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Transmission ratio distortion in the myotonic dystrophy locus in human preimplantation embryos

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One form of myotonic dystrophy, dystrophia myotonica 1 (DM1), is caused by the expansion of a $(CTG)_n$ repeat within the dystrophia myotonica-protein kinase (DMPK) gene located in chromosome region 19q13.3. Unaffected individuals carry alleles with repeat size $(CTG)_{5-37}$, premutation carriers $(CTG)_{38-49}$ and DM1 affected individuals $(CTG)_{50-6000}$. Preferential transmission both of expanded repeats from DM1-affected parents and larger DMPK alleles in the normal-size range have been reported in live-born offspring. To determine the moment in development when transmission ratio distortion (TRD) for larger normal-size DMPK alleles is generated, the transmission from heterozygous parents with one repeat within the $(CTG)_{5-18}$ range (Group I repeat) and the other within the $(CTG)_{19-37}$ range (Group II repeat) to human preimplantation embryos was analysed. A statistically significant TRD of 59% (95% confidence interval of 54–64) in favour of Group II repeats from both mothers and fathers was observed in preimplantation embryos, which remained significant when female embryos were considered separately. In contrast, no significant TRD was detected for repeats from informative Group I/Group I parents. Our analysis showed that Group II repeats specifically were preferentially transmitted in human preimplantation embryos. We suggest that TRD, in Group II repeats at the DMPK locus, is likely to result from events occurring at or around the time of fertilisation.
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Introduction

A fundamental principle of Mendelian genetics is the probability of equal transmission, from parent to offspring, of the two alleles at any given diploid locus. However, deviation from Mendelian 1:1 segregation ratios, also termed transmission ratio distortion (TRD), has been documented for different species and different genetic loci (reviewed by Lyttle).[1] In humans, allele-specific TRD, which refers to preferential transmission of a particular allele(s) has been reported for the repeat region near the insulin gene (INS-IGF2 VNTR),[2] and for larger trinucleotide repeats in the genes responsible for myotonic dystrophy (DMPK),[3–7] Machado–Joseph disease (ATXN3),[8–10] fragile X syndrome (FMR1),[11] spino-cerebellar ataxia type 1 (ATXN1),[9] spino-cerebellar ataxia type 7 (ATXN7),[12] and

dentatorubral-pallidoluysian atrophy (*ATN1*).[8] The common feature of these trinucleotide repeats is that they cause a progressive pathological phenotype when there is an expansion of the repeat and, in general, genetic anticipation is seen, with a progressively earlier onset and increased severity of disease, as the condition is transmitted to successive generations of a family.[13]

The human dystrophia myotonica-protein kinase (*DMPK*) gene is located in chromosome region 19q13.3 and contains a $(CTG)_n$ repeat in its 3′ untranslated region.[14,15] Expansion of this repeat causes dystrophia myotonica 1 (DM1 [MIM 160900] Online Mendelian Inheritance in Man http://www.ncbi.nlm.nih.gov/Omim), an autosomal dominant disorder, which is the most common form of adult muscular dystrophy. DM1 has variable phenotypic expression ranging from presenile cataracts in some mildly affected individuals to the severe congenital form with general muscular hypotonia, respiratory distress, increased neonatal mortality rate, delayed motor development, and mental retardation.[16] The phenotypic expression and age of onset are correlated with the length of the repeat expansion.[17,18] Individuals with $(CTG)_{>1000-6000}$ repeats develop the congenital disease.[14] Adult-onset patients carry *DMPK* alleles with $(CTG)_{100-1000}$ repeats, mildly affected or very late onset patients carry alleles with $(CTG)_{50-150}$ repeats, whereas premutation carriers have $(CTG)_{38-49}$ repeats, and unaffected individuals carry alleles with $(CTG)_{5-37}$ repeats.[14]

Preferential transmission of expanded $(CTG)_n$ repeats from DM1-affected parents to live-born offspring has been reported in DM1 families.[4,6,7] Moreover, preferential transmission of the larger of the two $(CTG)_n$ repeats in the normal-size range from parents to live-born offspring has been found in families with no DM1.[3,5,19]

The mechanism of such preferential transmission of larger *DMPK* alleles remains unclear. In principle, TRD may result from events that occur before (meiotic drive or preferential survival of gametes), at the time of, or after fertilisation (embryonic death). To determine the timing of TRD and thereby clarify its mechanism, transmission of *DMPK* alleles was studied in individual spermatozoa of three heterozygous males each with a $(CTG)_5$ and a $(CTG)_{20-37}$ repeat.[20] No segregation distortion was observed, leading to the conclusion that TRD resulted from events following sperm ejaculation. Thus, TRD is present among live-born offspring and apparently, at least in the male germline, results from events at the time or after fertilisation.

