Current status of preimplantation genetic diagnosis in Japan

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Abstract:
This is a retrospective study aimingto clarify the current status of preimplantation genetic diagnosis (PGD) in Japan. Our data were collected from 12 facilities between September 2004 and September 2012, and entered into a database. A majority of PGD in Japan was performed for balanced structural chromosomal abnormalities in couples with recurrent miscarriage. PGD for monogenic diseases was performed only in two facilities. The average maternal age was 38 years for monogenic diseases and 40 years for chromosomal abnormalities. Overall there have been671 cycles to oocyte retrieval reported. Of these cycles, 85% (572 cycles)were for chromosomal abnormalities, and 15% (99 cycles) for monogenic diseases. Diagnosis rates in the current study were 70.8% for monogenic diseases and 94.0% for chromosomal abnormalities. Rates of embryo transfer of PGD were 62.7% for monogenic diseases and 25.5% for chromosomal abnormalities. Clinical pregnancy rates per embryo transfer were 12.0% for monogenic diseases and 35.6% for chromosomal abnormalities. Our study is the first PGD report from all facilities which had the approval of the ethics committee of the Japanese Society of Obstetrics and Gynecology. We have built a basis for gathering continuous PGD data in Japan.

Keywords: Chromosomalabnormalities, Genetic counseling, Japan, Monogenic diseases, Preimplantation genetic diagnosis (PGD)

Background:
Preimplantation genetic diagnosis (PGD) is a rapidly developing procedure for embryo genetic analysis [1]. It allows couples carrying genetic diseases to have an unaffected child, without undergoing an invasive prenatal diagnosis and possible termination of pregnancy [2]. The number of cases employing PGDincreases annually [3, 4], and indications for PGD have expanded to include identification of a broad variety of genetic diseases [5]. Since 1997, the European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium has been collecting data internationallyfrom the corresponding facilities where PGD was performed [3]. Since 1999, 11 data sets of PGD-related information have been analyzed and published. The data of only a few Japanese facilities has been included [3].

PGD and prenatal diagnosis (PND) in Japan are not regulated by law but are governed by professional guidelines. In 1998,

the Japanese Society of Obstetrics and Gynecology (JSOG) approved the clinical research of PGD and issued "The Guidelines on Preimplantation Genetic Diagnosis" [6]. In 2003, 10 genetic-related medical societies published comprehensive guidelines to incorporate and expand on previously established guidelines. The "Guidelines for Genetic Testing" (2003) cover particular conditions for PND but do not mention PGD in particular [7]. According to these guidelines, PGD is conducted as a "clinical research," and approval is required on a case-by-case basis, not only by the ethics committee of the facility but also by the ethics committee of JSOG. The report "Guidelines on Preimplantation Genetic Diagnosis" (1998) was amended in 2006 and revised in 2010. These guidelines indicate that this diagnostic procedure should be applied only to serious hereditary disorders and balanced structural chromosomal abnormalities in couples with recurrent miscarriages. Because of long-term discussions involved and these severe preconditions, no case of PGD had been

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performed in Japan up to the end of 2004 despite the rapidly increasing number of PGD cycles worldwide **[8].** It has been 11 years since the initiation of clinical practices for PGD in Japan, and the number of live births has been increasing every year.

Although PGD data collection has been conducted by the ESHRE consortium, detailed data such as the technical background of facilities has not been evaluated. In addition, there is a need for continuous accumulation of information regarding the precision of genetic diagnosis, pregnancy outcome, and follow-up of deliveries. Furthermore, since PGD application in Japan is limited by JSOG, it is necessary to survey our present situation to expand PGD application. The aim of the present study was to monitor PGD treatment cycles and their outcome including the follow up of children born in order to clarify the current status of PGD in Japan.

Methodology:

Our data were collected retrospectively from 12 IVF laboratories. These facilities also have own PGD laboratories, and had the approval of the ethics committee of JSOG between September 2004 and September 2012, and entered into a FileMaker Pro 12 database (FilemakerInc, Santa Clara, California, USA). This included files for PGD referrals (patient history, PGD indications, and decisions taken by facilities and patients), ethical review and genetic counseling, PGD cycles (ART data and PGD analyses and results), and pregnancy and infant records. Indications for PGD are divided into two categories: (a) PGD for chromosomal abnormalities and (b) PGD for monogenic diseases. Data submitted were thoroughly assessed to identify omissions and any inconsistent data. Records with insufficient data (e.g., with no cycle, no patient identification, no clear indication, or from an incorrect time period) were excluded from subsequent data calculations. Following editing and correction of all data, the entire collection was separated into the two above-mentioned categories. We defined genetically transferable embryos as carrier and non-carrier embryos for monogenic diseases, and balanced and normal embryos for chromosomal abnormalities. Clinical pregnancies were defined as the presence of one or more fetal heartbeats by ultrasound at 6–7 weeks of gestation. Implantation rate was defined as the number of active fetal heartbeats per 100 transferred embryos. Delivery rate was defined as the percentage of pregnancies resulting in delivery per OR and per embryo transfer procedure. This clinical research was approved by the ethics committee of the Keio University School of Medicine (approval No. 20120089).

