Title

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Journal

JAMA Neurology, 71(4)

ISSN

2168-6149

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Publication Date

2014-04-01

DOI

10.1001/jamaneurol.2013.5884

Peer reviewed

Case Report/Case Series

Preimplantation Genetic Diagnosis (PGD) for Genetic Prion Disorder Due to F198S Mutation in the *PRNP* Gene

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IMPORTANCE To describe the first case of preimplantation genetic diagnosis (PGD) and in vitro fertilization (IVF) performed for the prevention of genetic prion disease in the children of a 27-year-old asymptomatic woman with a family history of Gerstmann-Sträussler-Sheinker syndrome (GSS).

OBSERVATIONS PGD and fertilization cycles resulted in detection of 6 F198S mutation-free embryos. Of these, 2 were selected for embryo transfer to the patient's uterus, yielding a clinical twin pregnancy and birth of healthy but slightly premature offspring with normal development at age 27 months.

CONCLUSION AND RELEVANCE IVF with PGD is a viable option for couples who wish to avoid passing the disease to their offspring. Neurologists should be aware of PGD to be able to better consult at-risk families on their reproductive choices.

JAMA Neurol. doi:10.1001/jamaneurol.2013.5884
Published online February 3, 2014.

Editorial

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Preimplantation genetic diagnosis (PGD) with in vitro fertilization (IVF) has emerged as an important option for at-risk couples wishing to conceive a healthy child without a fatal or severely debilitating inherited disorder.[1,2] PGD allows for transferring only embryos without the disease-causing mutation into the uterus.[1,2]

Prion diseases, also termed *transmissible spongiform encephalopathies*, are a group of fatal neurodegenerative disorders linked to abnormal folding of the prion protein. Genetic prion diseases (gPrDs) are divided into 3 forms based on clinicopathologic features: familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia. There is currently is no cure, and the illness is uniformly fatal. One genetic mutation linked to GSS is a phenylalanine to serine change at codon 198 (F198S) in the prion protein gene (*PRNP*), which has known high penetrance.[3] We describe the first application to our knowledge of PGD for a patient carrying the F198S mutation for the gPrD GSS.

Report of a Case

This case report was deemed exempt research by the Duke University School of Medicine institutional review board, and the patient gave written permission for this report.

A 27-year-old asymptomatic woman with a known family history of GSS chose to undergo predictive testing after genetic counseling and was identified with an F198S *PRNP* mutation with codon 129VM (V cis) polymorphism. The patient opted to be informed of the results of her genetic test. During prior genetic counseling, PGD had been presented as an option, and she and her husband chose to have PGD at a private experienced IVF and PGD center.

After providing written informed consent, the patient underwent IVF-PGD cycles, using methods reviewed elsewhere.[2,4] Twelve of 14 mature retrieved oocytes were fertilized by intracytoplasmic sperm injection and were available for testing (**Figure**). PGD by sequential polar body 1 (PB1) and polar body 2 (PB2) mutation analysis, followed by additional blastomere analysis of day-3 embryos and confirmation, identified 6 mutation-free embryos (Nos. 1, 2, 3, 7, 10, and 14) (Figure).