It has been suggested that larger normal-size $(CTG)_{19-37}$ repeats, unlike $(CTG)_{5-18}$ repeats, may be susceptible to intergenerational instability and, therefore, preferential transmission of the $(CTG)_{19-37}$ repeats provides a reservoir for future expanded alleles in the population.[21] Carey *et al*[3] selected a cutoff point of $(CTG)_{19}$ repeats for their study of transmission of *DMPK* alleles in the normal-size range.

Based on the functional importance of the $(CTG)_{19-37}$ repeats for the aetiology of DM1 and the evidence for their preferential transmission,[3] we have selected the same cutoff point. To simplify our terminology, we will refer to smaller normal-size *DMPK* alleles with $(CTG)_{5-18}$ as Group I repeats, and to larger normal-size alleles with $(CTG)_{19-37}$ as Group II repeats. To further investigate the timing of the occurrence of TRD of normal-size range repeats in the human *DMPK* locus and as a next step in the elucidation of its mechanism, we conducted an analysis of transmission of larger normal-size *DMPK* alleles, from parents to human preimplantation embryos. Parent-of-origin and gender-of-embryo effects have been previously described in TRD studies both on larger normal-size and expanded *DMPK* alleles to live-born offspring.[3–7,19] Therefore, we also analysed our results according to the sex of the transmitting parent and sex of the embryo.

Materials and methods
Subjects
The length of *DMPK* alleles was determined from consenting patients that underwent *in vitro* fertilisation (IVF) at the McGill Reproductive Centre from September 2001 to October 2003 (McGill University, Montreal, Quebec, Canada). No exclusion was made based on age of patients, cause of infertility, or ethnicity of couples. The research ethics review board of the Royal Victoria Hospital, McGill University Health Centre approved this study. Couples where at least one of the partners was heterozygous for one Group I and one Group II repeat were approached, after their embryos had been selected for transfer to the female and for embryo freezing, for permission to test the remaining embryos. If agreeable, each couple signed consent to permit the use of their embryos in this study.

Determination of the length of maternal and paternal *DMPK* $(CTG)_n$ repeats
The length of the *DMPK* alleles of the mother was determined by polymerase chain reaction (PCR) on the collected cumulus cells surrounding the oocyte. Shortly after the oocyte retrieval, cumulus cells (approximating 20% of the total cumulus mass) were removed from two oocytes using a sterile technique. These cumulus cells were disaggregated in 80 IU hyaluronidase (Vitrolife, Goteburg, Sweden) and washed in sterile phosphate-buffered saline (PBS) (Sigma, Oakville, ON, Canada). A 1.5 μl aliquot of cumulus cells was added to each of two labelled, sterile, 0.2 ml tubes containing 5 μl of alkaline lysis buffer (LB),[22] and overlaid with sterile oil. The allele size of the father was determined by PCR on spermatozoa from two 3 μl aliquots of the sperm preparation used for IVF, using LB and oil as above. Each aliquot of cumulus cells (from mother) and sperm (from father) was lysed by heating to 65°C for 15 min.

A fluorescent, hemi-nested, PCR was carried out to amplify the $(CTG)_n$ repeat region of the *DMPK* gene.[22] Reactions for the first round of PCR were carried out in a total volume of $50 \mu l$ and included 5% dimethylsulphoxide (DMSO), neutralisation buffer (900 mM Tris-HCl (pH 8.3), 300 mM KCl, 200 mM HCl), potassium-free PCR buffer (25 mM MgCl$_2$, 100 mM Tris-HCl (pH 8.3)), 100 μM of each dNTP, 1 U of Taq polymerase (Qiagen, Mississauga, ON, Canada), and 0.4 μM of *DMPK* primers, forward (DMF) and outer reverse (DMOR) (Intergrated DNA Technology, IA, USA). The DMF primer was labelled with CY5 fluorescent dye. The PCR was carried out on a PTC-200 thermocycler (MJ Research, Watertown, MA, USA) using in the first round the following program: 5 min denaturation at 96°C, followed by 15 cycles of 1 min at 96°C, 45 s at 60°C and 1 min at 72°C, followed by 5 min at 72°C.

For the second round of DNA amplification, 2 μl of the first-round products were added to reaction mixes in a total volume of 30 μl with PCR buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl, 15 mM MgCl$_2$), 100 μM dNTPs, 1 U of Taq polymerase (Qiagen, Mississauga, ON, Canada), and 0.4 μM of DMF and *DMPK* reverse (DMR) primers (Intergrated DNA Technology, IA, USA). The cycling conditions were 5 min denaturation at 96°C, followed by 10 cycles of 30 s at 96°C, 45 s at 62°C and 1 min at 70°C, then 15 cycles of 1 min at 94°C, 45 s at 62°C and 1 min at 70°C, and finally 5 min at 72°C.

