Outcomes of preimplantation genetic diagnosis in neurofibromatosis type 1

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Objective: To examine the effect of patient and facility level factors on the success of preimplantation genetic diagnosis (PGD) in patients with neurofibromatosis 1 (NF1).

Design: Retrospective review.

Setting: Large PGD reference laboratory.

Patient(s): All patients with NF1 referred from June 2004 to May 2013.

Intervention(s): None.

Main Outcome Measure(s): Embryos’ NF1 mutation status and live birth rates.

Result(s): Seventy-seven couples underwent 156 PGD cycles during the study period. The average maternal age at the time of embryo biopsy was 33.2 years. The majority of embryos had a day 3 single blastomere biopsy without aneuploidy screening. A diagnosis was obtained for 80% of biopsied embryos; 20% of biopsies were nondiagnostic due to technical failures. Diagnosis was more often obtained for embryos of parents with familial disease and for embryos biopsied at centers that referred multiple NF1 cases. Among diagnosed embryos, 483/1,060 (46%) were unaffected by the parental NF1 mutation. Twenty-two (14%) of the 156 cycles had a confirmed live birth; if the observed success rate is applied to cycles with unknown outcomes, 33/156 (21%) cycles are expected to have resulted in live birth. In multivariate logistic regression, having a live birth was significantly associated with having more unaffected embryos available for transfer (odds ratio 1.33 per additional embryo, 95% confidence interval 1.02–1.72).

Conclusion(s): Advances in biopsy and diagnostic techniques which increase the number of unaffected embryos identified may improve live birth rates for patients with NF1. Clinicians should counsel patients about their fertility and reproductive options early, with the use of disease-specific data, to set appropriate expectations for the PGD process. (Fertil Steril® 2015;103:761–8. ©2015 by American Society for Reproductive Medicine.)

Key Words: PGD, NF1, fertility, pregnancy, in vitro fertilization, genetic counseling

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Preimplantation genetic diagnosis is a molecular technique in which an embryo generated by in vitro fertilization (IVF) can be tested for a genetic condition before uterine transfer [1]. By amplifying and examining genetic material from a cellular biopsy of the embryo, a laboratory can determine which embryos may carry certain genetic abnormalities, and doctors can transfer only those embryos shown to be free of those abnormalities [2]. Preimplantation genetic diagnosis (PGD) is used to analyze embryos for specific genetic mutations found in one or both parents, whereas preimplantation genetic screening (PGS) tests embryos for chromosomal aneuploidies. Preimplantation genetic testing can be viewed as an early form of prenatal diagnosis and offers an alternative for couples who wish to increase their chances of giving birth to a healthy child but are unwilling to terminate an affected pregnancy [3].

Since its first application in preventing transmission of X-linked disorders [4] and cystic fibrosis [5], PGD has become an increasingly popular technique for couples affected by monogenic disorders to reduce their risks of having a similarly affected child [5, 6]. In particular, the number of PGD analyses being performed for patients with cancer predisposition syndromes has been steadily increasing over the past decade [7].
One such cancer predisposition syndrome is neurofibromatosis type 1 (NF1), an autosomal dominant monogenic disorder that displays full penetrance. NF1 is the most common neurogenic condition (with a birth prevalence of ~1 in 3,000) (8, 9), and the sixth most common monogenic disorder to be tested with the use of PGD (10). Clinical manifestations of NF1 include multiple benign nerve sheath tumors (neurofibromas), intellectual impairments, and bony abnormalities, and persons with NF1 have an increased risk of developing cancers such as sarcomas and gliomas (11). Comprehensive genetic analysis of the NF1 gene has been available since 2000 (12), and current testing can identify a causative genetic alteration in >95% patients who meet established clinical criteria, allowing many couples affected by NF1 the opportunity to pursue PGD.

