USE OF RFLPs For Detecting SNPs & Genes

"The Old Fashioned Way"

SNP that Generates An RFLP

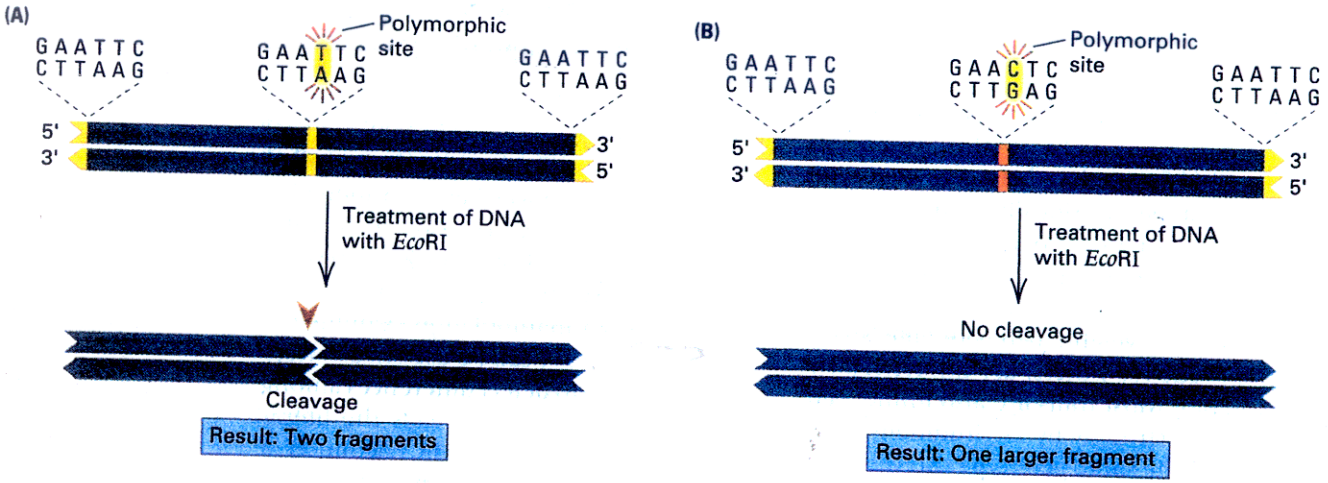
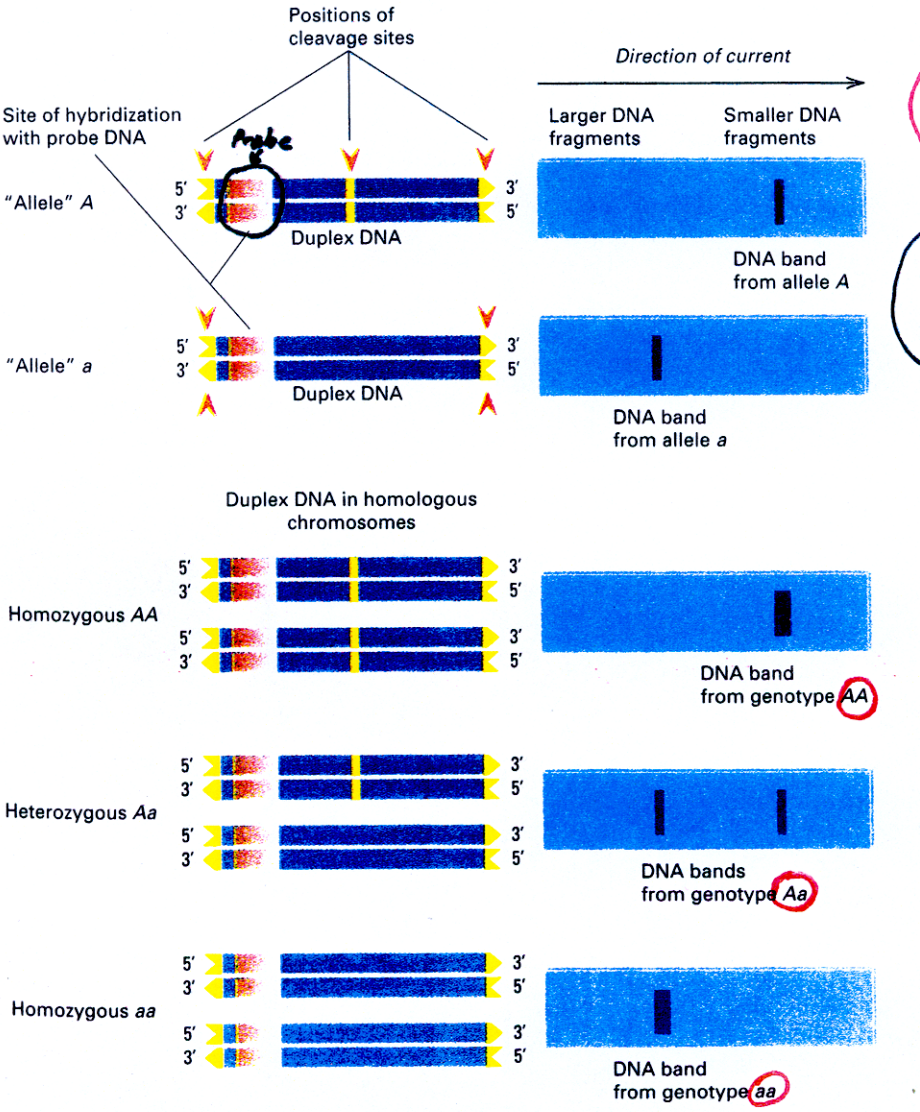


