CAG trinucleotide repeats in the androgen receptor gene of infertile men exhibit stable inheritance in female offspring conceived after ICSI

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The androgen receptor (AR) gene is located on the X chromosome and contains a polymorphic CAG tract. CAG repeat expansions in the AR have been associated with male infertility and the neuromuscular disease, spinal bulbar muscular atrophy (SBMA). Based on Mendelian inheritance patterns, moderate CAG expansions in infertile men treated by intracytoplasmic sperm injection (ICSI) would be vertically transmitted to female offspring. Should further elongation of the repeat region occur in the male germline, it is conceivable that longer expansions could also be transmitted by ICSI and may lead to an increased incidence of male infertility and SBMA in succeeding generations. To determine the degree of stability of the paternal AR CAG tract following ICSI, we compared the CAG repeat number in the AR alleles of 92 men presenting for ICSI and their 99 ICSI-conceived daughters. CAG repeat lengths in the AR alleles were determined by fluorescent polymerase chain reaction and Genescan analysis of amplification products separated on DNA sequencing gels. In the vast majority of cases (95 out of 99), we found that the AR CAG tracts ranging in size from 15–28 repeats exhibited stable inheritance in female offspring. However, in the remaining father–daughter pairs, there was a discordance in the expected inheritance pattern with evidence for both CAG expansion (20 → 24; 22 → 23) and contraction (26 → 18 or 22) of the paternal AR allele. The detection of a low frequency of CAG mutation in paternal AR alleles following ICSI would be consistent with gonadal mosaicism originating from meiotic DNA replication errors. These findings in a typical group of infertile men undergoing ICSI for a variety of indications tend to alleviate concerns that ICSI may promote the transmission of AR alleles with expanded CAG tracts and suggest that the risk of SBMA in second generation sons would be extremely low.

Key words: androgen receptor/CAG repeat/ICSI/male infertility/spinal bulbar muscular atrophy

Introduction

Androgens are key mediators of normal male sexual development and are continually required for maintenance of secondary sexual characteristics and fertility. These steroid hormones act through the androgen receptor (AR) which is a transcriptional factor that contains functional domains for DNA binding, ligand binding and transcriptional regulation (Jenster et al., 1991). The AR is encoded by a single copy gene on the X chromosome and contains, in exon 1, a polymorphic CAG tract (Lubahn et al., 1988) that varies in length between individuals with a normal range of 11–31 repeat units (La Spada et al., 1991; Edwards et al., 1992). Mutations in the AR gene have been associated with a wide variety of disorders in males, including androgen insensitivity syndrome, prostate cancer and Kennedy’s disease (Choong and Wilson, 1998).

Kennedy’s disease, also known as spinal and bulbar muscular atrophy (SMA), is a fatal X-linked neurodegenerative disease that occurs when the AR gene CAG repeat number is >40 (La Spada et al., 1991). A subgroup of patients with SMA also display other clinical features, e.g. progressive androgen insensitivity, severe oligozoospermia, testicular atrophy and gynaecomastia (Arbizu et al., 1983; Choong and Wilson, 1998). Like all related CAG repeat neurodegenerative disorders, e.g. Huntington’s disease (HD), dentato-rubro-pallidoluysian atrophy (DRPLA) and spinocerebellar ataxia (SCA) types 1, 2, 3, 6 and 7, the length of the AR CAG tract shows a tendency to expand over generations leading to an increase in the severity of SBMA pathology and a concomitant decrease in the age of disease onset (Choong and Wilson, 1998). The association between CAG repeat number in the AR gene and male infertility has also been the subject of several investigations in different ethnic populations. In American (Puscheck et al., 1994), Swedish (Giwercman et al., 1998) and German (Dadze et al., 2000) studies of infertile men, no significant increase in AR gene CAG repeat number above control subjects was found. However, in Chinese (Tut et al., 1997) and Belgian (Legius et al., 1999) studies, where a larger group of infertile men with spermatogenic defects and control male subjects were examined, longer CAG tracts were associated with infertility. More recently, in another study in which men with idiopathic infertility were classified according to their semen parameters (Dowsing et al., 1999), it was shown that a subgroup of azoospermic and oligozoospermic men had...
significantly longer AR gene CAG repeats than men of proven fertility, thus providing the most convincing evidence to date of a link between CAG expansion in exon 1 of the AR gene and defective spermatogenesis.