Currently, there are limited data available about the outcomes of PGD for NF1 patients. Earlier publications have documented aggregate outcomes of PGD for multiple monogenic diseases (10, 13–16), and earlier NF1-specific reports have been case series of a small number of patients (17–19). For this reason, we retrospectively evaluated the diagnostic and clinical outcomes for couples affected by NF1 who pursued PGD at a large international laboratory that provides PGD services.

**MATERIALS AND METHODS**

We retrospectively reviewed the medical records of an international laboratory providing testing for couples who pursued PGD to reduce the risk of transmitting NF1. We included all couples with NF1 referred to the laboratory from the start of NF1 testing through May 2013. This research was reviewed by the Partners Institutional Review Board and determined to be nonhuman subjects research.

**PGD Protocol**

Oocyte stimulation was conducted according to the referring IVF center’s individual protocols. Embryos were fertilized with the use of intracytoplasmic sperm injection (ICSI) to minimize the risk of subsequent sample contamination from supernumerary sperm embedded in the zona pellucida. DNA diagnostic reports from a Clinical Laboratory Improvement Amendment (CLIA)–approved laboratory and buccal swab samples from appropriate family members were sent to the laboratory to generate molecular probes by means of a standardized procedure.

Genetic material was obtained from embryos primarily (>95% of cases) by cleavage-stage biopsy of a single blastomere on day 3 of development. Biopsy procedures for PGS were identical to those used in PGD, and additional biopsies were not required when both procedures were performed. Biopsy samples were transported in individual tubes in a lysis buffer by courier to the laboratory for PGD analysis before transfer of embryos.

Before 2010, PGD analysis began with direct amplification of the DNA from the diploid cell(s). The cells were lysed and a polymerase chain reaction (PCR) reaction was performed to amplify the genetic region in question from each cell. An outer amplification reaction and an inner amplification reaction were performed to isolate the mutated region in the gene. All patient samples received after 2010 were subjected to whole-genome amplification (WGA) protocols rather than direct amplification. Samples were amplified with the use of the BlueGnome SurePlex system. Biopsy samples underwent genomic amplification followed by locus-specific standard PCR of 2–µL aliquots. Six fully informative NF1-flanking genomic markers were tested independently to determine the haplotype of each sample; this was done to evaluate the remote possibility of an intragenic recombination. After standard PCR of each locus, the amplified DNA was analyzed on an Applied Biosystems 3300 capillary electrophoresis instrument, with independent genotype analysis of each allele. Genotypes were then compared to the haplotype of the gamete providers to determine the NF1 status of each embryo.

For samples also undergoing PGS, amplified DNA was labeled and hybridized onto Illumina 24sure bacterial artificial chromosome microarrays. Microarrays were washed and scanned, and single-channel images were imported into the Illumina software. Fixed algorithms automatically determined whole-chromosome gains and losses, whereas segmental chromosome deletions or duplications >10 Mbp were examined manually. Reports from NF1 PGD analysis and, if applicable, PGS analysis were issued to the referring IVF center within 48 hours. Pregnancy test and birth outcomes were voluntarily reported by IVF centers back to the diagnostic laboratory.

**Data Collection**

**Demographics.** We collected demographic data on affected partner’s inheritance pattern for NF1 (sporadic or familial), maternal and paternal age, sex of the affected partner, history of infertility, and previous use of IVF. Referring IVF centers were categorized based on number of NF1 cases completed at this PGD laboratory by May 2013 (single referral vs. multiple referrals) and on practice type (academic vs. nonacademic affiliation).

**Embryo data collection.** We collected data on the number, quality, and stage (cleavage stage vs. trophectoderm) of all biopsied embryos within each IVF cycle for NF1 patients. Embryo quality was rated by referring centers with the use of a 3-point, 4-point, or 5-point scale. For consistency in our analyses, 4- and 5-point ratings were rescaled to a 3-point scale (good, fair, and poor).

