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Original Article

ORIGINAL ARTICLE

Preimplantation genetic testing for monogenic disorders (PGT-M) for monogenic nephropathy: a single-center retrospective cohort analysis

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ABSTRACT

Background. Hereditary nephropathy is an important cause of renal insufficiency and end-stage renal disease. Therefore, for couples with monogenic nephropathy, preventing transmission of the disease to offspring is urgent. Preimplantation genetic testing for monogenic disorders (PGT-M) is a means to prevent intergenerational inheritance by screening and transplanting normal embryos. We provide a clinical overview of patients with monogenic nephropathy who underwent PGT-M.

Methods. The single-center retrospective cohort study was conducted at the Center for Reproductive Medicine, Shandong University from January 2014 to December 2022. A total of 352 couples with nephropathy-related disease were included in the cohort totally.

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Results. Of the 352 couples with nephropathy-related disease, 180 accepted genetic screening. A total of 104 couples with monogenic nephropathy indications underwent PGT-M, including 90 of autosomal dominant inheritance, 10 of autosomal recessive inheritance, 4 of X-linked inheritance. 498 blastocysts were biopsied prior to testing, and 394 embryos underwent genetic testing, of which 76 were transferable, 247 were non-transferable and 71 were recommended for genetic counseling. Finally, 80 vitrified-thawed single blastocyst transfer cycles were performed in the cohort. Live births occurred in 38 women, of which 37 transferred embryos with non-pathogenic genotypes. The invasive prenatal diagnosis results of 18 women with live birth were obtained through follow-up, consistent with the PGT-M results of transferred embryos.

Conclusions. PGT-M is an effective means of preventing intergenerational inheritance of monogenic nephropathy. The absence of genetic abnormalities detected by prenatal diagnosis in healthy newborns without monogenic nephropathy also underscore its validity.

Keywords: genetic counseling, monogenic nephropathy, pregnancy outcome, preimplantation genetic testing for monogenic disorders, prenatal diagnosis

KEY LEARNING POINTS

What was known:

- Hereditary kidney disease refers to a group of kidney diseases related to genetic factors, accounting for 10%–15% of all kidney diseases and most of them have a very poor prognosis and often progress to end-stage renal disease.

This study adds:

- This study verified the effectiveness of preimplantation genetic testing for monogenic disorders (PGT-M) in preventing disease transmission to offspring from couples with monogenic nephropathy by screening and transplanting embryos with non-pathogenic phenotypes.

Potential impact:

- Study on the clinical application of PGT-M contributes to preventing transmission of the disease to offspring for couples with monogenic nephropathy at childbearing age.

INTRODUCTION

Hereditary nephropathy is a disease caused by chromosomal or gene variation, characterized by abnormal structure and function of kidney parenchyma, caused by single gene disease, polygene disease, mitochondrial disease and others [1–4]. A single-center study using whole-exome sequencing (WES) showed that a single gene cause could be identified in 24% of adults with chronic kidney disease (CKD) [5, 6]. In early onset-CKD (defined as CKD presenting before the age of 25 years), a single gene cause can be detected in approximately 20% of patients with early-onset CKD [7]. Furthermore, some patients with monogenic kidney disease may develop end-stage kidney disease (ESKD). For example, mutations in *COL4A3*, *COL4A4* and *COL4A5* may cause Alport syndrome, which usually leads to ESKD [8]. Therefore, for couples with monogenic nephropathy at reproductive age, preventing transmission of the disease to offspring is necessary to avoid renal insufficiency or even ESKD in their offspring.

Before the application of preimplantation genetic testing for monogenic/single-gene disorders (PGT-M), invasive prenatal diagnosis was the primary way to avoid the birth of an affected child [9]. However, the parents might be obliged to decide whether to terminate an affected pregnancy after spontaneous conception [10]. As a well-established alternative to prenatal diagnosis, preimplantation genetic testing (PGT) refers to analyzing the genetic materials from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for determining genetic abnormalities, including testing for monogenic/single-gene disorders (PGT-M), aneuploidy (PGT-A) and structural rearrangements (PGT-SR). PGT-M is known as an effective approach to avoid transmitting pathogenic nuclear DNA variant(s) causing monogenic disorders to the offspring [11]. In terms of technology, PGT-M clinical testing should be performed simultaneously with direct detection of pathogenic variant site(s) in genes associated with disorders and linkage analysis of genetic polymorphic sites (short tandem repeat or single nucleotide polymorphism) to avoid amplification failure, allele dropout and other factors leading to unclear diagnosis [12]. And given aneuploidy as a major cause of pregnancy wastage, the combined-PGT strategy involving PGT-M and testing for unrelated sporadic chromosomal abnormalities (namely PGT-A) in single trophectoderm biopsy is commonly used [11, 13].