Fluorescently labelled amplified DNA (3 μl) were mixed with 5 μl of loading dye and denatured for 3 min at 90°C prior to the samples being loaded onto a 9% polyacrylamide denaturing gel and run on an ALF Automated DNA Sequencer (Amersham Pharmacia Biotech, Montreal, QC, Canada). DNA was visualised as fluorescent peaks using the instrument's packaged computer software. A fluorescently labelled 50–500 bp marker (Amersham Pharmacia Biotech, Montreal, QC, Canada) was loaded onto each gel and used to size peak fragments.

Collection of embryos
Every donated embryo was tested with no selection being made on the basis of developmental stage. The inclusion criteria for the study were that the fertilised embryo was not selected to be transferred to the female or to be frozen. Hence, preimplantation embryos from the two-cell stage to blastocyst were included. Each donated embryo was incubated for 5 min in Ca^{2+} and Mg^{2+} free media (Cook Canada Inc., Stouffville, ON, Canada) to allow the decompaction of blastomeres. The zona pellucida was removed using acid Tyrode's solution (pH 2.5) (Sigma, Oakville, ON, Canada) and the cells washed through three drops of PBS containing 4 mg/ml bovine serum albumin (Sigma, Oakville, ON, Canada). The blastomeres were then disaggregated using a sterile pulled-glass pipette and pipetted into one to three tubes, depending on the number of cells present, which contained LB and were overlaid with sterile oil. Grouping of cells for the PCR assay ensured that results were obtained from the maximum number of embryos. After collection, all samples were centrifuged and kept at −80°C for at least 20 min before being incubated at 65°C for 15 min to lyse the cells. For each embryo, a sample of media from the last drop in which the blastomeres had been washed was placed in a PCR tube with 5 μl LB to act as a negative control for that embryo.

Genotyping of donated embryos
A multiplex, hemi-nested, fluorescent PCR was carried out to amplify the *DMPK* $(CTG)_n$ repeat region along with a sequence of the amelogenin gene (*AME*) to determine gender. The primer sequences to amplify the *AME* region were taken from Ray et al.[23] The *AME* primers allowed the detection of two sequences, one on the X and one on the Y chromosome that were different in size to distinguish the gender of each embryo by fragment length analysis. Females have one 115 bp amplified fragment, whereas males have two fragments of 115 and 121 bp. The PCR conditions in the first round were similar to those employed for the determination of the *DMPK* parental allele sizes except that 0.05 μM of *AME* primers, outer forward (AME-OF) and outer reverse (AME-OR) (Intergrated DNA Technology, IA, USA), were added. For the second round *DMPK* and *AME* were amplified separately. For *DMPK*, the reaction mixes were the same as above. For *AME*, 3 μl of the first-round products were added to reaction mixes in a total volume of 30 μl with PCR buffer, 50 μM dNTPs, 1 U of Taq polymerase (Qiagen, Mississauga, ON, Canada), and 0.25 μM of *AME* primers, inner forward (AME-IF) and AME-OR (Intergrated DNA Technology, IA, USA). The AME-IF primer was labelled with CY5 fluorescent dye. The PCR conditions for both reactions were similar to those used in the second round for the parental alleles except that 28 cycles were used in total, 10 cycles followed by 18 cycles. The samples were run on an ALF Automated DNA Sequencer as above using 3 and 5 μl of amplified *DMPK* and *AME* products, respectively.

The printouts from all aliquots amplified from each embryo were compared to ensure they gave the same result and each negative control was checked to ensure there was no amplification. For each embryo, the *DMPK* genotype and embryo gender were recorded.

Reliability of genotyping
Amplification rates for *DMPK* and *AME* were 95 and 94% and rates of allele dropout were 12 and 6%, respectively. Allele dropout is the failure of amplification of one allele in a heterozygous sample and is observed when single cells or very small quantities of DNA are amplified.[24] To confirm the genotypes, duplicate or triplicate samples were run for 86% (293/341) of the embryos, and complete results for the size of the inherited *DMPK* alleles and the gender were obtained for all but six of the embryos tested.

Statistical analysis

Deviation from 1:1 segregation was tested using the exact binomial test in R for Windows, version 1.8.0. (The R Project for Statistical Computing, http://www.r-project. org). Reported *P*-values were not corrected for multiple testing. Taking into account for at most nine comparisons, we considered a *P*-value of <0.0056 to be statistically significant. All other variables were analysed using a two-tailed *t*-test or a two-sided χ^2 test for independent variables. The exact test in GENEPOP, version 3.4 was used to test for Hardy-Weinberg equilibrium (GENEPOP, http://wbiomed. curtin.edu.au/genepop).[25]