Additional laboratory data retrieved included the NF1 status of each biopsied cell and the diagnosis of aneuploidy for any cycles also undergoing PGS. Embryos that displayed normal NF1 alleles were classified as unaffected, and embryos that displayed the mutant parental allele were considered affected. If the laboratory report indicated amplification failure of the probe or no molecular signal, the outcome was classified as “insufficient data.” Embryos were classified as “inconclusive results” if the laboratory report indicated allele drop-out, weak amplification, conflicting marker data, recombination, incomplete analysis, or multiple parental alleles observed. Outcomes of PGS were classified as euploid, aneuploid, or no signal.

**Clinical outcomes.** We collected all available data on clinical outcomes for all PGD cycles, including pregnancy test results.
Factors associated with ability to obtain a molecular diagnosis. Descriptive statistics of the patient cohort and embryo characteristics were tabulated. If two biopsies were performed on the same embryo, the most informative diagnosis was used for analysis (i.e., status as affected/unaffected rather than insufficient data or inconclusive result). Chi-square analyses were used to test for an association between the ability to obtain a molecular diagnosis on an embryo (as either affected or unaffected with NF1) with the following variables: embryo quality rating, familial or sporadic parental mutation, number of NF1 referrals from IVF center, IVF practice type, and use of whole genome amplification. We excluded from these analyses seven samples that could not be analyzed owing to breakage during shipping.

Factors associated with PGD resulting in live birth. Descriptive statistics were generated for the number of pregnancies and live births observed. We also generated estimated pregnancy and birth rates, taking into account the likely outcomes of cycles with missing data to predict what the true pregnancy and birth rates may have been in our cohort. To derive the estimated number of unobserved pregnancies, we multiplied the observed pregnancy rate in women with an embryo transfer by the number of cycles with unknown pregnancy outcome. To derive the estimated number of unobserved live births, we multiplied the observed birth rate in pregnant women by the number of observed and unobserved predicted pregnancies with unknown outcome.

We performed logistic regression analysis to determine which factors correlated with live birth. Dependent variables included number of unaffected embryos produced, maternal age, number of NF1 referrals from the IVF center to the diagnostic laboratory, and time elapsed since the first PGD analysis was performed (in months). Time elapsed since the first PGD analysis was included in our statistical analysis to control for improvements in PGD and IVF technique over time. We excluded from this analysis 24 cycles that did not produce any unaffected embryos, one cycle for which embryo transfer was known not to have occurred, and four cycles with incomplete data on number of unaffected embryos produced.

RESULTS
Patient Demographics and Clinic Characteristics
From June 2004 to May 2013, 81 couples affected by NF1 began the PGD process, and 77 couples completed at least one PGD cycle. Three couples did not undergo an IVF cycle because molecular probes could not be generated for PGD analysis, and one couple underwent an IVF cycle but did not undergo PGD analysis because no embryos of sufficient quality for biopsy were produced. A total of 156 PGD cycles were completed, with a median number of two cycles per couple (range 1–9 cycles). Thirty-three couples (43%) completed one cycle, 24 (31%) completed two cycles, 12 (16%) completed three cycles, and eight (10%) completed four or more cycles. 42 cycles (27%) were performed in women with advanced maternal age (age ≥ 35 years).

The affected partner was female in 51 couples (66%). There were 48 sporadic NF1 patients (62%) and 23 familial patients (30%); the inheritance pattern was unknown or not reported in six patients (8%). Fourteen (18%) couples had a history of infertility, and four (5%) had previously undergone IVF. The average maternal and paternal ages at the time of embryo biopsy were 33.2 years (range 23.7–43.1 years) and 35.6 years (range 25.8–57.5 years), respectively.

Couples were referred from 62 IVF centers from 18 states and 6 non–United States countries (Table 1); 51 centers referred one couple for NF1 PGD, and 11 centers referred more than one couple. The clinic with the highest number of NF1 cases referred eight patients for PGD, seven of whom completed a PGD cycle. Fourteen IVF centers were academically affiliated, and 47 were independent. Throughout the 9 years of data collection, the use of PGD by couples affected by NF1 steadily increased from three in 2004 to a peak of 38 cycles in 2010.