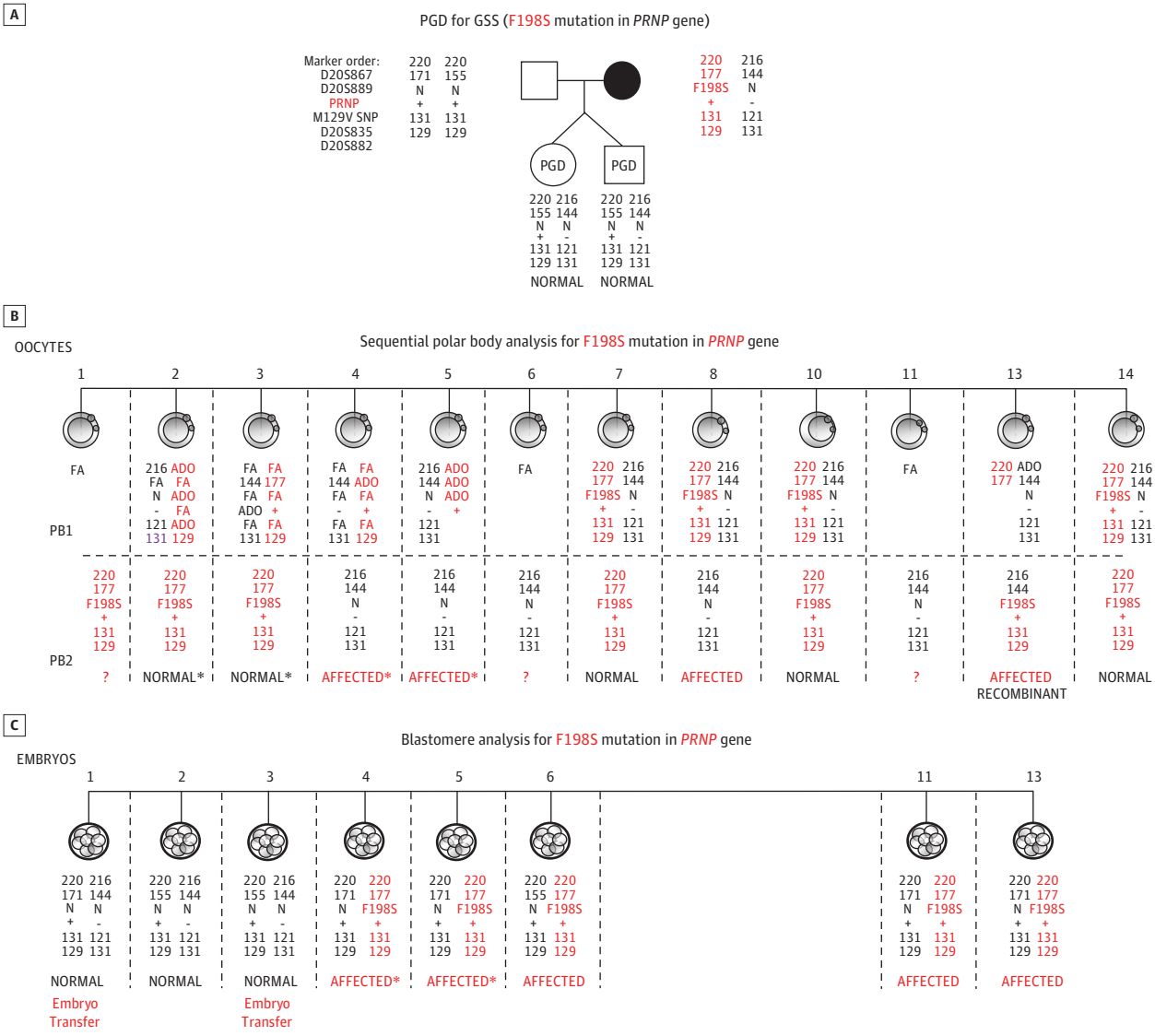
Elective single embryo transfer to prevent multiple pregnancy was discussed, and the patient elected to transfer 2 embryos. Based on PGD analysis, 2 mutation-free embryos (Nos. 1 and 3) (Figure) were chosen for fresh embryo transfer, with 3 remaining viable embryos designated for cryopreservation.

The 2 embryos implanted successfully, and the patient conceived twins. Healthy infants were delivered by a Cesarean section at 33 weeks and 5 days of gestation, each weighing more than 4 pounds. As expected, due to their prematurity, the infants were slightly below the curve for weight for age and for head circumference, both of which normalized by age 3 months. By age 27 months, the infants had consistently completed communicative, social, and emotional developmental milestones on schedule.

Discussion

To our knowledge this is the first published report of IVF with PGD for a genetic prion disease with 27-month normal follow-up of the offspring. Although the patient in our case chose to learn her genetic status, because of emotional risks associated with learning one's carrier status of a *PRNP* gene mutation, nondisclosure PGD (a specialized protocol in which the subject remains unaware of his/her genotype) was discussed as an option.[2]

Figure. Preimplantation Genetic Diagnosis (PGD) for Gertmann-Sträussler-Scheinker Syndrome (GSS) Determined by an Autosomal Dominant Mutation in the Prion Protein Gene (*PRNP*)