Results

Inclusion criteria and characteristics of participating couples

In total, 234 couples (468 individuals) undergoing IVF consented to enter the study and will be further referred to
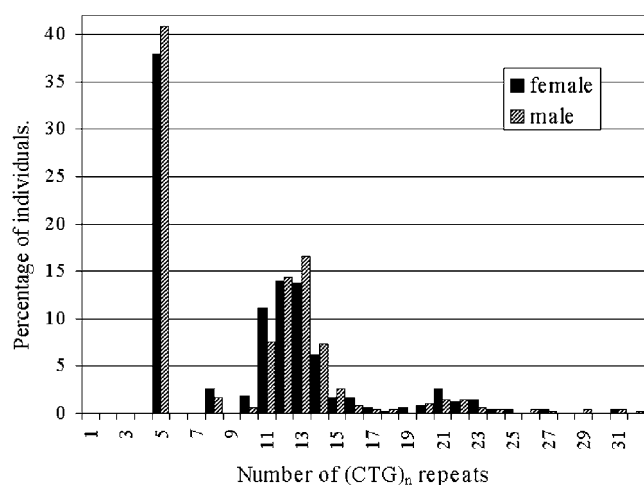
Figure 1 Distribution of *DMPK* $(CTG)_n$ repeat sizes observed in the 234 study couples (*N* = 936 alleles).

as study couples. Analysis of the *DMPK* alleles of these individuals showed a tri-modal distribution of $(CTG)_n$ repeat size similar to that previously observed in other populations of predominantly European ancestry (Figure 1).[21,26,27] The sample was found to be in Hardy–Weinberg equilibrium (*P* = 0.4). This suggests that although our study group was a selected group that had difficulty to conceive, it was not different from the general population with respect to the distribution of the *DMPK* alleles. Therefore, our study group could be considered as representing the general population with respect to transmission of *DMPK* alleles. Of the 234 couples, 62 (26%) had at least one partner with a Group I/Group II genotype. (Supplementary Information, see Table S1). The largest repeat size observed was $(CTG)_{32}$. Of the 62 couples, 54 donated embryos for research after their IVF cycle was completed (42 couples donated in one cycle, nine couples in two consecutive cycles, and three in three cycles).

The age and IVF parameters of the study couples were analysed. The characteristics of those couples in which there was at least one partner with Group I/Group II genotype were compared to those carrying two Group I repeats. No significant differences were found between the two groups with respect to the age of the male or female partner, fertilisation rate, *in vitro* blastocyst formation rate, implantation rate, or clinical pregnancy rate (Table 1).

In total, 341 embryos were donated and genotyping results were obtained for 335 embryos. The number of genotyped embryos per couple ranged from one to 16 with a mean and a median of six. Each embryo provided information for two transmissions, one maternal and one paternal, and therefore 670 transmissions were recorded (Table 2).

Transmission ratios of *DMPK* $(CTG)_n$ repeats

Among the 335 embryos genotyped, 59% (95% confidence interval of 54–64) inherited the Group II repeat from parents with Group I/Group II genotype (Table 3). There-

Table 1 Cycle parameters for the study couples carrying a Group II repeat compared to those who only carried Group I repeats

Genotypes of couples[a]	No. of couples	Average female age (range)	Average male age (range)	Fertilisation rate (%)	Blastocyst formation rate (%)	Implantation rate[b] (%)	Clinical pregnancy rate[c] (%)
At least one parent with a Group I and a Group II repeat	62	34.9 (22-43)	37.4 (23-51)	60.5	34	26	46
Both parents with Group I repeats	171	34.9 (20-43)	37.6 (22-51)	59.7	35	20	43

[a]*N* = 233 couples; one couple in which the paternal genotype was Group I homozygous and the maternal genotype was Group II/Group II was not included in this table. Group I repeat = *DMPK* $(CTG)_{5-18}$; Group II repeat = *DMPK* $(CTG)_{19-37}$.
[b]Implantation rate is defined as the total number of sacs seen at 6-week ultrasound divided by the total number of embryos transferred to the uterus of the female patient at embryo transfer.
[c]Clinical pregnancy rate is defined as ultrasound evidence of pregnancy seen at 6 weeks of pregnancy.

fore, we detected a statistically significant deviation from Mendelian 1:1 segregation in the embryos ($P = 0.0004$, exact binomial test). TRD in favour of the Group II repeat was observed following both maternal and paternal transmissions (60%, $P = 0.0055$ and 59%, $P = 0.03$, respectively), although with correction for multiple testing, the significance level ($P < 0.0056$) was reached only in maternal transmissions. When sex of the embryo was considered, a greater magnitude of TRD was observed among female rather than male embryos (65%, $P = 0.0001$ and 55%, $P = 0.2$, respectively).

To determine if the preferential transmission of the longer DMPK repeat in individuals with a Group I/Group II genotype was due to a relative repeat size effect (as proposed by Chakraborty et al[5]) or whether it was due to preferential transmission of the Group II repeat, the 225 transmissions from informative Group I/Group I parents, who were heterozygous for $(CTG)_{5-18}$ repeats, were analysed. The transmission of the Group I repeats to the embryos were recorded with the smaller repeat present designated as 'Short' and the larger as 'Long' irrespective of the actual size of the repeat. That is, the same allele could be designated as 'Short' or 'Long' dependent upon the length of the other parental repeat. For example, in an individual with a $(CTG)_5/(CTG)_{11}$ genotype, the $(CTG)_{11}$ was considered as 'Long'. However, in an individual with a $(CTG)_{11}/(CTG)_{14}$ genotype, the $(CTG)_{11}$ was considered as 'Short'. The 'Long' allele was transmitted to the embryo in 46% (104/225, $P = 0.3$) of transmissions (Supplementary Information, see Table S2). Thus, in our study, we observed TRD in favour of the larger allele only from individuals with Group I/Group II genotype.