PGD Cycle Outcomes
Over 156 PGD cycles, 1,356 embryos were biopsied (Fig. 1). The median number of embryos biopsied per cycle was eight (range 1–40 embryos) for all women and six (range 1–13 embryos) for women of advanced maternal age (≥ 35 years); 34 embryos (2.5%) were biopsied twice; 88% of biopsied embryos (1,193/1,356) were fresh, and 12% (163/1,356) were frozen. By IVF center self-report, 23% of biopsied cells were of good quality, 58% were of fair quality, 8% were of poor quality, and 11% were not rated. Whole-genome amplification was used to analyze 782 embryos (within 90 cycles) and was not used in 574 embryos (within 66 cycles).

PGD outcomes were available for 1,322/1,356 (97%) of the biopsied embryos, and a clear molecular diagnosis was obtained for 1,060/1,322 embryos (80%). Insufficient molecular data was observed in 161/1,322 embryos (12%), and 101/1,322 embryos (8%) produced inconclusive results. Of the embryos with a molecular diagnosis, 483/1,060 (46%) were unaffected with NF1. The number of unaffected embryos produced per PGD cycle ranged from 0 to 18, with a median number of three unaffected embryos per cycle; 135/156 cycles (87%) produced at least one unaffected embryo.
Definitive diagnoses were more likely to be obtained for embryos of parents with familial NF1 inheritance compared with embryos from parents with sporadic NF1 (84% vs. 76%; \( \chi^2 = 12.1; P < .001 \)). Diagnoses were also more likely to be obtained for embryos biopsied at centers with multiple NF1 referrals compared with embryos biopsied at centers with a single NF1 referral (83% vs. 77%; \( \chi^2 = 6.2; P = .01 \)). The ability to obtain a molecular diagnosis was not associated with embryo quality rating (\( P = .46 \)), academic affiliation of the referring medical center (\( P = .18 \)), or use of whole genome amplification in the probe development process (\( P = .98 \)).

### PGS Cycle Outcomes

Ten couples completing a PGD cycle also underwent PGS in a total of 15 cycles. The average maternal age for patients completing a cycle with PGS was 35.5 years (range 29.9–42.0 years). PGS was done in addition to PGD on 114 embryos. Of these, 44/114 (39%) were euploid, 56/114 (49%) displayed some form of aneuploidy, and 14/114 (12%) did not display a signal. Twenty-six embryos unaffected by NF1 were not transferred due to the presence of aneuploidy. In three cycles, even though embryos unaffected by NF1 were produced, no embryos were available for transfer owing to aneuploidies found during PGS.

### Pregnancy and Birth Outcomes

One hundred thirty-two of the 156 cycles (85%) had at least one embryo suitable for transfer (Fig. 2). Pregnancy test data were available for 80/132 cycles (61%) which had at least one embryo suitable for transfer. Of the cycles with pregnancy test data, 41/80 (51%) resulted in a positive serum pregnancy test. In 22 cycles, these pregnancies resulted in a live birth. Multiple births (twin or triplet) accounted for 23% of the live births, and a total of 28 infants were born to 21 different couples. No live births occurred in 13/41 cycles (32%) with a
positive pregnancy test, and there were no birth outcome data available for the remaining 6/41 cycles (15%).

Overall, 21/77 (27%) couples undergoing at least one IVF/PGD cycle achieved a live birth. Among couples that successfully had a live birth, the median number of cycles to achieve a live birth was two (range 1–5 cycles). Of these couples, 19/21 (90%) were successful within their first two cycles of PGD. By applying the observed pregnancy and birth rates to cycles for which an outcome was unknown, we estimate that 57/156 cycles (37%) resulted in a positive pregnancy test and that 33/156 cycles (21%) resulted in a live birth (Table 2).