A, Pedigree showing that the maternal partner is a 27-year-old asymptomatic woman with an F198S mutation identified by predictive testing in a family with a known history of GSS due to an F198S mutation in the *PRNP* gene (phenylalanine to serine substitution at codon 198). Marker order in relation to the gene is shown on the left. B, Sequential PB1 and PB2 mutation analysis in 12 oocytes, with the results available for 9 oocytes, 4 of which had the mutation, including 1 recombinant oocyte (oocyte 13). The remaining 5 oocytes with DNA results were free of the F198S mutation (oocytes 2, 3, 7, 10, and 14), 3 of which were from oocytes with heterozygous PB1 and hemizygous mutant PB2 (oocytes 7, 10, and 14). C. Blastomere analysis of 8 embryos deriving either from the oocytes with failed amplification of PB1, ADO of linked markers, or from affected oocytes for confirmation. This analysis allowed detecting 1 additional mutation-free embryo for transfer (embryo 1), deriving from a mutation-free oocyte and confirmed normal. Two healthy embryos were transferred, resulting in the birth of healthy twins with a very high likelihood (91%-98%) of being free of the F198S mutation, likely without predisposition to this familial fatal prion-related neurodegenerative disorder. ADO, allele dropout, refers to the inability to detect an allele during polymerase chain reaction (PCR) through amplification of linked markers; FA, failed amplification, the inability to amplify the gene of interest via PCR; PB1, the first polar body, extruded from the mature oocyte and the outcome of meiosis I, containing 2 copies of maternal DNA; PB2, the second polar body, extruded following fertilization of the oocyte and the outcome of meiosis II, containing 1 copy of maternal DNA.

Other forms of genetic prion disease and other inherited neurologic disorders are also candidates for PGD.[5,6] For example, guidelines from professional reproductive societies have been created for PGD in Huntington disease,[5] and similar guidelines for other neurologic conditions may be forthcoming.

In summary, PGD can serve as a viable reproductive option for patients faced with genetic prion disorders, such as GSS, and may affect their inclinations for predictive testing and consideration of nondisclosure PGD. Clinicians should discuss PGD as an option with patients genetically predisposed to prion disease.

ARTICLE INFORMATION

Accepted for Publication: November 13, 2013.

Published Online: February 3, 2014. doi:10.1001/jamaneurol.2013.5884.

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Author Contributions: Dr Tur-Kaspa had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Uflacker, Doraiswamy, Rechitsky, Geschwind, Tur-Kaspa.
Acquisition of data: Rechitsky, See, Geschwind, Tur-Kaspa.
Analysis and interpretation of data: Uflacker, Doraiswamy, Rechitsky, Geschwind, Tur-Kaspa.
Drafting of the manuscript: Uflacker, Doraiswamy, Tur-Kaspa.
Critical revision of the manuscript for important intellectual content: Uflacker, Doraiswamy, Rechitsky, See, Geschwind, Tur-Kaspa.
Obtained funding: Doraiswamy, Rechitsky, Geschwind, Tur-Kaspa.
Administrative, technical, and material support: Doraiswamy, Geschwind.
Study supervision: Doraiswamy, Rechitsky, Geschwind, Tur-Kaspa.

Conflict of Interest Disclosures: Dr Doraiswamy has received research grants (through Duke University) from Elan, Avid, Lilly, Novartis, Neuronetrix, Medivation, Wyeth, Janssen, Pfizer, and National Institutes of Health (NIH) over the past 3 years. He has received advisory or speaking fees in the past from Accera, Avid, AstraZeneca, Abbvie, Baxter, Cognoptix, Lundbeck, Takeda, Piramal, Genomind, Sonexa, Shire, Targacept, Grifols, Neuronetrix, TauRx, Medivation, Danone, Neurocog Trials, Alzheimer's Association, Alzheimer's Foundation, University of California, National University of Singapore, and University of Copenhagen. He owns shares in Maxwell Health, Sonexa, Clarimedix, and Adverse Events Inc, whose products are not discussed here. Dr Rechitsky is employed by the Reproductive Genetic Institute, where the PGD procedure was performed. Dr Tur-Kaspa is employed by the Institute for Human Reproduction, where the IVF was performed. No other disclosures were reported.

Funding/Support: Drs Uflacker and Doraiswamy did not receive any specific external support for this work. Drs Rechitsky and Tur-Kaspa were supported by the Reproductive Genetics Institute and the Institute for Human Reproduction, Chicago, Illinois, respectively. Dr Geschwind was supported in part by National Institute on Aging/National Institutes of Health (NIH) grants R01 AG031189 and P50 AG023501; NIH/National Center for Research Resources, University of California San Francisco Clinical and Translational Science Institute grant UL1 RR024131; The Michael J. Homer Family Fund; and Alzheimer's Disease Research Center of California grant 10-10030.