A definitive clinical diagnosis and adequate genetic counseling are required to obtain PGT-M indications. Prior to PGT-M treatment, it is necessary to inquire as to the clinical diagnosis of specialized diseases, collect disease-related clinical and genetic information of affected and non-affected family members, and finally draw a pedigree. The genetic counseling clinician should analyze and confirm the severity, heterogeneity and genotype–phenotype correlation of the condition [14]. After pursuing PGT-M protocol, another genetic counseling should be conducted to inform the results of embryo testing and risks of subsequent transfer and pregnancy [15]. Couples are also informed that a transferable embryo without the familial mutation(s) does not exclude other unrelated gene mutations. Ultrasound, as a non-invasive examination, is a reliable and effective method to preliminarily determine the specific types of nephropathy referring to ultrasonographic features and family history [16, 17]. In contrast, genetic testing enables the classification of clinical

features and histological diagnosis. For example, there is a correlation between the genotype and clinical phenotype for autosomal recessive inheritance Alport syndrome (ARAS). The clinical manifestations of homozygous mutations for ARAS are more serious than heterozygous mutations, and nonsense mutations are more serious than missense mutations. Therefore, genetic testing can provide an important reference for determining the clinical diagnosis [18]. Of note, PGT-M cannot be used for nephropathy patients with nondefinite gene variant(s), even those with a clear family history of renal diseases.

Recent data have highlighted the role of PGT-M for patients with monogenic nephropathy in significantly mitigating the risk for a pregnancy affected with the familial disease [19]. Prior studies focused on patients with specific types of monogenic nephropathy [e.g. autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD)], and investigated the value of genetic counseling and PGT-M in addressing disease burden [20, 21]. Mir Pardo *et al*. also explored the influencing factors of embryo detection results in ADPKD patients after combined-PGT, and found that advanced maternal age was associated with an increased risk of aneuploid embryos [22]. However, very few retrospective cohort studies have been performed to illuminate the application of PGT-M in patients with monogenic nephropathy in large reproductive centers [23–25].

Therefore, we retrospectively analyzed genetic counseling process for patients with nephropathy-related disease and provided a clinical overview of patients with monogenic nephropathy who underwent PGT-M, involving various patient demographics, pathogenic gene variant(s) causing monogenic disorders, embryo testing results, prenatal diagnosis and pregnancy outcomes, thus providing references for clinical decision-making.

MATERIALS AND METHODS

Ethical approval for this retrospective cohort study was obtained from the Institutional Review Board of Reproductive Medicine, Shandong University (IRB #2021-140). Given our study was a retrospective analysis of deidentified data, we granted a waiver of informed consent. We ensured compliance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

From January 2014 until December 2022, a total of 352 couples with nephropathy-related disease were included in our study. Finally, 104 couples who acquired their inclusion and applied PGT-M for monogenic nephropathy were included for subsequent analysis. Only the first PGT cycles following genetic counseling and screening were examined. The records of baseline characteristics, genetic counseling, genetic diagnosis, PGT parameters, prenatal diagnosis and pregnancy outcomes were reviewed for all enrolled couples. Patients who used egg/sperm donor cycles were excluded. The primary outcome was the cumulative live birth rate (LBR) which is calculated as the number of deliveries per oocyte retrieval cycle. Live birth referred to delivery of one viable infant after 28 weeks of gestation. The secondary outcome parameters were defined as follows: biochemical pregnancy was defined as a serum human chorionic gonadotropin level >25 IU/L at 12 days after blastocyst transfer; clinical pregnancy was regarded as an intrauterine gestational sac with fetal heart beating via ultrasound at 7 weeks of gestation; ongoing pregnancy was defined as a viable intrauterine pregnancy beyond 12 weeks' gestation; and pregnancy loss was denoted as spontaneous termination of pregnancy before 28 weeks of gestation, including biochemical, first trimester and second trimester pregnancy loss. In addition, neonatal outcomes and obstetrical complications were also analyzed in this study.