In multivariate logistic regression, having a higher number of unaffected embryos available for transfer was significantly associated with having a live birth after a given PGD cycle ($P = .03$; odds ratio 1.33 per additional embryo, 95% confidence interval 1.02–1.72). Maternal age, number of center referrals, and months since the first PGD analysis were performed were not significantly associated with birth outcome.

**Reasons for Failure of PGD Technology**

Four of the 81 couples (5%) affected by NF1 who wished to pursue PGD were not able to. Molecular probes could not be designed for three couples, owing to the specific nature of their de novo mutations. When attempting to develop molecular probes, it was found that each patient’s specific mutation in the \( \text{NF1} \) gene made it nearly identical to another area of DNA (called a pseudogene). During PCR, both the \( \text{NF1} \) gene and the pseudogene were amplified (known as pseudogene bleed-through), which can lead to embryos without the parental mutation presenting as false positives. Without genetic samples available from other affected family members, the risk of false positives was deemed to be unacceptably high to continue PGD. Additionally, for one couple, no embryos of sufficient quality to biopsy were produced in the only IVF cycle undertaken.

**DISCUSSION**

In this study, we reviewed the records of one of the largest diagnostic laboratories in the United States to evaluate the outcomes of PGD for patients affected by NF1. These data provide valuable disease-specific information for counseling couples about the potential benefit of using IVF and PGD to reduce the risk of transmitting NF1 to their offspring. The diagnostic laboratory was able to create molecular probes for most NF1 couples (95%). Of 1,322 biopsied embryos, 483 (37%) were determined to be unaffected by the parental NF1 mutation. We estimate that 37% of IVF/PGD cycles for

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**TABLE 2**

Clinical outcomes of IVF/preimplantation genetic diagnosis cycles for neurofibromatosis 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Observed, n (%)</th>
<th>Estimated, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles with embryo biopsy</td>
<td>156</td>
<td>156</td>
</tr>
<tr>
<td>Cycles resulting in positive pregnancy test</td>
<td>41 (26)</td>
<td>57 (37)</td>
</tr>
<tr>
<td>Cycles resulting in live birth</td>
<td>22 (14)</td>
<td>33 (21)</td>
</tr>
<tr>
<td>Cycles with embryo transfer</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Cycles resulting in positive pregnancy test</td>
<td>41 (31)</td>
<td>57 (43)</td>
</tr>
<tr>
<td>Cycles resulting in live birth</td>
<td>22 (17)</td>
<td>33 (25)</td>
</tr>
<tr>
<td>Percentage of live births with twins or triplets</td>
<td>5 (23)</td>
<td>7 (21)</td>
</tr>
</tbody>
</table>

*Includes biochemical pregnancy, spontaneous abortion, and/or elective termination.

NF1 result in a positive pregnancy test, and that 21% of all cycles result in a live birth. At least 27% of couples eventually achieved at least one live birth during treatment.

Analysis of disease-specific outcomes after PGD is increasingly important as the scope of reproductive counseling spreads beyond women and men affected by infertility to include patients with genetic abnormalities. In an age where the cost of genome sequencing is dramatically dropping, PGD is becoming available to more patient populations. From 1997 to 2007, parents used PGD in more than 10,000 IVF cycles to prevent the transmission of a known genetic disorder to a child (7). NF1 was the third most common autosomal dominant disorder analyzed among centers reporting to the European Society of Human Reproduction and Embryology (ESHRE) consortium in 2008 (10). For this reason, analysis of laboratory and clinical outcomes after PGD in patients with NF1 is needed to appropriately advise patients about their reproductive options.

In the present analysis, 80% of biopsied embryos could be classified as affected or unaffected by the parental NF1 mutation. This figure is slightly below the average figure observed from 1997 to 2007 for two other autosomal dominant disorders: 86.6% for myotonic dystrophy type 1 and 87.8% for Huntington’s disease (7). Given that the ability to identify the presence or absence of mutations for all monogenic disorders during PGD has improved from 83% in 1997 to 90% in 2007, it is likely that a higher percentage of embryos from couples affected by NF1 are able to be diagnosed today (7).