Results:

Data from 12 PGD facilities were included in this report. Any PGD data collected from facilities that did not have the approval of the ethics committee of JSOG were not included. In cases of PGD, ICSI was the most frequent method of fertilization, and cleavage-stage aspiration was the most common method of biopsy. Overall, laser drilling of the zona pellucida was most commonly performed.

Genetic counseling and the system of administrating genetic information

At three of 12 facilities, licensed medical geneticists were not in charge of genetic counseling. Four facilities had no separate room dedicated for genetic counseling. At seven of 12 facilities,

genetic information was recorded on regular medical charts. In addition, at five facilities, genetic records were stored in usual medical records, and at three facilities, all staff were allowed access to genetic information. Further, at nine facilities, genetic testing results were concurrently disclosed to couples.

PGD for monogenic diseases

Table 1 (see supplementary material) summarizes data from 99 OR cycles, for which PGD was performed to identify monogenic diseases. Most common indications for this treatment were Duchenne muscular dystrophy (DMD, 59 cycles) and myotonic dystrophy type 1 (DM1, 31 cycles). Other indications were Ornithine transcarbamylase deficiency (OTCD, 6 cycles) and Fukuyama congenital muscular dystrophy (FCMD), adrenoleukodystrophy (ALD), and Leigh syndrome of mitochondrial disease, all with one case each. ICSI was used in all cycles, and the use of a noncontact diode infrared laser was the preferred method for biopsy (94.3% PGD cycles). Day 3 cleavage-stage embryo biopsy was used in all PGD cycles. A total number of 1050 cumulus–oocyte complexes (COCs) were collected, and 63.0% (661/1050) mature oocytes were fertilized. A total of 83.8% (554/661) embryos were biopsied, of which, 97.7% (541/554) were successful. Further, of successfully biopsied embryos, 70.8% (383/541) presented a diagnostic result, of which, 62.7% (240/383) were genetically transferable On an average, 10.6 COCs were collected per cycle that yielded 6.7 embryos, of which, 5.6 were suitable for biopsy. In addition, diagnosis was achieved for 3.9 embryos per cycle, of which, 2.4 embryos were deemed genetically transferable. On an average, 1.7 embryos were transferrable per cycle. A positive hCG was obtained in 19 cycles, with a positive heartbeat in 14 cycles [14.1% per OR (14/99), 12.0% per embryo transfer (14/117), with 15 active fetal heartbeats, giving an overall implantation rate of 9.0% (15/167)]. The delivery rate was 10.1% per OR (10/99) and 8.5% per embryo transfer (10/117). Eight miscarriages (42.1% per positive hCG, 21.4% per clinical pregnancy) and one ectopic pregnancy were reported.

PGD for chromosomal abnormalities

Table 2 (see supplementary material) summarizes 572 OR, for which PGD was performed for chromosomal abnormalities. PGD for reciprocal translocations was performed more often compared with that for Robertsonian translocations. The percentage of female carriers was higher for both reciprocal translocations and Robertsonian translocations (65% and 79%, respectively). In 72% cycles, ICSI was used for fertilization. Zona breaching was achieved by either laser drilling or mechanical techniques, in equal numbers. The aspiration of blastomeres from cleavage-stage embryos was the preferred biopsy method (86%). A total of 4140 oocytes were collected (mean, 7.2 per cycle). Of these, 66.3% (2744/4140) were fertilized. A total of 66.4% (1822/2744) embryos were biopsied, and of these, 99.8% (1819/1822) were successful. Of successfully biopsied embryos, 94.0% (1710/1819) presented a diagnostic result, of which, 25.5% (436/1710) were genetically transferable. On an average, 7.2 COCs were collected per cycle. This yielded 4.8 embryos, of which, 3.2 embryos were suitable for biopsy. Diagnosis was achieved for 3.0 embryos, of which, 0.8 embryos were deemed genetically transferable. On an average, 0.5 embryos were transferred per cycle. A positive hCG was obtained in 101ET cycles, with a positive heartbeat in

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77 cycles [13.5% per OR (77/572), 35.6% per embryo transfer (77/216), with 80 active fetal heartbeats, giving an overall implantation rate of 27.4% (80/292)]. The delivery rate was 10.5% per OR (60/572) and 27.8% per embryo transfer (60/216). Moreover, 36 miscarriages (35.6% per positive hCG, 15.6% per clinical pregnancy) were reported, with approximately more than half of them (61%) occurring in the female reciprocal translocation group. In addition, two ectopic pregnancies were reported.

