



## Case Report

## Targeting myotonic dystrophy by preimplantation genetic diagnosis-karyomapping

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## ABSTRACT

**Objective:** To report a case with Myotonic dystrophy type I with successful preimplantation genetic diagnosis-karyomapping.**Case report:** A 34-year-old female carrier of myotonic dystrophy type I was treated at our clinic with a successful pregnancy after preimplantation genetic testing for monogenic disorders using karyomapping of her blastocysts.**Conclusion:** Myotonic dystrophy type I is an inherited autosomal dominant disease producing various neuromuscular disturbances. Offspring of carriers have a 50% chance of carrying CTG repeat sequences in the DMPK gene, and various time-consuming methodologies have been developed for genetic diagnosis. With a novel, efficient, and precise method by karyomapping using single nucleotide polymorphism arrays to diagnose single gene disorders, one could terminate the transmission of single gene disorder. Herein, we reported a 34-year-old female carrier of myotonic dystrophy type I achieve a successful pregnancy after preimplantation genetic testing for monogenic disorders using karyomapping method of her blastocysts.© 2019 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Myotonic dystrophy type 1 (DM1) is the most common inherited muscular dystrophy in adults with an estimated prevalence of 1 in 8000 [1]. Steinert first described this disease in 1909 and the gene defect responsible for myotonic dystrophy was elucidated in 1992 [2]. Clinical presentations of myotonic dystrophies include myotonia, muscular dystrophy, cardiac conduction defects, posterior iridescent cataracts, and endocrine disorders.

Myotonic dystrophy is caused by the expansion of an unstable CTG trinucleotide repeat in the 3' untranslated region of the gene encoding myotonic dystrophy protein kinase (DMPK; OMIM 605377), which codes for a myosin kinase expressed in skeletal muscle [2–4]. Clinically, DM1 should be suspected in neonates with symptoms of facial hypotonia, muscle weakness, generalized weakness, positional malformations including club foot, and

respiratory insufficiency. DM1 should be suspected in adults with symptoms of muscle weakness, myotonia, and posterior subcapsular cataracts. The DMPK gene is located on chromosome 19q13.32 and molecular genetic diagnosis for the numbers of CTG repeats in DMPK can enable definite diagnosis of DM1. Different CTG repeat numbers result in different penetrance for DM1: 5–34 repeats produce normal alleles; 35–49 repeats provide mutable normal alleles; and more than 50 repeats give full penetrance of the disease. The most severe form of DM1 with >1000 CTG repeats will lead to infertility, hypotonia, early onset respiratory defects, and early death. Thus, the severity of disease penetrance and phenotype is positively correlated with CTG repeat numbers. The offspring of female carriers of CTG repeats in the gene typically show a gain of repeat numbers: a phenomenon called anticipation.

Preimplantation genetic testing for monogenic disorders (PGT–M) is an assisted reproductive technology enabling clinics to select for transfer those embryos free from targeted genetic disorders [5,6]. Typically, 4–6 trophoblast cells from blastocysts in culture 5 days (D5) after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) are biopsied for PGT. Various methods have been developed for different purposes in genetic testing. Prior to PGT–M, preimplantation genetic testing for aneuploidy (PGT–A), a

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method to detect normal chromosome copy numbers was used to select euploid embryos for PGT–M. Conventional PGT–M using short tandem repeat (STR) sequences requires knowledge of the targeted genetic locus and custom design of primers for polymerase chain reaction (PCR) amplification. Karyomapping is a novel method using single nucleotide polymorphism arrays to build up a reference karyomap of any affected family. By linkage analysis of each embryo's karyomap to a reference one, a mutation-free embryo can be selected [7]. In contrast to conventional methods for detecting DM1, karyomapping is a faster and more reliable method for detecting single gene disorders.

In this case report, we used karyomapping to select unaffected embryos to achieve a healthy live birth. Using this novel PGT–M method can help prevent inheritance of DM1. This is the first reported case using karyomapping method for PGT–M of DM1 in Taiwan.