Definitive diagnoses were significantly more likely to be obtained for embryos from parents with familial NF1 inheritance compared with embryos from parents with sporadic NF1 (i.e., parent was the first in their family to have an NF1 mutation). In familial cases, genetic material could be solicited from additional family members, allowing for more sensitive probe preparation which enabled a greater diagnosis success rate. Diagnoses were also more likely to be obtained for embryos biopsied at centers with multiple NF1 referrals compared with embryos biopsied at centers with a single referral. This difference highlights the importance of consulting medical professionals experienced in PGD for the retrieval and biopsy of embryos.

Forty-six percent of embryos that were able to be diagnosed were unaffected with the parental NF1 mutation. Because NF1 is autosomal dominant, it is expected that an average of 50% of embryos would be unaffected by the parental mutation. However, in both our series and in earlier analyses of ESHRE data on muscular dystrophy 1 (43.4%) and Huntington’s disease (43.9%) (10), there were fewer unaffected embryos than expected. It is possible that technical difficulties in amplification and analysis are more frequent in unaffected samples, and that future improvements in laboratory analysis will increase the proportion of unaffected embryos observed.

The proportion of PGD cycles that resulted in a live birth in our sample was lower than the proportion of successful IVF cycles reported by the Society for Assisted Reproductive Technology. Given that many patients with NF1 and their partners do not have a history of infertility, patients may be surprised to learn that their reproductive chances are not equal to or even greater than other IVF couples (almost all of whom have experienced infertility). If so, education regarding the implications of selecting embryos based on mutation status rather than morphology may be warranted.

Although couples affected by NF1 pursuing PGD have lower success rates than couples with infertility only, analysis of clinical outcomes showed that a similar number of women in our cohort achieved a positive pregnancy test and live birth compared to women with other autosomal dominant and X-linked disorders (Supplemental Table 1, available online at www.fersttert.org). As expected, the pregnancy and birth rates for couples with NF1 and other autosomal dominant disorders are lower than those for autosomal recessive disorders (10), which are expected to have an average of 75% of embryos unaffected and available for transfer, as opposed to 50%. Thus, previously published data on PGD that has been aggregated across both dominant and recessive disorders would overestimate the success rate of PGD in NF1 and should not be used for comparison with our results.

Although the majority of embryos in our study were biopsied at the cleavage-stage, recent evidence suggests that higher live birth rates may be achieved with blastocyst-stage biopsy of the trophectoderm (20, 21). In a randomized controlled trial in 2013, the adverse effect of cleavage-stage biopsy compared with blastocyst biopsy was equivalent to two of every five reproductively competent embryos becoming incapable of sustained implantation (20, 22). Therefore, increased adoption of blastocyst biopsy could lead to even higher live birth rates in NF1 couples attempting PGD in the future.

In multivariate analysis, the only factor significantly associated with having a live birth was having a larger number of unaffected embryos available for transfer (OR 1.33, 95% CI 1.03–1.72). This result is unsurprising, because with more embryos to choose from for implantation, the higher the likelihood that there will be an optimally developing embryo available. Our results agree with those from Grace et al. in 2006 (23), who found that the only significant factor affecting live birth outcome was presence of two or more embryos genetically suitable for transfer.

Our data may be useful in future studies to calculate the cost-effectiveness of PGD in preventing transmission of NF1 to future generations. The average cost of an IVF cycle in the United States is from $12,400 to $13,775 (24, 25). In addition, ICSI costs an additional $1,500 per IVF cycle, and PGD costs $2,500–$5,000 per cycle (26). Given that the majority of couples in our study who achieved a live birth did so within the first two cycles, the direct medical costs of PGD compared with a natural birth are between $33,000 and $40,000. Comparisons of this figure to the lifetime costs of caring for a child with NF1 will likely reveal a significant savings to insurance companies (at least in the United States) from covering PGD and associated IVF expenses. Future cost-effectiveness analyses are imperative to investigate the impact of PGD on the care of patients with NF1 and other genetic disorders.