Discussion

We report here, for the first time, preferential transmission (59%) of larger DMPK alleles (Group II repeats, $(CTG)_{19-37}$) compared to smaller alleles (Group I repeats, $(CTG)_{5-18}$) to preimplantation embryos and showed this was specifically due to the presence of the Group II repeat. These results support the observation, of TRD in favour of Group II repeats, in the live-born offspring of couples with at least one parent with Group I/Group II genotype (56% Group II, $N = 150/266$ transmissions; $P = 0.04$, exact binomial test)[3] (Supplementary Information, see Table S3).

This finding advances our knowledge concerning the timing of TRD in Group II repeats. Leeflang et al.[20] had previously excluded segregation distortion for Group I versus Group II repeats in the male germline as a possible mechanism of TRD and hypothesized that TRD resulted from events following sperm ejaculation. Our results further narrow the time period when the TRD is generated, and suggest that TRD results from events preceding preimplantation development (Figure 2). In principle,

Table 2 Number of transmissions observed in our study by parental genotype

DMPK genotype of the parent[a]	No. of parents (mothers/ fathers)	No. of transmissions observed
Group I/Group II	61 (34/27)	371[b]
Group I/Group I heterozygous	33 (16/17)	225[c]
Group I/Group I homozygous	13 (4/9)	68[d]
Group II/Group II heterozygous	1 (0/1)	6[e]
Total	108 (54/54)	670

[a]Group I repeat = DMPK $(CTG)_{5-18}$; Group II repeat = DMPK $(CTG)_{19-37}$.
[b]Includes 36 embryos (72 transmissions) where both parents had a Group I/Group II genotype. These 371 transmissions were used to examine TRD in Group I versus Group II repeats (Table 3).
[c]These 225 transmissions were used to examine if TRD could be due a relative repeat size effect irrespective of the allele type, that is, transmission of 'Short' versus 'Long' allele.
[d]68 transmissions from Group I/Group I homozygous parents were uninformative because they could only transmit one particular $(CTG)_n$ repeat.
[e]Six transmissions in which the father had two different sized Group II repeats were excluded from the analysis due to the small sample size.

Table 3 Transmission of DMPK $(CTG)_n$ repeats to embryos[a]

Parental origin of the alleles	Female embryos				Male embryos				Embryos of both sexes			
	Group I	Group II	T_{GII} (%)	95% CI	Group I	Group II	T_{GII} (%)	95% CI	Group I	Group II	T_{GII} (%)	95% CI
Maternal	33	64	66[b]	56–75	51	61	54	45–64	84	125	60[b]	53–67
Paternal	25	44	64[c]	51–75	42	51	55	41–65	67	95	59[c]	51–66
Total	58	108	65[d]	57–72	93	112	55	48–62	151	220	59[d]	54–64

[a]Group I repeat = DMPK $(CTG)_{5-18}$; Group II repeat = DMPK $(CTG)_{19-37}$; T_{GII} = percent of embryos inheriting the Group II repeat; 95% CI = 95% confidence interval.
[b]Significant transmission ratio distortion from 1:1 at $P < 0.01$.
[c]Significant transmission ratio distortion from 1:1 at $P < 0.05$.
[d]Significant transmission ratio distortion from 1:1 at $P < 0.0005$.
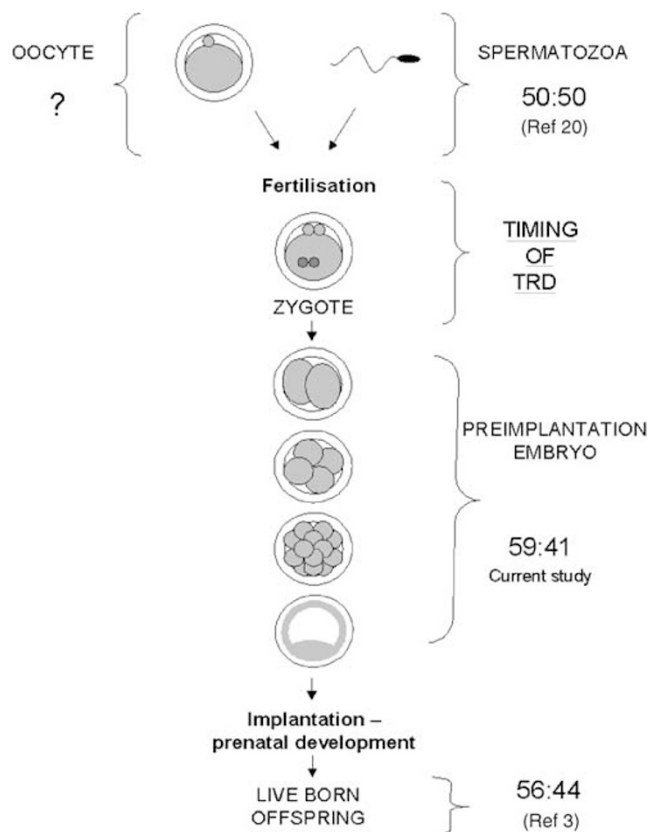
Figure 2 Transmission ratios for Group II repeats ($(CTG)_{19-37}$) versus Group I repeats ($(CTG)_{5-18}$) at the *DMPK* locus at different stages of human development including the findings of the current study. TRD in favour of Group II repeats at the *DMPK* locus is likely to occur at or around the time of fertilisation as indicated in the diagram.