Prenatal and postnatal diagnosis
Table 3 (see supplementary material) summarizes prenatal and postnatal diagnoses of pregnancies. Excluding unknown cases, confirmation of the diagnosis was performed prenatally (47%, 27/57) and/or postnatally (7%, 4/57). Amniocentesis was performed for 45% cases (26/57), and 100% them (26/26) were normal. No misdiagnoses were reported.

Method of delivery, gestational age, and infants born
Table 4 (see supplementary material) summarizes the pregnancy outcome data. There were 70 deliveries of 75 babies. Caesarean section was performed for about 50% deliveries (31/63). In seven cases, the delivery method was not recorded. The mean birth weight of all singletons was 2964 g. The percentage of females (56%) born was higher than that of males.

Discussion:
We have built a basis for gathering continuous PGD data from which the status of PGD implementation can be tracked in Japan. Our study is the first PGD report from all facilitieswhich had the approval of the ethics committee of JSOG. Our results help to clarify the current status of PGD in Japan. A majority of PGD in Japan (98%) was performed for balanced structural chromosomal abnormalities in couples with recurrent miscarriage. PGD for monogenic diseases was performed only in two facilities because PGD for monogenic diseases warranted genetic counseling and tailor-made diagnoses for each case, which may not be feasible for outpatientclinics.

At three of 12 facilities, licensed medical geneticists were not in charge of genetic counseling. Although the genetic counseling system in Japan has been on the way to establish, it is still insufficient. Nishiyama *et al.* examined the current state of providing genetic counseling in Japan to pregnant women before they elected amniocentesis for prenatal diagnosis. The data revealed that pre-amniocentesis genetic counseling was usually provided by the obstetrician alone (73.8 %), by geneticists (18.4 %), including obstetricians certified as geneticists (12.6 %) and geneticists with other specialties (5.8 %), and by an obstetrician and nurse/midwife (7.8 %). These data reveal that most of the certified genetic counselors in Japan are not involved with prenatal genetic testing. Thus, we should consider the appropriate involvement of certified genetic counselors to assess and improve mental stress of pregnant women **[9].** In addition, the handling and management of genetic information should be regulated more strictly and sufficiently in the clinical field in Japan **[10].** Although limited access should be established in the management of genetic records, at three facilities, all staff were allowed to access genetic information.

Advanced maternal age was identified in this study (38 years for monogenic diseases and 40 years for chromosomal abnormalities). These maternal ages are higher compared with those documented in a recent ESHRE report (34 years for monogenic diseases and 35 years for chromosomal abnormalities) **[3].** Prolongation of the period before PGD initiation is regarded as one of the causes of advanced maternal age in Japan, which may be attributed to the two-step ethics examination; it is required to shorten this period by timeous ethics examinations.

For monogenic diseases, our diagnosis rates data were lower than those documented in the ESHRE report **[3].** On the other hand, our data on chromosomal abnormalities were similar to those reported in the ESHRE report. The primary reason for low diagnosis rates for DM1 was difficulties in diagnosis. The expanded myotonic dystrophy protein kinase (DMPK) allele is refractory to PCR amplification; therefore, the diagnosis is based on detection of the affected individual's unexpanded allele in the embryo. However, for couples who are either uninformative (sharing all alleles) or semi-informative (sharing one of their alleles) for the DMPK region, two parental alleles transmitted to an embryo may be of equal size; therefore, it may not be possible to confirm that the affected partner's unexpanded allele has been inherited. In these cases, diagnoses have been facilitated by the incorporation of linked markers to allow detection of contamination and/or the presence of phase alleles **[11].** Rates of embryo transferof PGD were 62.7% for monogenic diseasesand 25.5% for chromosomal abnormalities in Japan. These data are similar to those previously reported in ESHRE reports.Furthermore, clinical pregnancy rates per embryo transfer in the current study were 12.0% for monogenic diseases and 35.6% for chromosomal abnormalities. For monogenic diseases, our data were lower than previous reports ranging between 13% and 29% **[3, 12, 13].** In contrast, for chromosomal abnormalities, our data were similar to other previous reportsranging between 23% and 39% **[3, 14, 15].** Pregnancy rate is highly influencedby women's age. It is thought that advanced maternal age and two cell biopsies from day3 embryosare the primary reason for lower pregnancy rates following PGD for monogenic diseases in Japan.The miscarriage rate after PGD for monogenic diseases (44%, 8/18) and chromosomal abnormalities (36%,36/101) was higher than other reports **[3, 16, 17]**. We speculated that this high miscarriage rate was due to advanced maternal age.