All analyses were performed using the Statistical Package for Social Science software, Release 26.0. Normally distributed continuous data were reported as mean ± standard deviation (SD), and continuous parameters in non-normal distribution were shown as median [the first quartile (Q1), the third quartile (Q3)]. Categorical variables were reported as frequencies and percentages.

Table 1: Baseline characteristics of monogenic nephropathy patients undergoing PGT-M.

Characteristic	Total couples, $n = 104$
Maternal characteristics	
Maternal age at first counseling—years, mean ± SD	31.12 ± 3.37
Nulliparity—*n* (%)	80 (76.92)
Multipara—*n* (%)	24 (23.08)
1 live birth—*n* (%)	22 (21.15)
2 live births—*n* (%)	2 (1.92)
BMI—kg/m^2, mean ± SD	22.98 ± 3.10
AMH—ng/mL, median (Q1, Q3)	3.68 (2.13, 6.35)
AFC—*n*, mean ± SD	15.61 ± 6.07
Genetic characteristics—*n* (%)	
Autosomal dominant disease	90 (86.54)
Autosomal recessive disease	10 (9.62)
X-linked disease	4 (3.85)
Source of pathogenic gene mutations—*n* (%)	
Paternal heredity	64 (61.54)
Maternal heredity	30 (28.85)
Paternal and maternal heredity	10 (9.62)

Values are presented as median (Q1, Q3), mean ± SD or *n* (%).
BMI, body mass index; AMH, anti-mullerian hormone; AFC, antral follicle count.

RESULTS

Over a 9-year period, 352 couples with nephropathy-related disease were included in our cohort as initial patients, of which 294/352 (83.52%) underwent genetic counseling; 180/294 (61.22%) of those couples following counseling accepted genetic testing. A total of 133 couples received PGT-M indications, of which 126 couples carried pathogenic or likely pathogenic variants (P/LP), and 7 couples carried variant of uncertain significance (VUS) which were later upgraded to pathogenicity by pedigree analysis. Finally, 104/133 (78.20%) couples chose to proceed with PGT-M after another genetic counseling in our hospital, and 6/133 (4.51%) couples turned for spontaneous pregnancy with invasive prenatal genetic diagnosis because of economic reasons. The rest (23/133, 17.29%) had not yet decided whether to utilize PGT-M. The median duration from the first genetic counseling to the beginning of PGT-M procedure was 146 days (Q1 = 101, Q3 = 250).

The baseline characteristics of patients with monogenic nephropathy who underwent PGT-M were presented in Table 1. The median maternal age at the first genetic counseling was 31.12 ± 3.37 years. The genetic patterns of monogenic nephropathy included autosomal dominant (AD), autosomal recessive (AR) and X-linked inheritance in the cohort. Some 86.54% of the cases suffered from AD disease. Among all the 104

Table 2: Referral indications on PGT-M for monogenic nephropathy.

Inheritance pattern	Initial clinical diagnosis of disease	Final genetic diagnosis of disease	*n* (%)	Genes (*n*)
Autosomal dominant inheritance	ADPKD	ADPKD	85 (81.73)	*PKD1* (80), *PKD2* (5)
	Renal dysplasia	HNF1B-related autosomal dominant tubulointerstitial kidney disease	3 (2.88)	*HNF1B* (3)
	Alport nephropathy	Alport nephropathy	1 (0.96)	*COL4A4* (1)
	Fibronectin glomerulopathy	Fibronectin glomerulopathy	1 (0.96)	*FN1* (1)
Autosomal recessive inheritance	ARPKD	ARPKD	4 (3.85)	*PKHD1* (4)
	Bardet-Biedl syndrome	Bardet-Biedl syndrome	1 (0.96)	*BBS12* (1)
	Nephronophthisis	Nephronophthisis	1 (0.96)	*ANKS6* (1)
	Pierson syndrome	Pierson syndrome	1 (0.96)	LAMB2 (1)
	nephrotic syndrome	Finnish congenital nephrosis	1 (0.96)	*NPHS1* (1)
	Primary hyperoxaluria	Primary hyperoxaluria	1 (0.96)	*AGXT* (1)
	Nephrotic syndrome	Primary CoQ10 deficiency	1 (0.96)	*COQ2* (1)
X-linked dominant inheritance	Alport nephropathy	Alport nephropathy	3 (2.88)	*COL4A5* (3)
X-linked recessive inheritance	Nephrogenic diabetes insipidus	Nephrogenic diabetes insipidus	1 (0.96)	*AVPR2* (1)

Values are presented as *n* (%).

couples with monogenic nephropathy, the cases in which the disease-causing gene was carried by the father accounted for 61.54%, and the cases in which the disease-causing gene was carried by the mother accounted for 28.85%.

