (0/16)</td>
</tr>
<tr>
<td>1.0–1.8%</td>
<td>0.1–1.8%</td>
<td>0.1–1.8%</td>
</tr>
<tr>
<td>&gt;5 × 10⁶/ml</td>
<td>2.4–7.4%</td>
<td>2.4–7.4%</td>
</tr>
<tr>
<td>5–20 × 10⁶/ml</td>
<td>5.7% (3/57)</td>
<td>5.7% (3/57)</td>
</tr>
<tr>
<td>&gt;20 × 10⁶/ml and motility&lt;50%</td>
<td>7.7% (8/104)</td>
<td>7.7% (8/104)</td>
</tr>
<tr>
<td>Motility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50%</td>
<td>2.7% (4/147)</td>
<td>2.7% (4/147)</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>5.7% (8/139)</td>
<td>5.7% (8/139)</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>14.3% (7/49)</td>
<td>14.3% (7/49)</td>
</tr>
<tr>
<td>&gt;20 × 10⁶/ml and motility&gt;50%</td>
<td>5.9–27.2%</td>
<td>5.9–27.2%</td>
</tr>
</tbody>
</table>

The proportions are given as percentage and N, 95% CIs are listed underneath. Significant differences in frequencies of the POLG polymorphism between subfertile patients and fertile controls are marked: *P = 0.0106; p = 0.0009; *P = 0.005; *P = 0.0001 compared with fertile controls.
harbour the POLG gene polymorphism which seems to impair sperm function in a discrete manner, without affecting traditional spermograms markedly.

In a previous analysis of the POLG polymorphism, Rovio et al. (Rovio et al., 2001) found a substandard motility of spermatozoa in a substantial subset of infertile patients, and hypothesized that the variant POLG might cause some defects in, for example, mtDNA repair or replication, leading to a reduced energy metabolism and impaired motility of spermatozoa. However, we could not corroborate this attractive hypothesis: the surprising finding of our study was that the majority (18 of 23) of the subjects homozygous for the POLG polymorphism in our series had sperm motility within the normal range. Depletion and rearrangements of mtDNA, including those caused by mutations in other regions of the POLG gene, are often manifested as serious diseases in humans, e.g. progressive external ophthalmoplegia (Van Goethem et al., 2001; Elpeleg et al., 2002), whereas variants of POLG limited to the CAG repeats were not associated with any systemic disorders and seem to cause solely an impairment in male fertility (Rovio et al., 1999; 2001; this study). The molecular mechanism of this impairment remains to be elucidated.

We found an association between sperm concentration and the POLG polymorphism, but in the opposite direction than we had expected. Nearly all #10/#10 homozygotes had reasonable sperm counts; most of them, in fact, exceeding the current normal cut-off value of $20 \times 10^6$ sperm/mL. Such an association was not observed among the heterozygotes, who were more or less evenly distributed across the sperm concentration strata, except the controls, who were less represented in the subgroup with the lowest sperm counts, most probably because these groups were very small. Rovio et al. (2001) observed a low number of morphologically normal spermatozoa in some patients with #10/#10, but always in conjunction with either poor motility or decreased sperm concentration. We could not confirm this in our study, and we found no correlation between the POLG polymorphism and sperm motility or morphology. This discrepancy, however, could simply be caused by technical differences between the laboratories. The assessment of these variables, especially morphology, is subjective and not very sensitive, thus discrete differences between the groups cannot be excluded and will require further studies using other methods and parameters of semen quality.

We investigated a possibility that the POLG polymorphism could impair the post-ejaculatory functions of spermatozoa, which include oocyte penetration and fertilization. We had recorded an in vitro sperm penetration test in some patients but we had too few polymorphic subjects to observe any associations. Another marker of biological fecundity is the TTP. We found a weak trend to longer TTPs in the control subjects heterozygous for the POLG polymorphism, but could not substantiate this observation in a more conclusive manner, because of the low number of subjects in this category. However, we had data on the outcome of ART in nearly all homozygous #10/#10 patients. In three cases treated with IVF, cleavage of embryos was obtained, but the oocyte fertilization rate tended to be low, thus we cannot exclude some impairment of the #10/#10 sperm ability to penetrate or fertilize the oocyte. When ICSI was used, fertilization of oocytes did not seem to be impaired, as nearly all couples conceived. So far, all children appear to be healthy.