In the CAG repeat diseases, DRPLA, HD and SMBA, paternal transmission results in a greater intergenerational increase in the length of the CAG repeat tracts than does maternal transmission (Ranen et al., 1995; Takiyama et al., 1995; Ikeuchi et al., 1996). Single sperm typing studies of disease alleles for DRPLA (Takiyama et al., 1999), HD (Leeflang et al., 1995) and SMBA (Zhang et al., 1995) have shown that a high proportion of individual spermatozoa contain larger intragenic CAG repeat tracts than those found in the corresponding lymphocyte DNA. To a much lesser extent, gonadal mosaicism is also observed in men carrying AR gene CAG repeat tracts within the normal range (Zhang et al., 1994). With the increased use of intracytoplasmic sperm injection (ICSI) to treat severe male infertility, moderately expanded AR gene CAG repeats within the upper normal range would be vertically transmitted to female offspring and, therefore, perpetuate male infertility in second generation sons. Further, given that the AR CAG tract can expand during spermatogenesis (Zhang et al., 1994), we postulated that transmission of the AR gene by ICSI may in some cases lead to an increased risk of infertility and even SMBA in future generations. To examine these risks, we compared the AR gene CAG repeat lengths in a large group of infertile men with those of their respective ICSI-conceived daughters using fluorescent polymerase chain reaction (PCR) and allelic sizing of the AR gene CAG tract on DNA sequencing gels.

Materials and methods

ICSI patients

In couples presenting to the Monash IVF Program for ICSI treatment, the male partners were invited to participate in an ongoing research programme to identify genetic causes of male infertility. This study has been approved by the Human Ethics and Research Committees of the Epworth Medical Centre, Richmond and the Monash Day Surgery Hospital, Clayton, Australia. Consent was obtained for collection of venous blood from prospective fathers and either cord blood (at delivery) or venous blood (at 4–12 months of age) from their ICSI children. Blood samples were obtained from 92 consecutive infertile men and their 99 daughters in EDTA blood tubes. Preliminary Y chromosome testing of the infertile men for the spermatogenic markers RBM and DAZ (McLachlan et al., 1998) eliminated Yq deletions in the AZFb and AZFc regions respectively, as a potential cause of their infertility. Male factor indications for ICSI and their clinical diagnoses are summarized in Table I. The assessment of semen was performed on two separate occasions at intervals of 6 weeks, according to World Health Organization guidelines (WHO, 1999). Sperm concentration was grouped into four categories: azoospermia, severe oligozoospermia (>0 to <5 × 10^6/ml), oligozoospermia (>5 to <20 × 10^6/ml) and normal (>20 × 10^6/ml). Sperm motility was assessed as total motility (WHO grades A, B and C) of <10, 11–40 and >40%. Sperm morphology was classified using strict criteria of <90, 91–95, 96–99 and 100% abnormal forms. In some cases of severe oligozoospermia, inadequate spermatozoa were available for assessment of motility and/or morphology. The diagnosis of idiopathic seminiferous tubule failure (STF) was made when one or more of semen count, motility or morphology was subnormal. Accordingly, some men (n = 5) had normal sperm counts but significant impairment of other sperm parameters indicative of a spermatogenic defect. An immunobead assay was used to assess the presence of sperm antibodies and a positive result was defined as >50% sperm binding to rabbit anti-human immunoglobulin (Ig)G and/or IgA beads (Irvine Scientific, CA, USA).

Isolation of genomic DNA

Unless otherwise stated, all reagents and chemicals were purchased from Progen Industries Ltd (Brisbane, Australia). Genomic DNA was purified from 2 ml blood samples. Red cells were lysed by the addition of 4 volumes of red cell lysis buffer (10 mmol/l Tris pH 8.0, 10% sucrose, 5 mmol/l MgCl2, 0.1% Triton X-100) for 5 min at room temperature. White cell pellets were resuspended in 0.5 ml of white cell lysis buffer (100 mmol/l Tris pH 8.0, 50 mmol/l EDTA, 50 mmol/l NaCl, 1% sodium dodecyl sulphate, 1 mg/ml of proteinase K) and incubated at 55°C for 1 h. Following two organic extractions with an equal volume of phenol/chloroform, DNA was precipitated by the addition of 1 ml of cold ethanol, dried and resuspended in TE buffer (10 mmol/l Tris pH 8.0, 1 mmol/l EDTA). The concentration of each genomic DNA preparation was measured by UV spectrophotometry (Lambda Bio 20; PE Applied Biosystems, Melbourne, Australia).