As presented in Table 2, the primary indication for referral of couples with monogenic nephropathy to our institution for PGT-M was ADPKD, with a total of 85 (81.73%) cases (causal gene: *PKD1*/*PKD2*). In addition, ARPKD and Alport syndrome were also common reasons for referral, with four ARPKD cases (causal gene: PKHD1) and four Alport syndrome cases (causal gene: *COLA4A4*/*COLA4A5*), of which one case carried *COL4A4* with AD inheritance, while the other three carried *COL4A5* with XD inheritance. Additionally, the rest of the monogenic nephropathy cases caused by X-linked gene variant(s) included one nephrogenic diabetes insipidus cases with XR inheritance (causal gene: *AVPR2*). The characteristics of pathogenic gene variant(s) in monogenic nephropathy patients undergoing PGT-M are described in Supplementary data, Table S1.

A total of 498 blastocysts were biopsied prior to testing in 104 couples who pursued PGT-M, of which 263 embryos underwent PGT-M combined with PGT-A, 129 embryos underwent only PGT-M, 7 failed-diagnosed embryos due to failed amplification, 2 embryos with unknown PGT-M result, and 97 embryos without testing considering economic costs (Table 3). Of the 394 biopsied blastocysts underwent genetic testing, 76 were transferable embryos with non-pathogenic genotypes and euploid. Of the 71 embryos recommended for genetic counseling, 56 noncarrier embryos were combined with subsegmental aneuploid, chromosomal mosaicism or both, and 13 carrier embryos were euploid or chromosomal mosaicism. Moreover, one mosaic embryo without PGT-M result and one embryo identified as uniparental dimorphism were also advised for genetic counseling. The remaining 247 non-transferable embryos included 60 embryos with non-pathogenic genotypes (53 noncarriers and 7 carriers) and aneuploid, 186 embryos with pathogenic genotypes, and 1 embryo with complex chromosomal abnormalities and unknown PGT-M result.

Finally, 80 vitrified-thawed single blastocyst transfer cycles were performed in the cohort (Table 4). The primary outcome of cumulative live birth occurred in 38 of 104 women (36.54%), including 36 women who yielded a healthy newborn and 1 woman who yielded two healthy newborns after two transfer cycles. One couple strongly requested to transfer an embryo with ADPKD pathogenic phenotype detected by PGT-M, and finally obtained a live-born baby infant suffering from ADPKD. The frequencies of cumulative biochemical pregnancy, clinical pregnancy and ongoing pregnancy were 56.73%, 49.04% and 7.69%, respectively. Among women subjected to pregnancy loss, the rates of cumulative biochemical pregnancy loss, first and second trimester clinical pregnancy loss were 11.86%, 6.78% and 3.39%, respectively. Neonatal outcomes were also assessed among women with singleton delivery, including gestational week (38.95 ± 1.59 weeks), birth weight (3.41 ± 0.51 kg) and newborn's sex ratio (male/female, 51.28%/48.72%). In addition, the incidences of adverse events were also presented in the cohort. Of the 38 women who yielded healthy newborns, 31 women underwent Down's screening or noninvasive prenatal testing for prenatal screening and 18 women underwent amniocentesis for prenatal diagnosis (Supplementary data, Table S2). Amniocentesis results showed that all genotypes of the fetuses were consistent with PGT-M results.

DISCUSSION

The entire process of PGT-M for monogenic nephropathy, including genetic counseling, embryo testing results, prenatal diagnosis and pregnancy outcomes, were studied in the cohort. The accuracy of genetic counseling is a prerequisite for accurate PGT-M results. The accuracy of genetic counseling refers to accurately diagnosing the etiology of nephropathy-related symptom in patients, determining the exact type of monogenic nephropathy in patients, and accurately finding the affected genes and pathogenic loci in patients. Besides, the genotypes of the pedigree members should be completely consistent with their clinical features. All patients who underwent PGT-M in the cohort had both adequate specialty diagnoses which were diagnoses from professional nephrologists and pathogenic gene variant(s) causing monogenic nephropathy. By applying PGT-M, all genotypes of the fetuses with amniocentesis results were consistent with the PGT-M results, which provides strong evidence that PGT-M is an effective tool for avoiding passing on these

Table 3: Results of embryos tested following combined PGT-M and PGT-A.