Figure 2.23 A minor difference in the DNA sequence of two molecules can be detected if the difference eliminates a restriction site. (A) This molecule contains three restriction sites for *Eco*RI, including one at each end. It is cleaved into two fragments by the enzyme. (B) This molecule has an altered *Eco*RI site in the middle, in which 5'-GAATTC-3' becomes 5'-GAACTC-3'. The altered site cannot be cleaved by *Eco*RI, so treatment of this molecule with *Eco*RI results in one larger fragment.

RFLPs/SNPs CAN BE USE TO detect Allelic Variability at a Locus

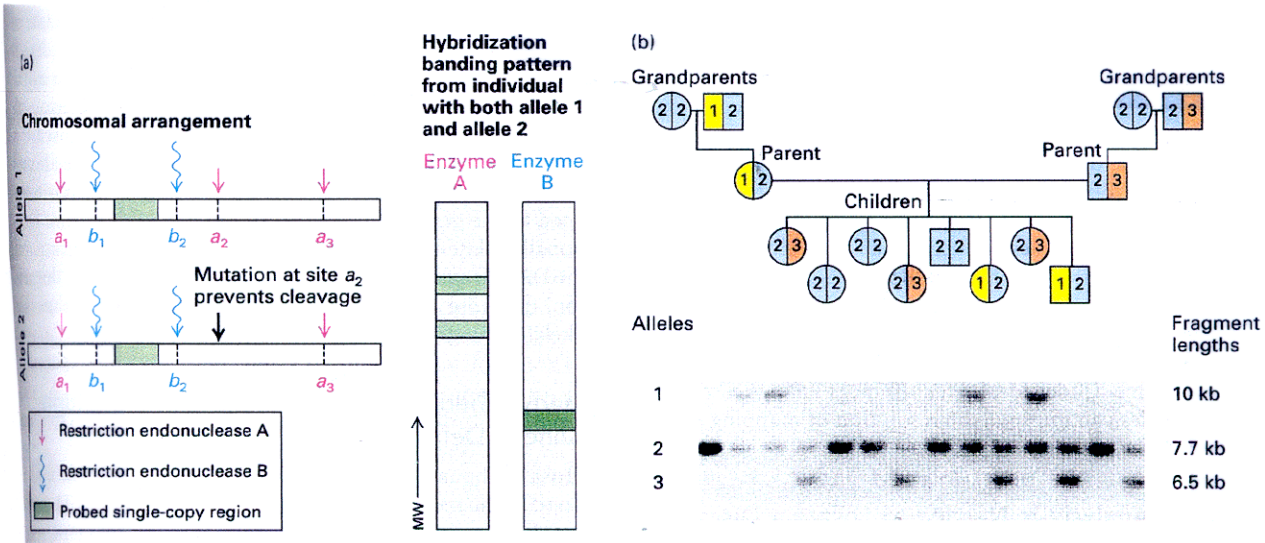


Probe Specific for left half of DNA only
∴ 1 band !!!
Pattern After Blotting

Figure 2.24 In a restriction fragment length polymorphism (RFLP), alleles may differ in the presence or absence of a cleavage site in the DNA. In this example, the *a* allele lacks a restriction site that is present in the DNA of the *A* allele. The difference in fragment length can be detected by Southern blotting. RFLP alleles are codominant, which means (as shown at the bottom) that DNA from the heterozygous *Aa* genotype yields each of the single bands observed in DNA from homozygous *AA* and *aa* genotypes.