Fluorescent PCR and gel electrophoresis

DNA encompassing the AR gene CAG tract in exon 1 was generated by PCR using primers: ARG1 5′-CAGAATCTGTTCAGAGCGTGC-3′ (sense) and ARG2 5′-AAGGTGTCTGGTCTCTCATCCAG-3′ (antisense) designed to conserved sequences flanking the trinucleotide repeat region. For fluorescent PCR, the ARG1 sense primer was labelled with 6-carboxyfluorescein (6-FAM) (Applied Biosystems, Foster City, CA). Autosomal microsatellite markers D1S199, D4S403, D9S1690 and D12S86 from Applied Biosystems (ABI PRISM Linkage Mapping Set Version 2) with heterozygosity indices of 0.83, 0.77, 0.78 and 0.89 and broad allelic size ranges of 99–125, 173–189, 230–244 and 132–172 bp respectively, were analysed with either 6-FAM or hexachlorinated analog of 6-FAM (HEX)-labelled primer sets to confirm ICSI father and daughter DNA pairs. Fluorescent PCR reactions were performed in 10 mmol/l Tris–HCl, pH8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl2 and 200 µmol/l dNTPs containing 100 ng of genomic DNA, 5–20 µmol/l of each primer and 0.2 IU of Taq polymerase (Amersham Pharmacia Biotech Australia Pty Ltd) in a final volume of 7.5 µl. Thermal cycling parameters were 95°C, 55°C and 72°C for 45 s each (30–35 cycles) followed by 72°C for 10 min to ensure complete addition of deoxyadenosine at the 3′ ends for accurate allele sizing.

Fluorescently-labelled PCR products were quantified on 1.5% agarose gels and diluted to optimal concentrations prior to polyacrylamide gel electrophoresis. Diluted PCR products (1 µl) were mixed with 2 µl of loading buffer (75% deionized formamide, 5 mmol/l EDTA, 5 mg/ml blue dextran) containing Genescan tetramethylrhodamine (TAMRA)-labelled internal standards (Applied Biosystems), heated for 3 min at 95°C and loaded directly onto 4% denaturing sequencing gels. Samples were electrophoresed (ABI 377 sequencer, Applied Biosystems) at 3000 V for 2 h and bands sized by Genescan Applied Biosystems software using the local Southern method to calculate the molecular size.

Analysis of AR gene CAG repeat lengths

The number of AR gene CAG repeats predicted by Genescan were compared with the actual number determined by direct dideoxy terminator cycle sequencing using the Big Dye Terminator Sequencing
Statistical analysis

Differences in mean AR gene CAG repeat lengths between the different male factor diagnostic groups were examined by the Wilcoxon two sample test. P < 0.5 was considered to be statistically significant.

Results

ICSI fathers and AR gene CAG repeat lengths

A cohort of 92 consecutive men who fathered 99 daughters (85 singletons and seven twins) by ICSI was selected for the study. The male factor diagnoses for these infertile men are shown in Table I. Half the men (46 of 92) were diagnosed with idiopathic seminiferous failure (STF). The remainder presented with other clinical indications for ICSI, particularly obstructive oligozoospermia (n = 20) and poor/failed fertilization in a previous IVF cycle (n = 14). The CAG repeat lengths in their AR gene were determined by fluorescent PCR and automated allelic sizing on DNA sequencing gels. As a group, the mean AR gene CAG repeat number of the ICSI fathers was 22.2 ± 0.3 with a range of 15–28. There was no significant difference in the mean AR gene CAG repeat number between men with STF and men with other male factor diagnoses (Table I).

Comparison of AR gene CAG repeat tracts in ICSI fathers and their female offspring

The length of the AR gene CAG tracts in the two alleles inherited by each of the 99 daughters conceived after ICSI were determined in the same gels with their respective fathers. As an example of our CAG repeat analysis strategy, the inheritance profile for father F697 who had twin daughters.