Embryo types	Total embryos, $n = 498$
Transferable embryos—*n* (%)	76 (15.26)
Noncarrier	
Euploid	76 (15.26)
Embryos recommended for genetic counseling—*n* (%)	71 (14.26)
Noncarrier	
Subsegmental aneuploid	5 (1.00)
Chromosomal mosaicism	49 (9.84)
Complex[a]	2 (0.40)
Carrier	
Euploid	8 (1.61)
Chromosomal mosaicism	5 (1.00)
Other[b]	2 (0.40)
Non-transferable embryos—*n* (%)	247 (49.60)
Noncarrier	
Monosomy	11 (2.21)
Trisomy	6 (1.20)
Fragment duplication/deletion	9 (1.81)
Complex[c]	27 (5.42)
Carrier	
Monosomy	3 (0.60)
Trisomy	1 (0.20)
Fragment duplication/deletion	1 (0.20)
Complex[c]	2 (0.40)
Pathogenic genotype	
Euploid	30 (6.02)
Monosomy	1 (0.20)
Trisomy	2 (0.40)
Subsegmental aneuploid	1 (0.20)
Fragment duplication/deletion	3 (0.60)
Chromosomal mosaicism	12 (2.41)
Complex[c]	8 (1.61)
Questionable[d]	129 (25.90)
Other[e]	1 (0.20)
Embryos without genetic testing—*n* (%)	97 (19.48)
Embryos with failed amplification—*n* (%)	7 (1.41)

Values are presented as *n* (%).
[a]A complex result was defined as the embryo which was mosaic and subfragmental aneuploid (<4 Mb).
[b]The embryos with other results for genetic counseling included one chromosomal mosaic embryo without PGT-M result and one embryo with the genetic testing result of uniparental dimorphism.
[c]A complex result was defined as a combination of more than one of the following features: monosomy, trisomy, fragment duplication/deletion (>4 Mb) or chromosomal mosaic.
[d]A questionable result was defined as the embryo with unknown chromosomal status only following PGT-M.
[e]The non-transferable embryo with other result was defined as complex chromosomal abnormalities without PGT-M result.

Table 4: Pregnancy and neonatal outcomes after single frozen embryo transfer.

Outcome	Total female patients, $n = 104$
Primary outcome	
Cumulative live birth rate—*n* (%)	38 (36.54)
Singleton	38 (36.54)
Twin	0 (0.00)
Secondary outcomes	
Cumulative biochemical pregnancy—*n* (%)	59 (56.73)
Cumulative clinical pregnancy—*n* (%)	51 (49.04)
Cumulative ongoing pregnancy—*n* (%)	8 (7.69)
Cumulative pregnancy loss—*n* (%)	13 (22.03)
Biochemical	7/59 (11.86)
First trimester clinical	4/59 (6.78)
Second trimester clinical	2/59 (3.39)
Features of live birth	
Duration of pregnancy—weeks, mean ± SD	38.95 ± 1.59
Birth weight—kg, mean ± SD	3.41 ± 0.51
Newborn sex (male/female)—*n* (%)	20/19 (51.28/48.72)
Cesarean section—*n* (%)	23/39 (58.97)
Adverse events—*n* (%)	
Gestational diabetes mellitus	4/51 (7.84)
Preeclampsia	1/51 (1.96)
Gestational hypertension	5/51 (9.80)
Premature rupture of membranes	1/51 (1.96)
Preterm delivery	3/51 (5.88)

Values are presented as mean ± SD or *n* (%).

single gene disorders to their offspring in couples with monogenic nephropathy in the reproductive age. The absence of genetic abnormalities detected by prenatal diagnosis in healthy newborns without monogenic nephropathy also underscores its validity. The increasing number of patients seen indirectly indicates the importance and popularity of PGT-M technology.