There were limitations to our study owing to its retrospective nature. There were missing data regarding pregnancy test and birth outcomes, and no data were available on clinical
pregnancy rates. For this reason, we report a possible range of outcomes (from the number of births actually observed, to the estimated number of births which actually occurred). In addition, our analysis spanned from 2004 to 2013, and although timing was not significant in our analysis of birth outcomes, our results may not reflect the outcomes that are obtained in current practice. Current use of blastocyst-stage (rather than cleavage-stage) biopsy may result in more favorable outcomes than those reported in this series.

Although having all cases from a single reference laboratory meant that probe preparation and analysis protocols were identical, there may be many differences between referring IVF centers that would affect live birth rates, including ovarian stimulation protocols. Similarly, our classification of centers based on number of NF1 case referrals is a crude marker of a center’s volume of PGD cases. We recognize that centers that referred only one NF1 case to this diagnostic laboratory may have referred other cases to other laboratories, and the analysis of centers may be confounded by other systematic differences between the two groups. For this reason, our finding that centers with more case referrals have higher success rates should be validated using stricter criteria. Finally, although embryo quality rating was transformed to a single numeric scale from primary data, the ratings were determined by each referring IVF center. This lack of standardization may have prevented us from recognizing an association between embryo quality and the ability to obtain a diagnosis on the embryo biopsy.

CONCLUSION

For many patients with NF1, PGD is an attractive option when starting a family. PGD is a useful for couples who want to avoid passing NF1 to the next generation and are not amenable to alternate methods of prevention (such as prenatal diagnosis followed by elective termination or use of donor gametes). Although fewer than one-half of patients in our dataset achieved a live birth, strategies that increase the number of unaffected embryos produced and identified in each cycle could improve live birth rates in the future. Identification of the optimal timing of embryo biopsy as well as laboratory improvements that decrease the rate of noninformative biopsy results should be especially helpful.

In addition, medical providers and genetic counselors should discuss PGD with couples early in the care process, so that they have time to consider their reproductive preferences before starting a family. Although overall pregnancy and birth rates were similar to those for other autosomal dominant disorders, our analysis revealed several factors that differ between NF1 and other disorders, as well as within NF1 patients. Clinicians can use this disease-specific data to guide their discussion with patients to provide more tailored advice and to set realistic expectations of the PGD process.

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REFERENCES


### SUPPLEMENTAL TABLE 1

Comparison of neurofibromatosis 1 (NF1) pregnancy outcomes after preimplantation genetic diagnosis (the present study) with published data from the European Society of Human Reproduction and Embryology.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Autosomal dominant</th>
<th>Autosomal recessive</th>
<th>X-Linked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Myotonic dystrophy type 1</td>
<td>Huntington disease</td>
</tr>
<tr>
<td>Mean maternal age (y)</td>
<td>33.2</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>No. of cycles with biopsy</td>
<td>156</td>
<td>552</td>
<td>516</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>26%–37%</td>
<td>26%</td>
<td>28%</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>–</td>
<td>19%</td>
<td>22%</td>
</tr>
<tr>
<td>Live birth</td>
<td>14%–21%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of cycles with embryo transfer</td>
<td>132</td>
<td>445</td>
<td>421</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>31%–43%</td>
<td>32%</td>
<td>34%</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>–</td>
<td>24%</td>
<td>26%</td>
</tr>
<tr>
<td>Live birth</td>
<td>17%–25%</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ranges represent observed and estimated results in the present study.

<sup>b</sup> Ranges represent lower and upper limits of possible results, given the amount of missing data.
