Preimplantation genetic diagnosis for fragile Xa syndrome: difficult but not impossible

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BACKGROUND: In this paper, we review our clinical preimplantation genetic diagnosis (PGD) programme for fragile Xa syndrome, analysing if PGD for these couples is still a valuable option, as it is particularly difficult for two reasons. First, the couples have to be informative (the number of triplet repeats on the healthy FMR-1 allele of the mother has to be different from the number of repeats on the healthy FMR-1 allele of the father) and second, women with a premutation are at increased risk of premature ovarian failure. METHODS: A total of 34 couples attended our genetics department between December 1998 and July 2001, requesting information about PGD for fragile Xa syndrome. RESULTS: Eight couples decided not to go further with the procedure and of the 26 remaining couples, 16 were informative (61.5%). Four couples have so far not started ovarian stimulation, one patient was totally refractive to stimulation and 11 couples have had a total of 19 oocyte retrievals. From these, there have been 13 embryo transfers with a clinical pregnancy rate per embryo transfer of 23%; the implantation rate was 13.6% and the live birth rate per couple was 27.3%. CONCLUSIONS: PGD for fragile Xa is feasible for a number of couples. A pre-PGD work-up should include a determination of the premutation or mutation carrier status, the maternal or paternal origin of the premutation and an estimation of the ovarian reserve of the patient. Fragile Xa premutation carriers should be advised not to postpone reproduction for too long.

Key words: controlled ovarian stimulation/fragile X syndrome/preimplantation genetic diagnosis/premature ovarian failure/premutation

Introduction

Fragile Xa syndrome, first described in 1969 (Lubs, 1969) and further delineated by several authors (Escalante et al., 1971; Turner et al., 1975; Cantu et al., 1976), is the most common single cause of severe mental retardation after Down’s syndrome. The disorder is inherited as an X-linked trait, but both males and (to a lesser extent) females can be affected. The prevalence of the syndrome is estimated to be 1 in 4000–6000 boys (Youings et al., 2000), 1 in 8000 girls (Crawford et al., 1999) and 1 in 250 unaffected female mutation carriers (Murray et al., 1997). The clinical phenotype comprises mental retardation of varying degrees, macro-orchidism in males, typical facies with prominent forehead and jaw, long face and large ears, joint laxity in some cases, and frequently behavioural problems (de Vries et al., 1998). A similar but milder clinical presentation is seen in 50–60% of the female carriers of fragile Xa syndrome (Bardoni et al., 2000).

The gene involved in fragile Xa syndrome, the fragile X mental retardation (FMR-1) gene, was identified in 1991 (Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991). The mutation causing fragile Xa syndrome most often involves an unstable expansion of a CGG trinucleotide repeat in the 5’ untranscribed region of the FMR-1 gene. The number of CGG repeats present in normal alleles varies from 6 to 52 (with an average of 30 repeats) and seems to be transmitted from parent to offspring in a stable manner (Fu et al., 1991). Fragile Xa mutations can be divided into premutations and full mutations. Premutations involve a limited increase in the number of CGG repeats ranging between 50–60 and 200 repeats. Full mutations involve larger expansions of >200 CGG repeats up to even several thousand repeats (Fu et al., 1991; Gilbert, 2001). Premutations are not associated with obvious clinical manifestations; they are found in unaffected carrier females and normal transmitting males. Premutations are unstable when transmitted by a carrier mother, and can become full mutations in both male and female offspring. The full mutation is nearly always associated with DNA hypermethylation of the CpG island in the 5’ untranscribed region of the FMR-1 gene and in this case leads to loss of expression and clinical manifestations in all males and ~50% of the females (Kaufmann and Reiss, 1999; Bardoni et al., 2000). Premutations do not expand when transmitted by a male to his daughters, who will be at risk of having affected children themselves (de Vries et al., 1998).