Accurate genetic counseling, clinical diagnosis and genetic testing are very significant. All patients in the cohort underwent adequate specialist diagnosis to exclude all nephropathy with unknown origin. As a center for reproductive medicine, we offer genetic counseling and testing for hereditary kidney disease in cases where definite clinical diagnoses are present. The diagnosis and classification of kidney diseases are conducted by specialists who mainly rely on anatomical and pathological mechanisms in another hospital. The advice to proceed with genetic testing is typically made by physicians and genetic counselors who have clinical experience medical genetic knowledge, and the professional title of physician-in-charge or above in our center. Before conducting genetic testing, we will ensure that the patient comprehends the purpose and potential outcomes of the test and provides informed consent as required. Moreover, we strictly adhere to pertinent policies and regulations on privacy and data security when processing genetic testing data. It must be mentioned that in recent years, with the development of next-generation sequencing and linkage analysis providing increasingly rich and extensive information, the applicability and complexity of PGT-M have also become increasingly prominent. Therefore, a multidisciplinary team that includes, but is not limited to nephrologists, geneticists, reproductive specialists and obstetrician-gynecologists is crucial for increasing the popularity and selection of PGT-M. Nephrologists provide expertise in hereditary kidney diseases, and assess individual and family disease backgrounds, while geneticists guide reproductive decisions through precise genetic analysis and genetic counseling. Just as crucially, reproductive specialists tailor individualized PGT strategies according to the patient's specific circumstances, and obstetrician-gynecologists safeguard the well-being of the pregnancy journey. Interdisciplinary collaboration not only deepens patients' understanding of genetic risks, but also promotes the development of personalized medicine and reduces the incidence of genetic diseases.

All 180 couples who received genetic testing underwent pedigree management. Higher diagnostic yields for disease were observed in family-based exome sequencing than when only

testing the proband's exome [26]. Moreover, sufficient family segregation study contributes to adequately avoiding misleading genetic test results in single relatives. Family segregation studies require sufficient family member samples. Due to sample limitations, there were very few VUS carriers patient who met the requirements for family segregation study. In 2018 and before, most of the variant site evaluations were based on the Human Gene Mutation Database, and some were based on the experience of the reproductive center database. Although two couples (ID 97 and 100 in Supplementary data, Table S1) carrying VUS mutations did not meet the conditions for family segregation study, their mutation pathogenicity was verified and upgraded by linkage analysis of the patients' parents and offspring. The female partner of one couple (ID 100) harbored the *ANKS6* gene (AR inheritance), c.1202T>G mutation, and the American College of Medical Genetics and Genomics (ACMG) evidence supported PM2 and PM3 criteria. For another couple (ID 97), the male partner carried the *PKHD1* gene (AR inheritance), c.2279G>T mutation, and the ACMG evidence supported PM2-P and PP3-M criteria; whereas the female partner carried the *PKHD1* gene (AR inheritance), c.11246C>T mutation, and the ACMG evidence supported PM2-P and PM5 criteria. Both couples sought PGT-M before 2018. Although their mutation pathogenicity was verified and upgraded by linkage analysis of the patients' parents and offspring, none of the newborn offspring has shown symptoms of renal disease during follow-up to date, which also reflected the accuracy of pathogenicity evaluation in early years and the effectiveness of PGT-M based on the old evaluation system. After 2018, our treatment indications are stringently in full compliance with ACMG standards. Upgrading the pathogenicity of VUS variants must be analyzed through family segregation study (ID 33 in Supplementary data, Table S1). The male partner in a couple (ID 33) carried the *PKD1* gene (AD inheritance), c.4349_4351del mutation, and the ACMG evidence was consistent with PM4, PM2-P and PP4 criteria. The couple upgraded the mutation pathogenicity through family segregation study and underwent PGT-M to obtain a healthy fetus. With the iterative updates of ACMG standards and the strict application of family segregation study, PGT-M has gradually been improved to further handle more difficult cases.