these events could be preferential fertilisation of oocytes by Group II repeat-bearing sperm, preferential retention of Group II repeats in the oocytes, preferential survival of Group II repeat-bearing gametes, or meiotic drive in the female germline at the time of the second meiotic division favouring generation of heterozygous embryos with a Group I/Group II genotype. Meiotic drive at the second meiotic division has been reported for maternal transmissions at the mouse *Om* locus.[28] We think that preimplantation embryo loss and postimplantation lethality are unlikely causes of TRD in our study group, as we included all donated embryos, even those arrested in their development, and demonstrated a magnitude of TRD (59%) in preimplantation embryos similar to the one reported for live-born offspring (56%).[3]

Our results showed TRD from both maternal and paternal transmissions (60 and 59%, respectively), which, for this work, reached the threshold for level of significance only in maternal transmissions. Carey *et al.*[3] also found TRD in Group II repeats to live-born offspring, in families with no known history of myotonic dystrophy, although it

was stronger from paternal (60%) than from maternal transmission (55%). On reanalysis of this data by another group, no evidence was found to support a male specific TRD but, similar to our results, a statistically significant TRD in favour of Group II repeats acting in both sexes was demonstrated.[29] In another study examining TRD in live-born offspring from CEPH pedigrees, which represent families selected for high fertility and longevity, no TRD was found in the small subset of transmissions with Group I/Group II genotype (49% Group II, $N = 46/94$ transmissions), which may be due to the sample size.[5] (Supplementary Information, see Table S3)

Three studies reported TRD in myotonic dystrophy pedigrees (251 Italian and Spanish pedigrees, 69 Brazilian families, and 59 pedigrees in Northern Ireland), which for purposes of comparison we consider as DM1.[4,6,7] (Supplementary Information, see Table S4) These studies reported the number of affected and unaffected offspring born to parents with DM1. In the combined data, TRD in favour of the *DMPK* allele was similar from affected fathers as compared to affected mothers (56 and 54%, respectively). Our results also demonstrate a similar degree of TRD for Group II repeats from both paternal and maternal transmissions, although the mechanism of TRD of Group II repeats may not be the same as for TRD of highly expanded repeats causing DM1.

When the male and female embryos in our study were considered separately, a statistically significant TRD in favour of Group II repeats was seen in female embryos following both maternal and paternal transmissions. These findings are in contrast to the greater degree of TRD in sons compared to daughters in the DM pedigrees[4,6,7] (Supplementary Information, see Table S4), but as previously mentioned the mechanism of TRD of Group II repeats may not be the same as in highly expanded repeats. Unfortunately, none of the studies looking at TRD of larger normal-size *DMPK* alleles reported their data by gender of offspring so we were unable to compare our results with the ratios occurring in live-born offspring. The absence of statistically significant TRD in our male embryos could result from limited numbers of embryos tested or alternatively, could be due to a difference in the mode of transmission of Group II repeats to males compared to females. One possible explanation for differences in the magnitude of TRD dependent on the sex of the offspring is the presence of modifiers on the X and/or Y-chromosomes affecting embryo survival or causing preferential transmission of a particular parental allele.[30-32] Another possibility is the genetic heterogeneity of individuals with respect to *trans*-acting factors involved in the genesis of TRD.[8,9]

The TRD in our embryos was due to the Group II repeat specifically rather than an overall relative allele size effect, as demonstrated by no significant TRD in transmissions from informative Group I/Group I parents. The low frequencies of individual Group II alleles and insufficient

number of observations for each particular Group II allele do not allow us to determine if a particular subgroup of Group II alleles drives the TRD. In contrast to our results, two other studies found preferential transmission of the 'Long' allele (compared to the 'Short' allele, irrespective of the absolute size) in maternal transmission.[5,19] (Supplementary Information, see Table S3) However, the repeat size in these studies ranged from $(CTG)_{5-30}$, which was different from the $(CTG)_{5-18}$, in our analysis.