Table I. Clinical indications for intracytoplasmic sperm injection (ICSI)

<table>
<thead>
<tr>
<th>Male factor diagnosis</th>
<th>No. of patients</th>
<th>AR gene CAG repeats mean ± SE (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic seminiferous tubule failure</td>
<td>46</td>
<td>22.1 ± 0.4 (15 ± 26)</td>
</tr>
<tr>
<td>Azoospermia (n = 2)</td>
<td></td>
<td>24, 25</td>
</tr>
<tr>
<td>Extreme oligozoospermia (n = 14)</td>
<td></td>
<td>21.7 ± 0.8 (15 ± 25)</td>
</tr>
<tr>
<td>Severe oligozoospermia (n = 14)</td>
<td></td>
<td>22.2 ± 0.5 (20 ± 26)</td>
</tr>
<tr>
<td>Oligozoospermia (n = 11)</td>
<td></td>
<td>21.9 ± 0.7 (18 ± 25)</td>
</tr>
<tr>
<td>Normozoospermia, astheno and teratozoospermia (n = 5)</td>
<td>26</td>
<td>21.6 ± 0.5 (21 ± 24)</td>
</tr>
<tr>
<td>Obstructive azoospermia – sperm retrieval</td>
<td>20</td>
<td>22.0 ± 0.6 (18 ± 27)</td>
</tr>
<tr>
<td>Vasectomy (FNA/TESE, ejaculated spermatozoa; n = 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital bilateral absence of the vas (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm antibodies</td>
<td>3</td>
<td>23.0 ± 1.1 (21 ± 25)</td>
</tr>
<tr>
<td>Poor semen quality (post-thaw motility, chemotherapy)</td>
<td>4</td>
<td>22.7 ± 2.3 (18 ± 28)</td>
</tr>
<tr>
<td>Normal male (oocyte/female indications)</td>
<td>5</td>
<td>21.0 ± 1.2 (18 ± 25)</td>
</tr>
<tr>
<td>Failed/poor prior IVF</td>
<td>14</td>
<td>22.7 ± 0.6 (18 ± 26)</td>
</tr>
</tbody>
</table>

FNA = fine needle aspiration; TESE = testicular sperm extraction.
Definitions: extreme oligozoospermia = >0 to ≤5.0 × 10⁶ spermatozoa/ml; severe oligozoospermia = 0.1 to <5.0 × 10⁶ spermatozoa/ml; oligozoospermia = ≥5.0 to 20 × 10⁶ spermatozoa/ml; normozoospermia = >20 × 10⁶ spermatozoa/ml.
Correlation of androgen receptor (AR) gene CAG repeat number in intracytoplasmic sperm injection (ICSI) father and daughter pairs. Diagonal numbers with an asterisk represent the frequency of concordance and discordance of AR gene CAG numbers (range of 15–28 repeat units) between related subjects.

Table II. Discordance between androgen receptor (AR) gene CAG repeat number in the father’s allele and the alleles of his female offspring conceived after intracytoplasmic sperm injection (ICSI). Numbers in bold indicate the paternal marker alleles inherited by each daughter

<table>
<thead>
<tr>
<th>Father/daughter pair</th>
<th>Male factor diagnosis</th>
<th>Father AR gene CAG repeat number</th>
<th>Daughter AR gene CAG repeat number</th>
<th>Father marker alleles D1S199 D4S403 D9S1690 D12S836</th>
<th>Daughter marker alleles D1S199 D4S403 D9S1690 D12S836</th>
<th>Change in paternal AR gene CAG repeat number after ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F437/D854 STF</td>
<td>26</td>
<td>22, 18</td>
<td>102 180 232 –</td>
<td>102 176 238 –</td>
<td>4 or 8 CAG</td>
<td></td>
</tr>
<tr>
<td>F972/D822 STF</td>
<td>20</td>
<td>19, 24</td>
<td>106 182 232 –</td>
<td>102 182 240 –</td>
<td>1 CAG contraction</td>
<td></td>
</tr>
<tr>
<td>F1416/D1415 STF</td>
<td>20</td>
<td>24, 24</td>
<td>– 182 232 146 –</td>
<td>106 186 240 –</td>
<td>4 CAG expansion</td>
<td></td>
</tr>
<tr>
<td>F1432/D1427 V AS REV</td>
<td>22</td>
<td>23, 23</td>
<td>– 186 240 140 –</td>
<td>– 186 242 146 –</td>
<td>1 CAG expansion</td>
<td></td>
</tr>
</tbody>
</table>

STF = seminiferous tubule failure; V AS REV = vasectomy reversal.