Compared with previous studies, more types of monogenic nephropathy, and four genetic patterns involving more comprehensive disease-causing genes were included in the retrospective cohort [23–25]. Patients with polycystic kidney disease accounted for 81.73%, the majority of genetic counseling. However, different from the Dutch and American cohorts, where PKD and Alport syndrome were the most prevalent diseases referred for PGT-M, few patients with Alport nephropathy were included in our cohort [23, 24]. Our cohort only included one patient with *COL4A4* mutation and three patients with *COL4A5* mutation among the couples who underwent PGT-M. Additionally, there were two patients with *COL4A3* mutation who met the criteria for PGT-M but opted not to undergo the procedure. But another Chinese cohort also exhibited a similar pattern in which Alport syndrome was not frequently observed as a monogenic renal disease referred for PGT-M consistent with our research [25]. Therefore, the regional disparities might result in variations in distribution of the monogenic kidney diseases included at the study. In addition, patients with Alport syndrome exhibit a spectrum of clinical presentations, which may encompass isolated hematuria, hematuria concomitant with proteinuria [27, 28], and progressive renal insufficiency [29] which may or may not be accompanied by extrarenal manifestations [30, 31]. For instance, AD Alport syndrome arises from heterozygous pathogenic variants within the *COL4A3* or *COL4A4* genes, typically manifesting with a delayed onset and a gradual clinical symptom progression [32]. Due to the mild clinical phenotype, these patients usually receive specialist treatment in the Department of Nephrology and will not consult for reproductive genetics and other related issues in our center. Besides, compared with previous studies, we included all nephropathy-related patients in the genetic counseling clinic, and provided detailed genetic counseling process and subsequent pregnancy outcomes. Different from the Dutch cohort, the prenatal screening and diagnosis results of patients who obtained clinical pregnancy through PGT-M were elaborated in our cohort [23]. The follow-up information for pregnancy outcomes, especially LBRs, in patients with monogenic nephropathy administered by PGT-M were not collected in one USA cohort [24]. The PGT-M process for patient with nephropathy-related disease were also reviewed in another China cohort, excluding the genetic counseling process [25].

It was difficult for patients with monogenic nephropathy to obtain the most suitable embryos. Noncarrier and euploid embryos were the best detection results for patients underwent PGT-M combined with PGT-A. For 80 vitrified-thawed single blastocyst transfer cycles performed in the cohort, only 59 noncarrier and euploid embryos were transferred. Moreover, 13 couples selected embryos recommended for genetic counseling for transfer, mainly including 4 carrier and euploid embryos, 9 noncarrier and mosaic embryos. One couple obtained only an embryo with ADPKD pathogenic phenotype detected by PGT-M, and strongly requested to transfer this embryo after genetic counseling about undergoing another PGT-M cycle for selecting embryos with non-pathogenic genotypes for transfer or using sperm donor cycles. Finally, the couple obtained a live-born baby infant suffering from ADPKD. Of the eight couples who selected chromosomal mosaic embryo with non-pathogenic genotypes for transfer, six yielded healthy newborns. Although previous studies have demonstrated mosaic embryo transfer is associated with a lower LBR compared with euploid embryo transfer, patients who only obtained mosaic embryos with non-pathogenic genotypes after combined-PGT could also be counseled regarding this alternative option [33]. However, for patients with monogenic nephropathy, whether or not the types of transferred embryos affect pregnancy and neonatal outcomes after PGT-M requires further investigation. Of note, the ethical challenges brought by transferring embryos with non-pathogenic genotypes also requires further discussion.

We also investigated the fluctuations in patient visits over the years and conducted a maximum follow-up on 104 patients who underwent PGT-M. During the period from 1 January 2014 to 31 December 2022, the number of genetic counseling outpatients and PGT-M cycles in the hospital gradually increased. But due to the impact of the epidemic prevention policy against COVID-19, the number of genetic counseling outpatients and PGT-M cycles fluctuated from the end of 2019 to 2022, which is in line with the current situation of the first generation of COVID-19 strains at the end of 2019 [34] and the Omicron mutant strain in 2022 [35]. We further updated the follow-up information of the 104 couples to 20 October 2024. Among the 38 couples who achieved live births, 7 underwent PGT-M again in our hospital, and 3 of them received live births again. Among patients who underwent frozen embryo transfer but did not obtain live births before 31 December 2022, 14 couples received live births in the first controlled ovarian hyperstimulation (COH) cycle, and 22 couples obtained live births in the second/third COH cycles. Thus, a total of 74 (71.15%) couples achieved live births, but 30 couples still did