To women who are carriers of the fragile Xa pre- or full...
A total of 34 couples contacted our genetics department between December 1998 and July 2001, requesting information about PGD for fragile X syndrome. Sixteen were from Belgium, four from France, two from Germany, Greece and Israel, and one from Yugoslavia, Morocco and the USA. Eight couples refrained from further action due to the complexity of the procedure.

Twenty-six couples were tested for informativity as previously described (Sermon et al., 1999); couples with only one repeat difference between the healthy alleles of the mother and father were considered not-informative due to possible difficulties in identifying the single cell PCR products. Sixteen couples were informative, but so far only 11 patients have had ovarian stimulation and reached the stage of oocyte collection. The genotypes and a short clinical history of these couples are given in Table I.

Before PGD, patients were counselled by a psychologist and underwent a minor subfertility work-up. Ovarian stimulation was carried out by pituitary desensitisation with GnRH analogues (buserelin, Suprefact; Hoechst, Brussels, Belgium) combined with hMG (Humegon; Organon, Oss, The Netherlands) or recombinant FSH (Gonal-F; Serono, Brussels, Belgium or Puregon; Organon). hCG

### Materials and methods

A total of 34 couples contacted our genetics department between December 1998 and July 2001, requesting information about PGD for fragile X syndrome. Sixteen were from Belgium, five from Spain, four from France, two from Germany, Greece and Israel, and one from Yugoslavia, Morocco and the USA. Eight couples refrained from further action due to the complexity of the procedure.

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### Table I. Clinical and molecular characteristics of the 11 couples prior to their PGD cycles for fragile Xa syndrome

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age of wife (years)</th>
<th>Repeat length + origin mutation length (wife)</th>
<th>Repeat length (husband)</th>
<th>Reproductive history</th>
<th>Clinical history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>30/100 paternal</td>
<td>42</td>
<td>G1P1</td>
<td>PM found because of family history and affected son; no fertility problem</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>27/150 paternal</td>
<td>22</td>
<td>G2P0A2</td>
<td>PM found because of family history; 2 TOP after CVS; no fertility problem</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>60/PM maternal</td>
<td>23</td>
<td>G1P1</td>
<td>PM found because of family history and affected son; no fertility problem</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>29/68 maternal</td>
<td>31</td>
<td>G0P0</td>
<td>PM found at routine screening before IVF; 2 years of male infertility</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>31/112 paternal</td>
<td>20</td>
<td>G3P0A3</td>
<td>PM found because of family history; had 2 TOP after amniocentesis; no infertility problem</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>30/84 maternal</td>
<td>23</td>
<td>G2P0A2</td>
<td>PM found because of family history; had 2 TOP after amniocentesis; no fertility problem</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>30/85 paternal</td>
<td>35</td>
<td>G0P0</td>
<td>PM found because of family history; mild male subfertility</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>34/66 maternal</td>
<td>30</td>
<td>G0P0A5</td>
<td>PM found because of one affected son; mother also carries balanced reciprocal translocation explaining miscarriages; no fertility problem</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>29/98 paternal</td>
<td>33</td>
<td>G0P0</td>
<td>PM found because of family history; no fertility problem</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>32/57 not known</td>
<td>22</td>
<td>G0P0</td>
<td>PM found at routine screening before ICSI; 1.5 years of severe male infertility</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>30/90 not known</td>
<td>19</td>
<td>G2P1A1</td>
<td>PM found because of one affected son; no fertility problem</td>
</tr>
</tbody>
</table>

PM = premutation (in case the exact length of the PM could not be determined by PCR); G = gestation; P = partus; A = abortus; TOP = termination of pregnancy; CVS = chorion villus sampling.