### Case report

A 36-year-old woman, Gravida 3 Para 1 Spontaneous Abortion 2, carrier of DM1 (*DMPK* gene with 667 CTG repeats) came to our fertility center requesting assisted reproductive technology. Her disease onset began at 9 years of age with symptoms of muscle stiffness. Her family history of DM1 included her father as a pre-symptomatic carrier (*DMPK* with 166 CTG repeats) and her affected aunt died early in life. At age 33, she underwent conventional STR analysis with IVF/PGT–M to screen for normal embryos. Ten embryos were screened, with five normal and five abnormal. She received five frozen–thawed embryo transfer cycles with a single embryo transferred in each cycle. Treatment outcomes were as follows: two chemical pregnancies, one no pregnancy, and one live birth (a healthy son). Reasons for two abortion were not further investigated. This time, she came to our clinic to initiate a new PGT–M cycle using karyomapping. Family history and genetic testing reports (of the patient and her father) provided a family tree. Swab DNA samples of five family members including the patient, patient's father, patient's mother, patient's son, and patient's partner were collected and sent to Genesis Genetics (Plymouth, MI, USA) for analysis (Fig. 1). Though there were more family member potentially affected by this disease, we were unable to acquire further result because she lived in Hong Kong.

She had antral follicle counts of 11 (six on the right and five on the left) on the second day of menstruation. She had received controlled ovarian hyperstimulation with gonadotropin-releasing hormone antagonist protocol using agonist triggering of

ovulation. The gonadotropin doses were 225 IU per day for 11 days (human menopausal gonadotropin, Menopur; Ferring Pharmaceuticals, St-Prex, Switzerland). Ten oocytes were retrieved including seven metaphase II-stage oocytes, one immature germinal vesicle-stage oocyte, and two lysed oocytes. We used ICSI to achieve fertilization. Five of the seven fertilized embryos reached the blastocyst stage and were cryopreserved. They were subsequently thawed for NGS-based PGS and PGT–M [8]. Two of them were eligible for trophectoderm biopsy and were sent for PGT–A and PGT–M karyomapping. Two embryos were euploid: one of these was normal and the other was possibly affected. We transferred this normal embryo and she gave birth to a live baby.

Karyomapping involved the use of the Infinium Human Karyomap-12 DNA Analysis kit, (Cat# 1500055; Illumina, San Diego, CA, USA). The protocol essentially followed that described in the kit instructions of Illumina. Genomic DNA samples from the family members' oral swabs were tested using a HumanKaryomap-12 BeadChip (Illumina) and analyzed using BlueFuse Multi software (Illumina). This allowed genotyping of single nucleotide polymorphisms (SNPs) within and flanking the targeted *DMPK* gene on chromosome 19, and allowed phasing of SNP alleles (i.e., revealing which of the patient's two copies of the *DMPK* repeat region contained each SNP allele), to construct a family tree (Fig. 1). Aliquots of the same whole-genome amplified samples from embryos, used for NGS-based PGS, were also analyzed using VeriSeq PGS kits (Illumina) and protocol. NGS-based PGS and karyomapping data analyses were done by GGA Corp. (Taipei, Taiwan) and Genesis Genetics, respectively (Fig. 2).

### Discussion

DM1 is an inheritable autosomal dominant disease with no treatment available currently. Management for affected patients is to treat the symptoms and provide genetic counseling for couples wishing to conceive. It is very difficult to predict the severity of the disease based on molecular diagnosis. Furthermore, anticipated gain of repeats numbers may potentially worsening the disease to offspring of carrier [9]. Transmission of CTG repeats in the *DMPK* gene in human oocytes and preimplantation embryos is unstable and hard to predict [10]. Therefore, it is difficult for DM1-affected individuals to decide on having a family. In this case, the patients gave birth to two healthy children consecutively from conventional STR method and from karyomapping.