D696 and D698 are shown in Figure 1. In this case, the AR gene CAG repeat number of 22 in the father’s allele was also found in one of the two daughter alleles. This inheritance pattern indicates that each daughter inherited an identical paternal AR allele with 20 CAG repeats but different maternal AR alleles with 14 and 22 GAG repeats respectively. To determine the degree of stability of the CAG repeat tract of paternal AR alleles following ICSI, the CAG repeat number in each matching ICSI father and daughter pair were similarly compared and results are summarized in Figure 2. In 95 of 99 cases (95%) an AR allele with the same number of CAG repeats was found in each father/daughter pair indicating the transmission of an unchanged paternal allele to the daughters. However, in the remaining four father/daughter pairs F437/D854, F972/D822, F1416/D1415 and F1432/D1427, an identical AR CAG repeat allele was not found (Figure 2 and Table II), suggesting the presence of an altered paternal allele in these daughters. To eliminate the possibility that these discordant results were due to mislabelled DNA samples or non-paternity, father and daughter DNAs were subjected to microsatellite analysis using the polymorphic markers D1S199, D4S403, D9S1690 and D12S86. In all cases, at least one of the paternal alleles for each of three markers was inherited by the daughters (Table II), thus validating the expected relationship between each pair.

For F437/D854, the two AR alleles of the daughter contained a CAG number less than the 26 repeat paternal allele, suggesting a contraction of the CAG tract by either four or eight repeat units. In contrast, for F972/D822, the daughters AR alleles were one CAG repeat shorter and four CAG repeats longer than the 20 repeat paternal allele suggesting either a one CAG contraction or a four CAG expansion of the paternal allele. For both F1416/D1415 and F1432/D1427, the daughters were biallelic for the AR repeat region with CAG numbers that were four and one CAG repeat unit longer than that of the respective 20 and 22 repeat paternal alleles indicating an expansion of the CAG tract in each case. Together, these findings provide evidence for a low frequency of contraction and expansion of the AR CAG tract in paternal alleles of infertile men transmitted by ICSI.

Discussion
In this study of 92 infertile men who fathered 99 daughters by ICSI, we examined the transmission pattern of the paternal AR gene CAG tract by comparing the CAG repeat regions in
the AR allele of the father and the two AR alleles in the daughter. In the vast majority of cases, there was no trend towards an increase in paternal AR CAG repeat lengths following ICSI. In four female offspring, minor expansions and contractions of the paternal AR gene CAG repeat region were found. In light of the fact that a low level of gonadal mosaicism has been reported in normal healthy men (Zhang et al., 1994), it is likely that the altered paternal AR alleles inherited by these daughters originated in the fathers’ spermatozoa and was transmitted by ICSI. These findings from a reasonably representative group of patients undergoing ICSI for a variety of indications suggest that the procedure is relatively safe for this type of population although some concern still remains for infertile or sterile men.

An important issue in this study was the identification of the paternal AR allele in the female offspring. In view of our failure to co-amplify the adjacent GCC polymorphic region and the unavailability of maternal DNA to assist in determining the origin of the parental AR alleles, we simply applied Mendelian principles to deduce the paternal allele in each ICSI daughter. Seven of the fathers had twin daughters which allowed us to additionally analyse the maternal AR alleles, see Figure 1. In each of the 14 twin daughters, one of the two AR alleles had the same number of CAG repeats as the paternal AR allele. In six pairs, each of the twin daughters had a different second AR allele, identifying the two maternal alleles and one pair had the same second allele, identifying only one of the maternal alleles. The six ‘non-identical’ twin daughter pairs and one ‘identical’ twin daughter pair with respect to the AR alleles demonstrate that the paternal allele was most likely inherited unchanged. In 11 other father/daughter pairs, only one AR allele was found in the daughter (bi-allelic) and it had the same number of CAG repeats as the paternal allele. These 11 cases again demonstrate that the paternal allele must have been inherited unchanged. In 70 other cases (e.g. paternal 25 CAGs, daughter 25, 20 CAGs) an allele with exactly the same number of CAG repeats was found in the daughter and strongly suggests inheritance of an identical paternal allele. However, in this example, the possibility that the paternal allele had changed from 25 to 20 and the daughter actually inherited a maternal AR allele with 25 repeats (like the father) cannot be excluded, even though the probability of these two independent events occurring by chance would be extremely low. Therefore based on these analyses, the inheritance patterns would strongly indicate that in 95 of 99 cases, the paternal AR allele was transmitted unchanged to the female offspring.