Mutation, genetic counselling should be offered before they start to reproduce in order to inform them about the risks of transmitting the disease and possible preventive measures. Until recently, couples at risk who wished to prevent the birth of an affected child could remain childless, opt for a spontaneous pregnancy with prenatal diagnosis (PND) and termination of pregnancy (TOP) if the fetus was shown to be affected, use donor oocytes or adopt. Psychological evaluation of couples undergoing TOP after PND revealed that abortion performed for a genetic condition is often experienced as an emotional burden (Blumberg et al., 1975). Moreover, both oocyte donation and adoption result in the absence of a genetic parental relationship and are consequently seldom observed as the first choice option.

The development of preimplantation genetic diagnosis (PGD) techniques has offered an extra option for these couples. PGD can be considered as an early form of PND carried out before embryonic implantation. Hence, the physical and psychological trauma associated with a possible TOP is avoided.

Carrying out PGD for fragile Xa syndrome is difficult for two reasons. The first has to do with the analytical part of the PGD procedure (Sermon et al., 1999). The principle of the procedure is to amplify the normal, shorter CGG triplet repeats present in the biopsied single cells from the embryos to be diagnosed. The aim is to select unaffected embryos. To be able to do this, couples at risk have to be informative for their CGG repeats, i.e. the number of triplet repeats on the healthy FMR-1 allele of the mother has to be different from the number of repeats on the healthy FMR-1 allele of the father. This way, non-carrier female embryos and unaffected male embryos can safely be selected for transfer. Premutation carrier embryos will only be identified if the numbers of repeats do not exceed 75. Larger premutations or mutations will not be seen because they will not be amplified by PCR, this means that a number of embryos carrying a premutation will be lost for transfer. Moreover, only 63% of couples are informative (Fu et al., 1991). However, a number of non-informative couples can be helped by using informative polymorphic DNA markers linked to the FMR-1 gene (Dreesen et al., 2000; Apessos et al., 2001).

The second reason has to do with the observation that women carrying a premutation are at increased risk of premature ovarian failure (POF) (Murray et al., 1999; Marozzi et al., 2000; Hundscheid et al., 2001) and a reduced ovarian response to stimulation protocols results in few embryos for biopsy and diagnosis, rendering PGD an unrealistic option (Vandervorst et al., 1998). Some centres even stopped offering PGD diagnosis for fragile Xa syndrome following a few initial cycles (Howard-Peebles, 1996; Apessos et al., 2001).

In this paper, we review our clinical PGD programme for fragile Xa syndrome, analysing if PGD for these couples is still a valuable option.
(10 000 IU, Pregnyl; Organon or Profasi; Serono) was administered when at least three follicles of 17 mm diameter were seen on vaginal ultrasound scan. Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 h after hCG administration. The luteal phase was supplemented by 600 mg micronized progesterone daily, administered intravaginally (Utrogestan; Piette, Brussels, Belgium).

ICSI rather than IVF was used to fertilize the oocytes to prevent residual contamination with sperm (Liebaers et al., 1998). The details of the ICSI procedure have been described previously (Joris et al., 1998; Van Steirteghem et al., 1998). At 16–18 h after the injection procedure, all oocytes were evaluated for intactness and fertilization. The quality of the embryos was assessed 1 day later. According to the number of anucleate fragments, the embryos were subdivided into grades A, B, C and D as described previously (Vandervorst et al., 2000). In the morning of day 3, grade A, B and C embryos were biopsied. Two blastomeres were removed from those embryos that contained seven or more blastomeres. Embryo biopsy was accomplished by making a hole in the zona pellucida by a stream of acidic Tyrode with a fine needle or by laser as previously described (De Vos et al., 2001). PGD diagnosis was assigned only if two blastomeres gave the same result (Sermon et al., 1998). The cell lysis and PCR procedures were performed as previously described (Sermon et al., 1999). As in regular IVF cycles, the age of the patient, the rank of trial and embryo quality determined the number of embryos transferred, usually two if available. Supernumerary unaffected embryos were cryopreserved.