Several problems about PGD in Japan were clarified in this study. First, it is imperative to reduce the prolonged period before implementation of PGD because of the two-step ethics examination. In addition, the genetic counseling system in Japan is still insufficientdue to the lack of medical geneticists and certified genetic counselors. Thus, we need to establish the system as quickly as we can.Finally, the introduction of the microarray-based PGD for chromosomal abnormalitiesshould be considered in the technical aspect **[17, 18].** To date, studies investigating neonatal outcomes following PGD have reported reassuring findings **[19, 20].** However, long-term effects of embryo biopsy have not been clarified. It is necessary to investigate the health of infants born following PGD to assess long-term effects of embryo biopsy. We wish to clarify the long-term negative effects of PGD in future by continuing investigation. Though the JSOG has so far approved only PGD,

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clinical studies on preimplantation genetic screening (PGS) are likely to be launched by the JSOG. Behind its decision is the fact that the technical level of PGS has been improved remarkably, and that an increasing number of people have been calling for application of the latest genetic profiling.

Conclusion:
We have established a basis for gathering continuous PGD data from which the future status of PGD implementation can be tracked in Japan. Moreover, this system can be used to track the health of infants born following PGD. These data represent a valuable information resource with regard to the clinical application of PGDnot only from the social and ethical aspects but also from the technological and medical aspects.

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Supplementary material:

Table 1: Summary of the PGD data for monogenic diseases.

Indications	DMD	DM1	OTCD	FCMD	ALD	Leigh syndrome	Total
Cycles to OR	59	31	6	1	1	1	99
Number infertile	24	9	1	1	1	1	37
Female age (average)	38	36	42	38	41	39	38
Cancelled before IVF/ICSI	1	1	0	0	0	0	2
ART method							
IVF	0	0	0	0	0	0	0
ICSI	58	28	6	1	1	1	95
IVF+ICSI	0	0	0	0	0	0	0
Unknown	0	2	0	0	0	0	2
Cancelled after IVF/ICSI	1	0	1	0	0	0	2
Cycles to PGD	57	30	5	1	1	1	95
Zona breaching							
AT drilling	0	0	0	0	0	0	0
Laser drilling	57	23	0	1	1	1	83
Mechanical	0	0	0	0	0	0	0
Laser drilling + Mechanical	0	0	5	0	0	0	5
Unknown	0	0	0	0	0	0	0
Biopsy method							
Polar body biopsy	0	0	0	0	0	0	0
Cleavage aspiration	57	23	5	1	1	1	88
Cleavage extrusion	0	0	0	0	0	0	0
Blastocyst	0	0	0	0	0	0	0
Indications	**DMD**	**DM1**	**OTCD**	**FCMD**	**ALD**	**Leigh syndrome**	**Total**
Embryology							
COCs (mean / OR)	614 (10.4)	360 (11.6)	52 (8.7)	8 (8.0)	13 (13.0)	3 (3.0)	1050 (10.6)
Fertilized	395	224	29	5	7	1	661
Biopsied	331	186	25	4	7	1	554
Frozen before biopsy	0	0	0	0	0	0	0
Successfully biopsied	330	174	25	4	7	1	541
Diagnosed (mean / OR)	254	101	22	-	5	1	383
Transferable (mean / OR)	175	48	12	-	5	0	240
Transferable rate (% / diagnosed)	68.9	47.5	54.5	-	100	0	62.7
Transferred	125	30	7	-	5	0	167
Clinical outcome							
Cycles to ET (% / OR)	90 (153.0)	19 (61.3)	5 (83.3)	0 (0.0)	3 (300.0)	0 (0.0)	117.0 (118.2)
hCG positive	14	3	0	0	2	0	19
Positive FHB	12	1	0	0	1	0	14
Number of FHB	13	1	0	0	1	0	15
Clinical pregnancy rate (% / OR)	20.3	3.2	0	0	100	0	14.1
Clinical pregnancy rate (% / ET)	13.3	5.3	0	0	33.3	0	12.0
Implantation rate (FHB / embryos transferred)	10.4	3.3	0	0	20	0	9.0
Deliveries	9	0	0	0	1	0	10
Miscarriages	5	2	0	0	1	0	8
Ectopic pregnancies	0	1	0	0	0	0	1
Twins	1	0	0	0	0	0	1