In the remaining four cases, it was clear that the paternal AR allele inherited by the daughter had either lost or gained CAG repeats. Notably, there were two expansions, one of a 22 and one of a 20 CAG allele involving one and four repeats respectively and one contraction of a 26 CAG allele involving either a four or eight CAG contraction. The other change to a 20 CAG allele could have been either an expansion of four repeats or a contraction of one repeat. Given that the normal length of AR gene CAG tract lies between 11 and 31 repeats (La Spada et al., 1991; Edwards et al., 1992), there was no case where the mutation of AR CAG repeat region shifted the CAG repeat tract beyond the limits of the normal range. Interestingly, three of the four paternal alleles that exhibited CAG expansion/contraction were from men diagnosed with STF who had extreme or severe oligozoospermia. The other paternal allele which increased by only a single CAG repeat unit was from a man who had undergone vasectomy reversal. Further analysis of a larger group of men with STF and a variety of other male factor diagnoses is therefore essential to determine if there is any significant association between defective spermatogenesis and a propensity of the paternal AR allele to undergo a shift in CAG number following ICSI.

The overall stability of the somatic CAG repeats (Sutherland and Richards, 1995) would suggest that the most likely origin of AR gene CAG repeat mosaicism results from DNA replication errors during meiosis in the male germ line. The molecular mechanisms underlying the susceptibility of CAG repeats to expansion and contraction are not known but are believed to involve either unequal crossover or single strand slippage of the DNA polymerase during meiotic DNA replication (Hancock and Santibanez-Koref, 1998). From single sperm analyses of alleles involved in the CAG repeat diseases HD, DRPLA and SBMA, a high level of gonadal mosaicism with respect to the CAG tract has been reported (Leeflang et al., 1995; Zhang et al., 1995; Takiyama et al., 1999). For example, HD alleles show an increase in the frequency of CAG expansion as the size of the CAG repeat tract increases from a premutation length to a disease length, as well as a concomitant increase in the extent of the triplet expansion (Leeflang et al., 1995). For highly expanded HD alleles, the frequency of spermatozoa with increased CAG repeats of >95% (Leeflang et al., 1995). In contrast, the frequency of contraction of disease alleles and expansion and contraction of normal alleles was only in the order of 5%. Studies on a large number of X-bearing spermatozoa from men with AR gene CAG repeat sizes within the normal range also showed a low frequency of change relative to their lymphocyte DNA with a four-fold increase in contraction over expansion (Zhang et al., 1994). The range of CAG contraction varied from 1–16 triplets whereas the range of CAG expansions varied by 1–6 triplets. For alleles in the lower range of normal (20, 22 CAG repeats) the frequency of change was ~1.5% but doubled for moderately expanded alleles (28, 29, 30 and 31 CAG repeats). Based on these single sperm analyses, the predicted frequency of transmission of an altered paternal AR allele would be in the order of 1.5–3%. Our results from 92 infertile men and their ICSI-conceived daughters showed an overall mutation frequency of 4% at the AR gene CAG repeat locus which closely reflects the pattern of AR gene CAG repeat mosaicism reported in single spermatozoa from subjects with similar AR gene CAG lengths. Together, these data tend to alleviate concerns that paternal transmission by ICSI significantly increases the frequency of mutations in the AR gene CAG repeat region inherited by female offspring above that predicted for natural conception.

If moderately expanded AR alleles are the cause of the spermatogenic defect in some of the patients analysed in this study, ICSI will undoubtedly perpetuate a similar phenotype in approximately half of the second generation sons. Further, given the overall stability of the paternal AR gene CAG repeat
region following male meiosis (Zhang et al., 1994) and ICSI, the risk of further increases in the severity of male infertility and that of SBMA is extremely low. However, as a cautionary note, none of the patients in this study had AR alleles with >30 CAG repeats. Bearing in mind that premutation length CAG repeats in HD alleles have a much higher propensity to expand in the male germline (Leeflang et al., 1995), infertile men with an AR gene CAG number of >31 could be at a somewhat higher risk of transmitting an expanded disease allele. Whilst female offspring would be protected from disease by X inactivation (La Spada et al., 1991) half of her future sons could be at risk for developing SBMA. Single sperm studies of men carrying AR alleles with CAG repeat numbers of 32–40 are therefore warranted to more clearly delineate this risk. Based on this knowledge, men who fall into this higher risk category could be more effectively counselled to inform them of the potential risks of assisted reproduction. For couples that elect to have children, pre-implantation genetic diagnosis could also be offered as a viable option to avoid the transfer of embryos with the genetic potential for developing SBMA.

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