Implantation was confirmed when two serum hCG concentrations at least 10 days after embryo transfer showed a gradual increase. A clinical pregnancy was noted when an intrauterine gestational sac was seen on vaginal ultrasound at least 5 weeks after the embryo replacement. An ongoing pregnancy was defined as a clinical pregnancy with a fetal heartbeat >12 weeks. In these cases, the couple was advised to undergo PND in order to confirm the PGD diagnosis.

Normally distributed (Kolmogorov–Smirnov test with Lilliefors correction) variables were tested with the t-test for independent samples, while not-normally distributed variables were analysed with the Mann–Whitney U-test. All tests were two-tailed with a confidence level of 95% (P < 0.05), while values were expressed as mean ± SEM.

**Results**

Of the 26 couples that wanted further testing, 16 were informative (61.5%). Of these, four couples have, so far, not started ovarian stimulation as they found the procedure too complicated, too expensive or preferred to wait for a spontaneous pregnancy combined with PND. One patient was totally refractory to the ovarian stimulation. So far, 11 couples have had PGD. Of these, five wanted to avoid TOP, three had already had a TOP after prenatal diagnosis and three had a coinciding male factor infertility problem.

Details of the individual PGD cycles for fragile Xa syndrome are shown in Table II. Patients 3 and 10 had no biopsy as they found the procedure too complicated, too expensive or preferred to wait for a spontaneous pregnancy combined with PND. One patient was totally refractory to the ovarian stimulation. So far, 11 couples have had PGD. Of these, five wanted to avoid TOP, three had already had a TOP after prenatal diagnosis and three had a coinciding male factor infertility problem.

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all the ICSI cycles performed in our centre in 2000 are shown in Table III. Fragile Xa patients needed significantly more ampoules of FSH for ovarian stimulation.

Sixty-one embryos were biopsied (55% of all normally fertilized oocytes): 27 (44.26%) were unaffected male or female embryos based on the presence of the ‘healthy’ FMR-1 allele of the mother; 21 (34.42%) were affected male and female embryos based on the absence of the ‘healthy’ FMR-1 allele of the mother; 4 (6.5%) were female premutation carrier embryos and 9 (14.5%) of the embryos remained without diagnosis.

Twenty unaffected and two female premutation carrier embryos (after discussion with the couple) were transferred.

Three healthy babies were born at term after uncomplicated pregnancies and a normal vaginal deliveries; the two boys were confirmed to be unaffected (both with a birth weight of 3400 g) and the girl (3000 g) was a carrier of a stable premutation (as already known and discussed with the parents at the time of embryo transfer).

**Discussion**

PGD for fragile Xa syndrome may not be an option for all at-risk women due to technical limitations of the diagnostic procedure on the one hand and poor ovarian response on the other. However, as reported in this study, it is definitely feasible for a selected group of women.

The technical problem concerning the non-informativity of the CGG repeat, i.e. the healthy allele (the CGG repeat on the healthy X chromosome) of the female carrier has the same length as the allele of the male partner, which was 38.5% in our group of patients and comparable with what has been described in the literature (Fu et al., 1991), can be reduced to 10% when linked polymorphic markers are used (Dreesen et al., 2000; Apessos et al., 2001). This figure could even be lowered in the future by developing a multiplex PCR for the CGG repeat together with polymorphic markers in patients where there is only one CGG repeat difference in the healthy alleles of both partners. We have currently applied PGD for fragile Xa syndrome using two flanking linked markers (FRAXAC2 and DXS548) in a patient (with an affected son) who carried 30 repeats on her healthy allele and whose husband also carried 30 repeats. Three other couples have been tested for informativity for these same markers, but were shown to be not-informative for at least one of the two markers. This indicated that more markers will have to be used.