Role of the Sponsor: The supporting institutions had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We are grateful to our patient for allowing her case to be presented in this publication.

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EDITORIAL

Presymptomatic and Preimplantation Genetic Diagnosis
Neurology, NextGenetics, and the Next Generation

Golder N. Wilson, MD, PhD

The well-documented article by Uflacker et al[1] shows the 2 sides of genetic progress with presymptomatic diagnosis[2] foretelling tragic prion disease[3,4] and preimplantation genetic diagnosis (PGD)[5,6] allowing selection of unaffected offspring. This 2-edged sword also cuts across modern genetic testing, in which the benefits of comprehensive screening by microarray analysis[7] and rapid NextGen sequencing[8] are tempered by high costs, unequal access, and the uncertain consequences of nucleotide change. One major challenge is to distinguish disease-causing (pathogenic) mutations from benign variations (polymorphisms), an issue not faced by the patient of Uflacker et al,[1] since her prion protein *PRNP* F198S amino acid substitution (phenylalanine to serine at position 198) had been observed in other patients with spongiform encephalopathy (SE).[3,4] Applicable to their report[1] is the even greater challenge for gene test interpretation posed by multifactorial determination (interaction of multiple genes plus environment to cause disease), the usual mechanism for common diseases like epilepsy[9] but also operative when identical single-gene mutations cause variable outcomes within and among families.

Related article

Multifactorial determination of prion diseases includes environmental influences shown by the scrapie of hamsters or sheep, atypical Creutzfeldt-Jakob disease (CJD) from ingesting meat from cows with bovine SE, and kuru from cannibalism in New Guinea.[3,4,10-12] Hereditary diseases account for 15% of cases and include Gerstmann-Sträussler-Scheinker disease (GSS) (OMIM 137440), fatal familial insomnia (FFI) (OMIM 600072), familial CJD (OMIM 123400), Huntington disease-like 1 (OMIM 603218), and prion disease with protracted course (OMIM 606688).[10] Striking variable expression of prion disease is exemplified by individuals with the same *PRNP* D117V alteration (alanine to valine) that manifested variously as GSS, Alzheimer disease, multiple sclerosis, or mental illness.[11] The variable outcomes go beyond misdiagnosis: the effect of a pathogenic *PRNP* D178N mutation (aspartic acid to asparagine) is determined by the status of a *PRNP* V129M polymorphism (valine to methionine): FFI results when the mutation is coupled with *PRNP* 129M; and CJD results when the mutation is coupled with *PRNP* 129V.[12] The 129M form is present in all humans who contract mad cow disease (bovine SE).

Multifactorial determination of SE fits with the role of PRNP as part of a signal transduction pathway critical for neurogenesis. A membrane-bound glycoprotein, PRNP activates a specific tyrosine kinase when key domains are in α-helix conformation but not when altered to their pathogenic β-helical form. Heritable forms of SE encode the altered PRNP, while transmissible forms incorporate it from the environment, each further modified by *PRNP* gene polymorphism and no doubt other variations in the signal cascade. The patient of Uflacker et al[1] was heterozygous for this modifying polymorphism V129M with 129V within the same gene as the F198S mutation (in *cis*) and 129M on the other *PRNP* allele. Selecting embryos without the F198S mutation meant obligate presence of the SE-promoting 129M polymorphism that should remain silent in the absence of unhealthy dietary practices.