It could be suggested that the TRD in favour of Group II repeats, as demonstrated in embryos donated from IVF patients, was an effect of testing infertile couples rather than the general population. However, the distribution of the *DMPK* alleles within our study, which was not selected for ethnicity, but was largely French Canadian, showed a similar tri-modal pattern and prevalence of $(CTG)_5$ repeats as previously observed in other populations of predominantly European ancestry (Figure 1).[21,26,27] If infertility were associated with a change in transmission patterns, one would expect this to be reflected in the distribution of allele frequencies.

Furthermore, we did not find any differences in the embryos from couples where at least one partner had Group I/Group II genotype and couples where both partners had Group I/Group I genotype with respect to age or measurable IVF outcomes (Table 1). In particular, the quality of donated embryos as assessed by the percentage that developed to the blastocyst stage on day six of *in vitro* culture was similar in both genotypic groups demonstrating that the presence of a Group II repeat did not influence preimplantation embryo development (Table 1). From this finding we assume that the *DMPK* allele distribution in the donated embryos was similar to the distribution of embryos transferred to the female patient. Therefore, it is unlikely that the TRD observed in our IVF embryos was caused by the infertility of the parents or that the presence of a Group II repeat was linked to infertility. It is also unlikely that the IVF hormonal treatment undertaken by the women influenced the allelic transmission. However, it is not possible to exclude the effects of additional genes downstream of *DMPK*, which could influence the transmission process of *DMPK* alleles. A lack of association of *DMPK* genotype with fertility parameters is consistent with normal fertility for women carrying *DMPK* $(CTG)_{50-150}$ repeats.[33,34] Although fertility can be compromised in males with mild or severe DM1,[16,35] the fathers in our study carried repeat sizes within the normal range, and therefore, no reduction in fertility due to $(CTG)_n$ repeat size would be expected in our study group.

In conclusion, TRD in favour of Group II repeats from both mothers and fathers with two normal-size *DMPK* $(CTG)_n$ repeats, one Group I and one Group II, was observed in human preimplantation embryos. The TRD was stronger in female compared to male embryos. This TRD was due to the presence of the Group II repeats

specifically and not the relative size of the $(CTG)_n$ repeats. It is likely to have occurred at or around the time of fertilisation. Further studies of the transmission of the *DMPK* $(CTG)_n$ repeats are needed to identify the factors responsible for the TRD observed at the *DMPK* locus and the compensatory factors to maintain the normal-size alleles in the population.

References

1 Lyttle TW: Cheaters sometimes prosper: distortion of Mendelian segregation by meiotic drive. *Trends Genet* 1993; **9**: 205–210.
2 Eaves IA, Bennett ST, Forster P *et al*: Transmission ratio distortion at the INS-IGF2 VNTR. *Nat Genet* 1999; **22**: 324–325.
3 Carey N, Johnson K, Nokelainen P *et al*: Meiotic drive at the myotonic dystrophy locus? *Nat Genet* 1994; **6**: 117–118.
4 Gennarelli M, Dallapiccola B, Baiget M, Martorell L, Novelli G: Meiotic drive at the myotonic dystrophy locus. *J Med Genet* 1994; **31**: 980–982.
5 Chakraborty R, Stivers D, Deka R, Yu L, Shriver M, Ferrell R: Segregation distortion of the CTG repeats at the myotonic dystrophy locus. *Am J Hum Genet* 1996; **59**: 109–118.
6 Zatz M, Cerqueira A, Vainzof M, Passos-Bueno MR: Segregation distortion of the CTG repeats at the myotonic dystrophy (DM) locus: new data from Brazilian DM families. *J Med Genet* 1997; **34**: 790–791.
7 Magee AC, Hughes AE: Segregation distortion in myotonic dystrophy. *J Med Genet* 1998; **35**: 1045–1046.
8 Ikeuchi T, Igarashi S, Takiyama Y *et al*: Non-Mendelian transmission in dentatorubral-pallidoluysian atrophy and Machado–Joseph disease: the mutant allele is preferentially transmitted in male meiosis. *Am J Hum Genet* 1996; **58**: 730–733.
9 Reiss O, Epplen JT, Amoiridis G, Przuntek H, Schols L: Transmission distortion of the mutant alleles in spinocerebellar ataxia. *Hum Genet* 1997; **99**: 282–284.
10 Iughetti P, Otto PA, Zatz M, Passos Bueno MR, Marie SK: Different behavior in the paternally *vs* maternally inherited mutated allele in Brazilian Machado–Joseph (MJD1) families. *Am J Med Genet* 1998; **77**: 246–248.
11 Drasinover V, Ehrlich S, Magal N *et al*: Increased transmission of intermediate alleles of the FMR1 gene compared with normal alleles among female heterozygotes. *Am J Med Genet* 2000; **93**: 155–157.
12 Monckton DG, Cayuela ML, Gould FK, Brock GJ, Silva R, Ashizawa T: Very large (CAG)(n) DNA repeat expansions in the sperm of two spinocerebellar ataxia type 7 males. *Hum Mol Genet* 1999; **8**: 2473–2478.
13 Harper PS, Harley HG, Reardon W, Shaw DJ: Anticipation in myotonic dystrophy: new light on an old problem. *Am J Hum Genet* 1992; **51**: 10–16.
14 Brook JD, McCurrach ME, Harley HG *et al*: Molecular basis of myotonic dystrophy: expansion of trinucleotide (CTG) repeat at

3′ end of a transcript encoding a protein kinase family member. *Cell* 1992; **68**: 799–808.