Preimplantation genetic testing can detect many single gene disorders, including muscular dystrophies (e.g., myotonic dystrophy, Duchenne muscular dystrophy, and spinal muscular atrophy) and Charcot–Marie–Tooth syndrome (a neuromuscular disorder that causes varying degrees of muscle weakness, atrophy, and decreased sensation), hemoglobin disorders (e.g.,  $\beta$ -thalassemia and sickle cell anemia), and cystic fibrosis [11]. Using PGT–M, single gene disorders with targeted genetic loci can be detected before embryo transfer.

Karyomapping is a novel method for PGT–M. The conventional PCR–STR mapping method takes 6 months to construct a proper family tree and design probes to detect specific genetic locus. However, SNP processing and karyomapping analysis can be completed in 24–48 h [7] and can be used to detect various types of genetic disease, including single gene disorders, meiotic trisomies, monosomies, and deletions [12] (Table 1). Karyomapping can also generate information on chromosome copy numbers and genetic disorders simultaneously in a single assay. The test is quick to complete compared with the convention STR method and some report 24 h to 2 weeks [12]. However, karyomapping has several limitations. First, it needs DNA from affected close relatives to construct haploblocks and if there is a life-shortening disease, there

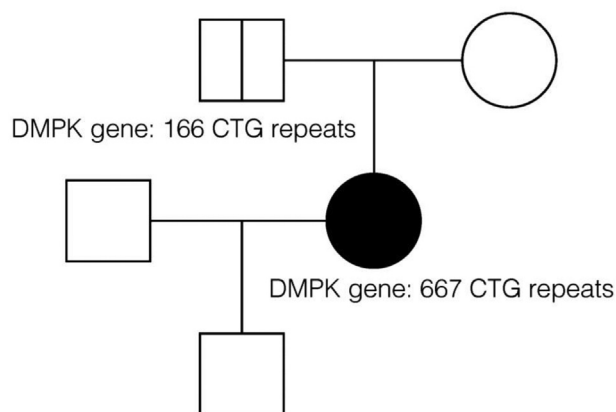
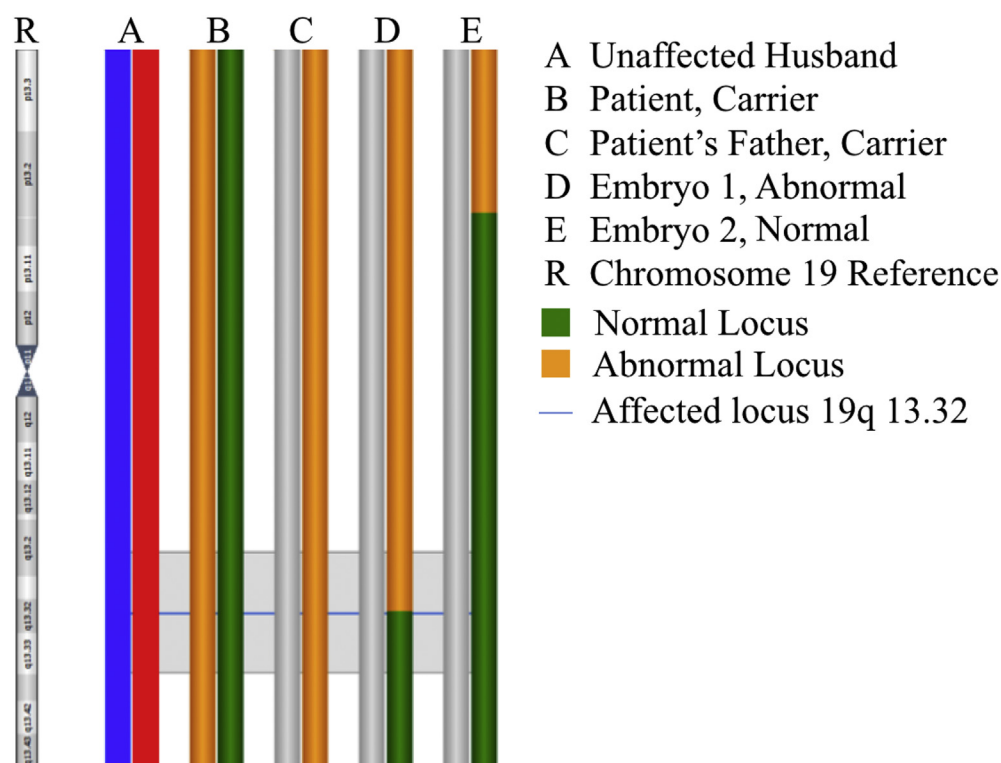