DMD, Duchenne muscular dystrophy; DM1, myotonic dystrophy type 1; OTCD, Ornithine transcarbamylase deficiency; FCMD, Fukuyama congenital muscular dystrophy; ALD, Adrenoleukodystrophy; OR, oocyte retrieval; AT, acid Tyrode's solution; COC, cumulus–oocyte complex; ET, embryo transfer; ART, assisted reproduction technology; FHB, fetal heart beat.

Table 2: Summary of the PGD data for chromosomal abnormalities.

Indications	Rob, male carrier	Rob, female carrier	Rcp, male carrier	Rcp, female carrier	Total
Cycles to OR	13	48	179	332	572
Number infertile	4	16	67	87	174
Female age (average)	43	39	39	39	40
Cancelled before IVF/ICSI	0	2	8	14	24
ART method					
IVF	0	11	61	76	148
ICSI	13	37	105	239	394
IVF+ICSI	0	0	5	3	8
Unknown	0	0	0	0	0
Cancelled after IVF/ICSI	1	5	11	20	37
Cycles to PGD	12	41	160	298	511
Zona breaching					
AT drilling	0	1	5	13	19
Laser drilling	10	20	61	156	247
Mechanical	2	20	94	129	245
Unknown	0	0	0	0	0
Biopsy method					
Polar body biopsy	0	0	0	3	3
Cleavage aspiration	10	38	130	262	440
Cleavage extrusion	2	3	30	33	68
Blastocyst	0	0	0	0	0
Indications	**Rob, male carrier**	**Rob, female carrier**	**Rcp, male carrier**	**Rcp, female carrier**	**Total**
Embryology					
COCs (mean / OR)	63 (4.8)	350 (7.3)	1322 (7.4)	2405 (7.2)	4140 (7.2)
Fertilized	34	225	922	1563	2744
Biopsied	11	166	636	1009	1822
Frozen before biopsy	13	0	80	228	321
Successfully biopsied	11	166	635	1007	1819
Diagnosed (mean / OR)	11	148	626	925	1710
Transferable (mean / OR)	3	42	177	214	436
Transferable rate (% / diagnosed)	27.3	28.4	28.3	23.1	25.5
Transferred	2	23	114	153	292
Clinical outcome					
Cycles to ET （% / OR)	2 (15.4)	16 (33.3)	83 (46.4)	115 (34.6)	216 (37.8)
hCG positive	0	5	45	51	101
Positive FHB	0	4	40	33	77
Number of FHB	0	5	41	34	80
Clinical pregnancy rate （% / OR)	0	8.3	22.3	9.9	13.5
Clinical pregnancy rate （% / ET)	0	25	48.2	28.7	35.6
Implantation rate （FHB / embryos transferred）	0	21.7	36.0	22.2	27.4
Deliveries	0	3	28	29	60
Miscarriages	0	2	12	22	36
Ectopic pregnancies	0	0	0	2	2
Twins	0	1	1	1	3

Rob, Robertsonian translocation; Rcp, Reciprocal translocation; OR, oocyte retrieval; AT, acid Tyrode's solution; COC, cumulus–oocyte complex; ET, embryo transfer; ART, assisted reproduction technology; FHB, fetal heart beat.

Table 3: Prenatal and postnatal diagnosis.

Methods	Total	Results		
		Normal	Abnormal	Failed
Prenatal diagnosis				
Amniocentesis	26	26	0	0
CVS	0	0	0	0
Cordocentesis	1	1	0	0
Unexamined	30			
Unknown	13			
Postnatal diagnosis				
Karyotype	3	3	0	0
DNA test	1	1	0	0

CVS, chorionic villus sampling.

Table 4: Method of delivery, gestational age, and infants born.

	Singletons	Twins	Total
No. deliveries	65	5	70
Method of delivery			
Vaginal	32	0	32
Caesarian	26	5	31
Unknown	7	0	7
Term at delivery			
Preterm	6	4	10
Term	57	1	58
Post term	0	0	0
Unknown	2	0	2
Sex			
Male	30	2	32
Female	32	8	40
Unknown	3	0	3
Mean birthweight (g)	2964	2356	2919