15 Mahadevan M, Tsilfidis C, Sabourin L *et al*: Myotonic dystrophy: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science* 1992; **255**: 1253–1255.

16 Harper PS: Myotonic dystrophy; in Rimoin DL, Connor JM, Pyeritz RE (eds): *Emery and Rimoin's principles and practice of medical genetics*. New York: Churchill Livingstone, 1997, 3rd edn, pp 2425–2443.

17 Buxton J, Shelbourne P, Davies J *et al*: Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature* 1992; **355**: 547–548.

18 Harley HG, Rundle SA, MacMillan JC *et al*: Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet* 1993; **52**: 1164–1174.

19 Shaw AM, Barnetson RA, Phillips MF, Harper PS, Harley HG: Evidence for meiotic drive at the myotonic dystrophy locus. *J Med Genet* 1995; **32**: 145A (Abstract).

20 Leeflang EP, McPeek MS, Arnheim N: Analysis of meiotic segregation, using single-sperm typing: meiotic drive at the myotonic dystrophy locus. *Am J Hum Genet* 1996; **59**: 896–904.

21 Imbert G, Kretz C, Johnson K, Mandel JL: Origin of the expansion mutation in myotonic dystrophy. *Nat Genet* 1993; **4**: 72–76.

22 Dean NL, Tan SL, Ao A: The development of preimplantation genetic diagnosis for myotonic dystrophy using multiplex fluorescent polymerase chain reaction and its clinical application. *Mol Hum Reprod* 2001; **7**: 895–901.

23 Ray PF, Vekemans M, Munnich A: Single cell multiplex PCR amplification of five dystrophin gene exons combined with gender determination. *Mol Hum Reprod* 2001; **7**: 489–494.

24 Findlay I, Ray P, Quirke P, Rutherford A, Lilford R: Allelic drop-out and preferential amplification in single cells and human blastomeres: implications for preimplantation diagnosis of sex and cystic fibrosis. *Hum Reprod* 1995; **10**: 1609–1618.

25 Raymond M, Rousset F: GENEPOP (version 1.2): population genetics software for eact tests and ecumenicism. *J Hered* 1995; **86**: 248–249.

26 Zerylnick C, Torroni A, Sherman SL, Warren ST: Normal variation at the myotonic dystrophy locus in global human populations. *Am J Hum Genet* 1995; **56**: 123–130.

27 Deka R, Majumder PP, Shriver MD *et al*: Distribution and evolution of CTG repeats at the myotonin protein kinase gene in human populations. *Genome Res* 1996; **6**: 142–154.

28 Wu G, Hao L, Han Z *et al*: Maternal transmission ratio distortion at the mouse Om locus results from meiotic drive at the second meiotic division. *Genetics* 2005; **170**: 327–334.

29 Hurst GD, Hurst LD, Barrett JA: Meiotic drive and myotonic dystrophy. *Nat Genet* 1995; **10**: 132–133.

30 Naumova AK, Leppert M, Barker DF, Morgan K, Sapienza C: Parental origin-dependent, male offspring-specific transmission-ratio distortion at loci on the human X chromosome. *Am J Hum Genet* 1998; **62**: 1493–1499.

31 de la Casa-Esperon E, Pardo-Manuel de Villena F, Verner AE *et al*: Sex-of-offspring-specific transmission ratio distortion on mouse chromosome X. *Genetics* 2000; **154**: 343–350.

32 Zollner S, Wen X, Hanchard NA, Herbert MA, Ober C, Pritchard JK: Evidence for extensive transmission distortion in the human genome. *Am J Hum Genet* 2004; **74**: 62–72.

33 Dao TN, Mathieu J, Bouchard JP, De Braekeleer M: Fertility in myotonic dystrophy in Saguenay-Lac-St-Jean: a historical perspective. *Clin Genet* 1992; **42**: 234–239.

34 Lavedan C, Hoffman-Radvanyi H, Shelbourne P *et al*: Myotonic dystrophy: size and sex dependent dynamics of CTG meiotic instability and somatic mosaicism. *Am J Hum Genet* 1993; **52**: 875–883.

35 Hortas ML, Castilla JA, Gil MT *et al*: Decreased sperm function of patients with myotonic muscular dystrophy. *Hum Reprod* 2000; **15**: 445–448.

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