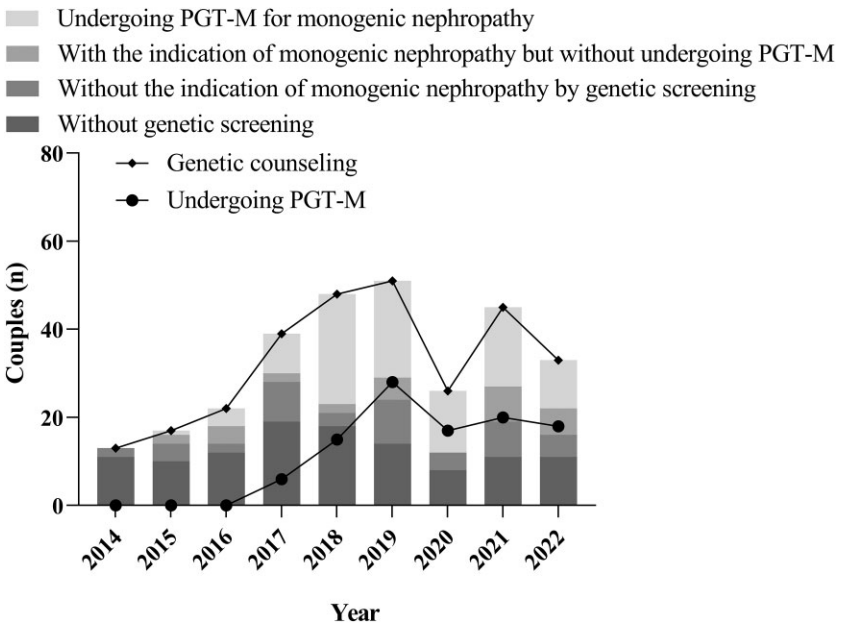

Figure 1: Couples with genetic counseling and PGT-M for monogenic nephropathy from 2014 to 2022.

not achieve live births after suffering from multiple PGT failures. Prolonging the follow-up time will obtain more valuable follow-up information, but it is undeniable that economic factors and factors such as poor ovarian reserve, high childbearing age, or abnormal gestation and birth history have led to multiple failures, reducing patients' confidence in assisted pregnancy and trust in technology, which may help to explain why patients give up PGT-M-assisted pregnancy [23, 24, 36]. Taking into account all COH cycles of patients, the cumulative ongoing pregnancy (OP)/LBR of the Shanghai cohort in China from January 2011 to December 2021 was 54.69% [25], while the cumulative LBR of the Dutch cohort from January 1995 to June 2019 was 65% [23]. After including all COH cycles of our 104 patients from January 2014 to December 2022, the cumulative OP/LBR was up to 67.31% in our cohort. Credit should be given to the safer and more standardized technical requirements of our center. For example, all frozen embryo transfers were performed with single blastocyst, which can reduce the miscarriage and stillbirth rates. The Dutch cohort did not specify combined PGT, so the LBR cannot be further compared with our cohort [25]. In short, we obtained good assisted pregnancy outcomes, which also suggests the effectiveness of PGT-M in monogenic kidney disease.

Overall, our study was unique in that it reviewed the genetic counseling process for patients with nephropathy-related disease and provided a clinical overview of patients with monogenic nephropathy who underwent PGT-M. Moreover, our data were more applicative than previous studies to current genetic counseling by employing combined-PGT strategy for monogenic nephropathy cases, thus providing references for clinical decision-making. Still, our study did have several limitations. Only 352 couples with nephropathy-related disease were included from our center in this cohort, and the universality of our data may thus be limited by the small sample size. Moreover, this was a retrospective study that did not further explore the influence of monogenic nephropathy process on pregnancy outcomes, due to the lack of specific specialist consultation information for patients with monogenic nephropathy in genetic counseling clinic. Therefore, further studies are required to guarantee the safety reliability and clinical value of PGT-M, especially combined-PGT strategy on monogenic nephropathy.

To summarize, our study contributed to the existing PGT-M literature by revealing that PGT-M is an effective means of preventing transmission of monogenic nephropathy to offspring for couples with monogenic nephropathy at childbearing age. The consistency between amniocentesis and PGT-M results in genotypes also underscore its validity. However, patients with nephropathy-related disease were at an increased risk of other factors associated with adverse pregnancy outcomes, such as renal disorders, renal hypertension and immune dysfunction. Further work is necessary to assess the impact of those above-mentioned factors, also including disease patterns and genetic characteristics, on PGT-M outcomes in patients with monogenic nephropathy (Fig. 1).

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

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DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of individuals who participated in the study. The data will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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