Since the late 1950s when particular chromosome changes were associated with conditions like Down syndrome, genetic diagnosis could bypass the intricacies of transcription, translation, and clinical expression to define disease as DNA change. DNA diagnosis expanded testing to all cells including amniocytes regardless of their protein expression. Chromosomal diseases could be diagnosed at 10 to 12 weeks of gestation by chorionic villus sampling (CVS) or at 14 to 16 weeks by amniocentesis, and soon altered ultrasonographic shapes were combined with maternal serum proteins (triple/quad screen) to modify fetal trisomy risks. Recombinant cloning techniques expanded prenatal DNA diagnosis to many mendelian disorders through nucleotide sequencing and polymerase chain reaction (PCR) amplification; advances ranged from detection of specific mutant alleles to scanning entire genomes for dose excess or deficiency using microarray analysis[7] or for protein-encoding (exonic) mutations in all ~23 000 genes using NextGen (exome) sequencing.[8] Present trisomy diagnosis using altered ratios of fetal cell-free DNA in maternal serum[13] will soon be extended to fetal genomic scanning, first for mutations targeted by family history and then as prenatal or newborn genetic screening. Exome sequencing for postnatal disease currently examines over 50 genes such as those for breast-ovarian cancer (*BRCA*), eliciting patient consent to report these findings incidental to the testing indication.[8] While study of fetal DNA in maternal blood or CVS/amniocentesis samples still forces the *Sophie's Choice* of accepting abnormality vs pregnancy termination, PGD chooses embryos before implantation, allowing, in the case of Uflacker et al,[1] a predestined mother to keep SE from her child.

First able to determine the sex of rabbit blastocysts in 1967, then female embryos destined to escape human X-linked disease, PGD gained momentum in the 1990s when PCR allowed detection of mutant alleles from single cells—a polar body within the oocyte or a blastomere from 30- to 60-cell embryos (blastocysts).[5,6] In vitro fertilization (IVF) to enhance conception for infertile couples was adapted to PGD using the same

techniques of gonadotropin-directed ovarian stimulation and fostering of ultrasonographically visualized follicles by human chorionic gonadotropin and progesterone. Harvested oocytes are now fertilized by more efficient intracytoplasmic sperm injection rather than IVF after oocytes are cleansed of cumulus cells—these and adhering sperm from IVF could contaminate embryos with maternal or paternal genotypes. Fertilization and cleavage are monitored daily with selected oocytes or embryos frozen until the status of its biopsied polar body or blastomere is determined by PCR-based DNA diagnosis (both polar body and blastomere DNA testing were conducted for the patient of Uflacker et al[1]). The diagnostic techniques are remarkably sensitive, with even microarray analysis giving results in 97% of embryos with a lower than 2% error rate.[6] Embryos with normal results are transferred to prepared women at day 3 to 5 after fertilization, and evidence so far suggests that embryo failures (20% do not survive freezing) rather than subsequent birth defects are the major adverse effects of this invasive technique (although mouse studies have observed weight gain and cognitive decline with brain changes similar to Alzheimer disease in embryo-biopsied offspring).[14]

Special diagnostic aspects of PGD are exemplified in the summary figure of Uflacker et al,[1] showing that flanking markers must be examined to be sure that one *PRNP* allele is not preferentially amplified by the many required PCR cycles (allele dropout). Ethical issues surrounding PGD include usual societal and religious objections to artificial reproductive technology and abortion, with access to care very pertinent (usual self-pay costs of $10 000-$20 000 for PGD), as it is to all DNA testing (targeted DNA analysis, ~$2000; microarray, $1800; and exome sequencing, ~$9000 compared with ~$450 for routine karyotype). A specific concern for neurologists is PGD for disorders presenting later in life,[2] and it is possible to avoid individual presymptomatic diagnosis but eliminate transmission of their potential disease by examining grandparental alleles. The larger concern, especially as exome screening detects multiple gene polymorphisms and mutations in parents and progeny, is to recognize that focus on one gene or disease outcome may not prevent others, just as selection of a normal *PRNP* allele brought with it a polymorphism causing increased susceptibility to bovine SE.[1] Multifactorial outcomes and the genomic instability[15] that guarantees new mutation must be recognized so that future genetic technology does not recapitulate the reductionist eugenics of the past.[16]

Will the prevalent model of laboratory to masters-level genetic counselor to patient be the NextGenetics of neurology? Futurists envision a "Doctor Algorithm" that replaces physicians entirely,[17] but perhaps the future neurologist, uniquely familiar with molecules and magnetic resonance images, can offer a different sequence to their presymptomatic patients: first, establish rapport with age-old assessments of sense and sensibility; then reassure of present normality and of presence when the DNA-augured storm begins; and finally, heal by validating the humanity that transcends any single gene.

I hope that neurologists will realize, like the Bradbury travelers who find their elusive Martians in a reflecting pool, that they are the NextGeneticists, necessary to peruse, predict, and astutely prevent the multifaceted sequences of neurological disease.

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Published Online: February 3, 2014. doi:10.1001/jamaneurol.2013.5834.

Conflict of Interest Disclosures: None reported.

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