All patients who are referred for a PGD cycle should be counselled about the success rate, which depends largely on the number of cumulus–oocyte complexes retrieved (Vandervorst et al., 1998). Patients who carry an FMR-1 premutation and not a full mutation (Murray et al., 1999) are at increased risk of a reduced ovarian reserve and subsequently a poor response to ovarian stimulation. It is therefore important for the clinician to know the repeat size of the patient’s FMR-1 gene. Furthermore, it is still unclear if the origin of the premutation is important, as women with a paternally-inherited premutation might be more prone to POF, whereas women with a maternally-inherited premutation are not (Hundseheid et al., 2000a,b; Murray et al., 2000; Sherman, 2000; Vianna-Morgante and Costa, 2000). All premutation carriers should therefore undergo at least one of the following preliminary medical examinations: early follicular FSH or inhibit B, clomid or GnRH agonist challenge test, ovarian volume or number of antral follicles visible on vaginal ultrasound (Sharara et al., 1998; Syrop et al., 1999; Welt et al., 1999; Ng et al., 2000), in order to assess, as far as possible, the chances of a successful response to ovarian stimulation and to counsel them appropriately.

Although the predictive value of these tests is limited (Corson et al., 1999; Gulekli et al., 1999; Creus et al., 2000), they give the IVF clinician an indication about the down-regulation protocol to use and the FSH dose to start with in order to yield a maximum number of oocytes and minimize the risks of ovarian hyperstimulation syndrome. Our results confirm, as expected, that ovarian stimulation of fragile Xa premutation carriers is difficult; our group of patients needed significantly more gonadotrophins ($P \leq 0.016$) to yield the same number of oocytes compared with our overall ICSI patients. Because of the low number of patients, we do not have enough data to draw conclusions about any influence of the parental origin of inheritance of the premutation on the ovarian stimulation.

Apart from a risk of early menopause, fragile Xa premutation carriers have also a tendency towards a high rate of dizygotic twinning due to multiple ovulations as a result of the elevated FSH concentrations (Marozzi et al., 2000). No data are yet available as to an increased risk of aneuploid embryos or offspring as these patients near the menopause.

Only 55% of all normally fertilized oocytes reached the stage of embryo biopsy, which is low compared with our overall PGD programme (Vandervorst et al., 2000). This could indicate that the embryo quality is reduced, perhaps due to an initial oocyte problem. A further follow-up of more patients in the future might confirm this finding.
Of the 61 biopsied embryos, 27 (44.26%) were unaffected male or female as expected in this condition. There were nine (14.75%) embryos without diagnosis due to problems at the biopsy, blastomere collection, cell lysis or PCR stages. This high rate can be explained by our prudent approach, ruling that a diagnosis could only be established if the two biopsied cells gave the same result.

We always aimed to transfer unaffected male or non-carrier female embryos, except in one case where only female carrier embryos were available, with the same, apparently stable premutation size (68 repeats) as in the mother on the day of biopsy. The small risks of evolution to a full expansion at a later stage of the embryo’s development were discussed and the couple agreed to the transfer. The premutation carrier status of the fetus was confirmed after prenatal diagnosis on amniotic cells and a healthy girl with the same premutation size of 68 repeats was born. In the other two pregnancies, PGD was also confirmed by chorion villus sampling. The three children were born at term and their birth weight, length and head circumference, were in the normal range. A neurodevelopmental follow-up until 26 weeks revealed no abnormalities.

We expect that the demand for PGD for fragile Xa syndrome will increase in the future, as more patients will become aware of their carrier status through screening programmes prior to IVF or even generally in young female adults (Kallinen et al., 2000; Toledano-Alhadef et al., 2001).

In conclusion, we think that PGD for fragile Xa is feasible for a number of couples. A pre-PGD work-up should include determination of the informativity of the CGG repeat, the linked markers or both, determination of the size of the expanded CGG repeat (premutation or full mutation), the maternal or paternal origin of the premutation and an estimation of the ovarian reserve of the patient. With this information, we can then counsel the couple about their chances for a successful PGD cycle.

In any case, an important recommendation to carriers of the fragile Xa syndrome premutation is not to postpone reproduction for too long.

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References


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