Finally, this study found a similar distribution of the normal and polymorphic POLG alleles in the Danish population to that reported in several other populations, including Finland (Rovio et al., 1999; 2001). In Finland, semen quality appears to be better than in Denmark and the incidences of genital malformations and testicular cancer are low (Jørgensen et al., 2001; 2002; Toppari et al., 2001). The differences in the male

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### Table II. Summary of the clinical information and the outcome of ART treatment in all subfertile cases homozygous for the POLG gene polymorphism

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (CAG)</th>
<th>Mean sperm conc. ($\times 10^6$/ml)</th>
<th>Mean sperm motility (%)</th>
<th>Female infertility factor present (age)</th>
<th>Treatment (IVF, ICSI, IUI-H)</th>
<th>Cleavage rate after IVF (%)</th>
<th>Cleavage rate after ICSI (%)</th>
<th>Clinical pregnancy</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>24</td>
<td>11/12</td>
<td>21</td>
<td>44</td>
<td>No (24)</td>
<td>ICSI</td>
<td>36</td>
<td>Yes</td>
<td>Yes (twice)</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>8/11</td>
<td>27</td>
<td>46</td>
<td>Yes (37)</td>
<td>ICSI</td>
<td>40</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>11/12</td>
<td>31</td>
<td>61</td>
<td>No (33)</td>
<td>ICSI</td>
<td>43</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>9/9</td>
<td>8.6</td>
<td>60</td>
<td>No (28)</td>
<td>ICSI and IVF-D</td>
<td>0</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>11/12</td>
<td>18</td>
<td>96</td>
<td>No (38)</td>
<td>ICSI</td>
<td>68</td>
<td>Yes*</td>
<td>Yes</td>
</tr>
<tr>
<td>6*</td>
<td>47</td>
<td>11/11</td>
<td>6.2</td>
<td>55</td>
<td>No (35)</td>
<td>ICSI</td>
<td>13</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>9/11</td>
<td>29</td>
<td>48</td>
<td>No (32)</td>
<td>IUI-H</td>
<td>–</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>11/12</td>
<td>27</td>
<td>36</td>
<td>Yes (34)</td>
<td>IVF + ICSI*</td>
<td>75</td>
<td>20</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>8/9</td>
<td>26.5</td>
<td>35</td>
<td>No (37)</td>
<td>IVF + ICSI*</td>
<td>11</td>
<td>62</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>9/9</td>
<td>88</td>
<td>32</td>
<td>Yes (32)</td>
<td>IVF + ICSI*</td>
<td>20</td>
<td>30</td>
<td>Yes*</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>9/11</td>
<td>46</td>
<td>37</td>
<td>Unknown (26)</td>
<td>Not treated</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>29</td>
<td>11/11</td>
<td>2</td>
<td>50</td>
<td>No (31)</td>
<td>ICSI</td>
<td>–</td>
<td>50</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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* Cleavage rate defined as proportion of embryos per aspirated oocytes.
* The pregnancy was achieved by IVF with donor sperm fertilization.
* Patient not Danish.
* The second cycle was changed to ICSI due to <1 $\times 10^6$ sperm cells with progressive motility after density gradient separation.
* ICSI was done in subsequent cycles due to low fertilization rate after IVF.
reproductive health between the two countries cannot be explained by the clustering of the $10/10$ genotype in Denmark; however, it is theoretically possible that the POLG polymorphism may render spermatozoa more sensitive to certain adverse environmental or lifestyle factors to which the Danish population is exposed.

In conclusion, our study confirmed an association between the POLG gene polymorphism and male subfertility. The $\neq 10$/10 patients had better average semen parameters than infertile men in general, thus the POLG gene polymorphism should be considered as a possible contributing factor in cases of idiopathic subfertility with normal spermograms. Molecular mechanisms leading to the impairment of fertility in patients with this polymorphism remain to be elucidated, but we found that spermatozoa of $\neq 10$/10 patients may have a somewhat impaired ability to penetrate or fertilize intact oocytes. This problem can be solved by ICSI and the clinical results of this treatment are promising.

Acknowledgements

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