Preimplantation Diagnosis for Early-Onset Alzheimer Disease Caused by V717L Mutation

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According to the most recent review, preimplantation genetic diagnosis (PGD) has been applied to at least 50 different genetic conditions in more than 3000 clinical cycles. In addition to traditional indications, similar to those in prenatal diagnosis, PGD was performed for an increasing number of new indications, such as late-onset disorders with genetic predisposition and HLA testing combined with PGD for pre-existing single-gene disorders. These conditions have never been an indication for prenatal diagnosis because of potential pregnancy termination, which is highly controversial if performed for genetic predisposition alone. With the introduction of PGD, it has become possible to avoid the transfer of the embryos carrying the genes that predispose a person to common disorders, thereby establishing only potentially healthy pregnancies and overcoming important ethical issues in connection with selective abortions.

To our knowledge, this article presents the first experience of PGD for early-onset Alzheimer disease (AD), representing a rare autosomal dominant familial predisposition to the presenile form of dementia. Three different genes have been found to be involved in this form of AD, including presenilin 1 located on chromosome 14, presenilin 2 on chromosome 1, and amyloid precursor protein (APP) on chromosome 21, which is well known for its role in the formation of amyloid deposits found in the characteristic plaques of patients with AD. The early-onset dementias associated with APP mutations are nearly completely penetrant and, therefore, are potential candidates for not only predictive testing but also PGD. Of the 10 APP mutations currently described, mutations in exons 16 and 17 have been...
reported in the familial cases with the earliest onset. One of these mutations, with onset as early as the mid or late 30s, is due to a single G-to-C nucleotide substitution in exon 17, resulting in a valine-to-leucine amino acid change at codon 717 (V717L). This mutation was identified in 3 of 5 family members (siblings) tested, 1 of whom presented for PGD.

METHODS

The patient who presented for PGD was a 30-year-old woman with no signs of AD who carried the V717L mutation. The patient had been tested because her sister developed symptoms of AD at age 38 years and was found to be carrying this mutation. This sister is still alive, but her cognitive problems progressed to the point where she was placed in an assisted living facility. The patient's father had died at age 42 years and had a history of psychological difficulties and marked memory problems. The V717L mutation was also detected in one of her brothers, who experienced mild short-term memory problems as early as age 35 years, with a moderate decline in memory, new learning, and sequential tracking in the next 2 to 3 years. Other family members, including 1 brother and 2 sisters, were asymptomatic, although predictive testing was done only in the sisters, who appeared to be free of the APP gene mutation.

Two PGD cycles were performed, involving 2 standard in vitro fertilization cycles, coupled with micromanipulation procedures, including removal of polar body 1 (PBI) and polar body 2 (PB2) and intracytoplasmic sperm injection, for which the patient gave informed consent. The study was approved by the institutional review board of the Illinois Masonic Medical Center, Chicago. Testing for the maternal mutation was done by DNA analysis of PBI and PB2, which were removed sequentially following maturation and fertilization of oocytes. A multiplex nested polymerase chain reaction (PCR) was performed, involving the mutation testing simultaneously with the linked polymorphic marker, representing the short tandem repeat in intron 1 ([GA]n . . . [GT]n).10

The first-round amplification cocktail for the multiplex nested PCR system contained outer primers for both the APP gene and linked marker, whereas the second-round PCR used inner primers for each gene. We designed the outer primers APP-1 (5’-GTTGCTTTGGCAGAGATG-3’) and APP-102 (5’-CATGGAAGCACACTGATT-3’) for performing the first-round amplification and the inner primers APP-101 (5’-GTTCACAAAAAGTGCAATC-3’) and APP-103 (5’-TCTTACACAAGTCAATC-3’) for the second round of PCR. As shown in Figure 2, second-round PCR produces a 115-base pair (bp) product, undigested by MnlI restriction enzyme, corresponding to the normal allele, and 2 restriction fragments of 72 and 43 bp, corresponding to the mutant allele. There was also an invariant fragment of 84 bp produced in both normal and mutant alleles, which was used as a control.

To perform nested PCR for specific amplification of the linked marker (GA)n . . . (GT)n in intron 1, we designed the outer primers In1-1 (5’-CCTTATTTCAATCCCCTAC-3’) and In1-2 (5’-GATTGGAGTTAAGTCTTG-3’) for the first round and the inner primers In1-3 (5’-CAGCATCGTCACTCAAG-3’) and In1-4 (5’-AATATTGTCTACATTCTC-3’) for the second round of amplification. The haplotype analysis, based on the PB genotyping, demonstrated that the affected allele was linked to the 10 and the normal one to the 6 repeats.

The patient was counseled and gave consent for unaffected embryos that resulted from oocytes determined to be mutation-free, based on both mutation and short tandem repeat analysis, to be preselected for transfer back to her and those predicted to be mutant to be exposed to the confirmatory analysis using the genomic DNA from these embryos to evaluate the accuracy of the single cell–based PGD. (We did not counsel the patient about her decision to undergo the PGD testing itself.) The patient was also informed about the expected number of embryos to be transferred to achieve a pregnancy and the risks of multiple gestation, the misdiagnosis rates depending on the availability of the marker information in addition to mutation analysis, and the need for confirmation of PGD by prenatal diagnosis.