Fig. 1. Family tree analysis: Listed family member were subjected for oral swab DNA testing, where patient's father was found to carry 166 CTG repeats.



**Fig. 2.** Karyomapping analysis: DM1 repeats at chromosome 19q 13.32 (blue line). Abnormal locus detected by karyomapping is labelled yellow, whereas normal locus is labelled green. In embryo 1, affected locus at 19q 13.32 is detected. In embryo 2, 19q 13.32 is normal.

**Table 1**  
Comparison of STR analysis and Karyomapping [7].

	STR analysis	Karyomapping
Description	Uses DNA repeats of 2–6 base pairs adjacent to a specific locus to identify a defective gene	Uses SNPs and genome-wide linkage data to inform the presence or absence of a defective gene
Marker	Multiallelic; measures variation in repeat length	Biallelic; measures variation at a single base
Coverage	Limited to a single locus in each set of STR markers	Able to screen multiple loci in parallel
Location	Requires knowledge of the location of the affected gene	Requires knowledge of the location of the affected gene
Preparation Time	Typically 3–6 months to work up and validate multiple STR markers	None; off-the-shelf solution
Workflow	Customized set of primers for each case	Standard workflow for all studies
Linkage analysis	Performed manually	Automated data interpretation using BlueFuse Multi analysis software
Aneuploidy	Not available	Not currently offered

might not be DNA suitable for testing. Second, karyomapping cannot detect *de novo* mutations because it will not construct parental chromosome linkages. Third, recombination occurring adjacent to a position of interest will lead to an inconclusive diagnosis.

In assisted reproduction technology, PGT–M is the ultimate precision medicine method for intervening and preventing the transmission of single gene disorders leading to a defective child. It can also reduce the economic, social, and psychological burden for affected family. Here we report a first case of a family affected by DM1 showing a novel and feasible method to target *DMPK* CTG repeats by PGT–M and karyomapping in Taiwan.

#### Ethics approval and consent to participate

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Protocol Title: Targeted a Single Gene Disorder for Myotonic Dystrophy *DMPK* CTG Repeats by Preimplantation Genetic Diagnosis-karyomapping

Principal Investigator: Chi-Huang Chen  
Study Site: Taipei Medical University Hospital  
Protocol Version/Date: Verion\_1.0/2018/08/29  
Informed Consent Forms: Waiver of Informed Consent  
Case Report Forms: Version 1.1/2018-10-01

#### Availability of data and material

Not applicable.

#### Funding

Not applicable.

#### Conflicts of interest

The authors declare that they have no conflict of interests.

## Synopsis

Understanding a novel PGT-M approach to tackle single gene disorder. By PGT-M we can terminate single gene disorder at this generation.

## List of abbreviations

DM1	Myotonic dystrophy type 1
DMPK	Myotonic dystrophy protein kinase
ICSI	Intracytoplasmic sperm injection
IVF	<i>in vitro</i> fertilization
PCR	Polymerase chain reaction
PGT–A	Preimplantation genetic testing for aneuploidy
PGT–M	Preimplantation genetic testing for monogenic disorders
SNP	Single nucleotide polymorphism
STR	Short tandem repeat
UTR	Untranslated region

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NGS-based PGS and karyomapping data analysis were done by GGA Corp. in Taipei, Taiwan and Genesis Genetics in Plymouth, MI, USA respectively.

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