RESULTS

In the first in vitro fertilization cycle, 8 oocytes were available for testing, of which 2 were tested by both PBI and PB2; both were affected. In the second in vitro fertilization cycle, 15 oocytes were available for testing, of which 13 were tested by both PBI and PB2. The mutation and linked marker analysis in intron 1 revealed 6 normal and 7 affected oocytes. The results of the second cycle, resulting in the embryo transfer, are presented in Figure 2. As shown...
in this figure, oocytes 4, 9, 14, and 15 were clearly normal because both mutant and normal genes were present in their PB1, with the mutant gene further extruded with the corresponding PB2, leaving only the normal gene in the resulting oocyte. In addition, oocytes 3 and 13 were also normal because their corresponding PB1s were homozygous mutant, suggesting that the resulting oocytes should have been normal, as further confirmed by the presence of the normal gene in the extruded PB2s, also in agreement with the linked markers analysis. However, because only 1 linked marker was available for testing, a .05 probability of allele dropout of the normal gene in the corresponding PB1 could not be excluded, as established in our previous observations.11

The remaining oocytes were predicted to be mutant, based on heterozygous PB1 and normal PB2 in 4 of them (oocytes 1, 8, 10, and 11; the heterozygous status of PB1 in oocyte 10 was based on the presence of markers linked to both normal and mutant alleles, which is not shown in Figure 2) and homozygous normal PB1 and mutant PB2 in 3 (oocytes 2, 6, and 7). The follow-up study of the embryos that resulted from these oocytes confirmed their affected status in all but 1 (oocyte 7). The latter may be explained by allele dropout of the mutant allele in the apparently heterozygous PB1, which was left undetected because of the amplification failure of the linked marker in this case.

To exclude any probability of misdiagnosis, the priority in the embryo transfer was given to 4 of the 6 normal embryos, resulting from the oocytes with heterogeneous PB2 and mutant PB2. However, only 3 of these embryos developed into the cleavage stage and

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**Figure 2. Preimplantation Diagnosis for V717L Mutation in the Amyloid Precursor Protein (APP) Gene**

**A.** Map of human APP gene, showing sites and location of V717L G-to-C mutation and polymorphic markers. **B.** Restriction map for normal and abnormal alleles. **C.** Polyacrylamide gel analysis of MnlI restriction digestion, showing 6 unaffected (N) oocytes (3, 4, 9, 13, 14, and 15) and 7 mutant (M) oocytes (1, 2, 6, 7, 8, 10, and 11). Unaffected oocytes are identified by the gel showing normal (top band) and affected (lowest 2 bands) genes in PB1. After extrusion of the affected genes in PB2, only normal genes remain. Four of these oocytes (4, 9, 14, and 15) had homozygous PB1 and mutant PB2, and only 2 (3, 13) had homozygous mutant PB1 and normal PB2, leaving a .05 probability for misdiagnosis, noted as N*. Oocyte 10 had heterozygous PB1 detected by marker analysis (not shown).

**ET** indicates embryo transfer; **L,** ladder (size standard); **bp,** base pair; **PB,** polar body; and **uncut,** the undigested polymerase chain reaction product. Arrows indicate fully nested primer sets. A, Map of human APP gene, showing sites and location of V717L G-to-C mutation and polymorphic markers. B, Restriction map for normal and abnormal alleles. C, Polyacrylamide gel analysis of MnlI restriction digestion, showing 6 unaffected (N) oocytes (3, 4, 9, 13, 14, and 15) and 7 mutant (M) oocytes (1, 2, 6, 7, 8, 10, and 11). Unaffected oocytes are identified by the gel showing normal (top band) and affected (lowest 2 bands) genes in PB1. After extrusion of the affected genes in PB2, only normal genes remain. Four of these oocytes (4, 9, 14, and 15) had homozygous PB1 and mutant PB2, and only 2 (3, 13) had homozygous mutant PB1 and normal PB2, leaving a .05 probability for misdiagnosis, noted as N*. Oocyte 10 had heterozygous PB1 detected by marker analysis (not shown).
could be transferred (4, 14, and 15), so an additional embryo (3) was preselected, originating from the oocyte with homozygous mutant PB1 and the normal PB2, since these results were also confirmed by the linked marker analysis. These 4 embryos were transferred back to the patient, yielding a singleton clinical pregnancy, confirmed to be unaffected by chorionic villus sampling and birth of a mutation-free child confirmed after birth by a blood test.

**COMMENT**

The results presented herein demonstrate the feasibility of PGD for early-onset AD, providing a nontraditional option for patients who wish to avoid the transmission of the mutant gene that predisposes their potential children to early-onset AD. For some patients, this may be the only reason for undertaking pregnancy, since the pregnancy may be free of an inherited predisposition to AD from the onset. Because the disease never presents at birth or early childhood and even later may not be expressed in 100% of cases, the application of PGD for AD is still controversial. However, because there is currently no treatment for AD, which may arise despite presymptomatic diagnosis and follow-up, PGD seems to be the only relief for at-risk couples, such as the presented case and the previously reported cases of PGD for p53 tumor suppressor gene mutations. 2

Therefore, prospective parents who are determined by strong genetic predisposition to be at risk for producing progeny with severe disorders should be informed about this emerging technology so they can make a choice about reproduction. 12, 13 This seems to be ethically more acceptable than suppressing information on the availability of PGD. Despite raising important ethical issues, 14, 15 the results presented herein, together with previously described cases of PGD for late-onset disorders with genetic predisposition and HLA typing, demonstrate the extended practical implications of PGD, such as providing prospective couples at genetic risk with more reproductive options for having unaffected children.

**REFERENCES**