As well as Genotypes

RFLPs CAN BE USED TO IDENTIFY Individuals



EXPERIMENTAL FIGURE 9-46 Restriction fragment length polymorphisms (RFLPs) can be followed like genetic markers. (a) In the example shown, DNA from an individual is treated with two different restriction enzymes (A and B), which cut DNA at different sequences (*a* and *b*). The resulting fragments are subjected to Southern blot analysis (see Figure 9-26) with a radioactive probe that binds to the indicated DNA region (green) to detect the fragments. Since no differences between the two homologous chromosomes occur in the sequences recognized by the B enzyme, only one fragment is recognized by the probe, as indicated by a single hybridization band. However, treatment with enzyme A produces fragments of two different lengths (two bands are seen), indicating that a mutation has caused the loss of one of the *a* sites in one of the two chromosomes. (b) Pedigree based on RFLP analysis of the DNA from a region known to be present on chromosome 5. The DNA samples were cut with the restriction enzyme *Taq*I and analyzed by Southern blotting. In this family, this region of the genome exists in three allelic forms characterized by *Taq*I sites spaced 10, 7.7, or 6.5 kb apart. Each individual has two alleles; some contain allele 2 (7.7 kb) on both chromosomes, and others are heterozygous at this site. Circles indicate females; squares indicate males. The gel lanes are aligned below the corresponding subjects. [After H. Donis-Keller et al., 1987, *Cell* **51**:319.]

RFLPs CAN BE USED AS Markers FOR disease Genes if Linked to the Mutant Allele

RFLPs are caused by SNPs!

Molecular Diagnosis of Human Diseases

Sickle Cell Anemia

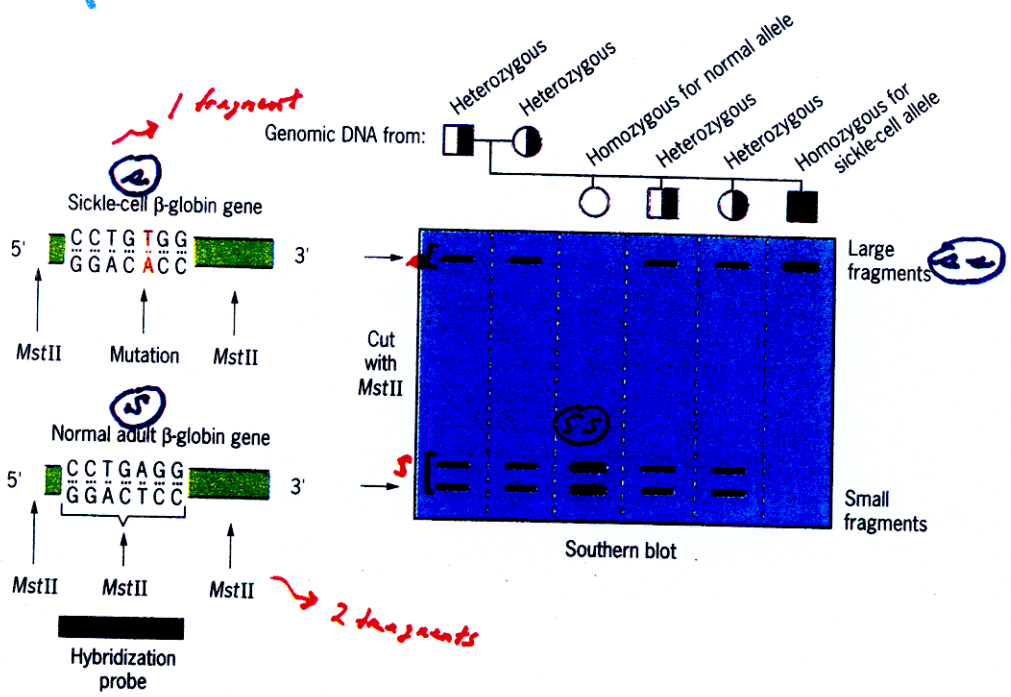


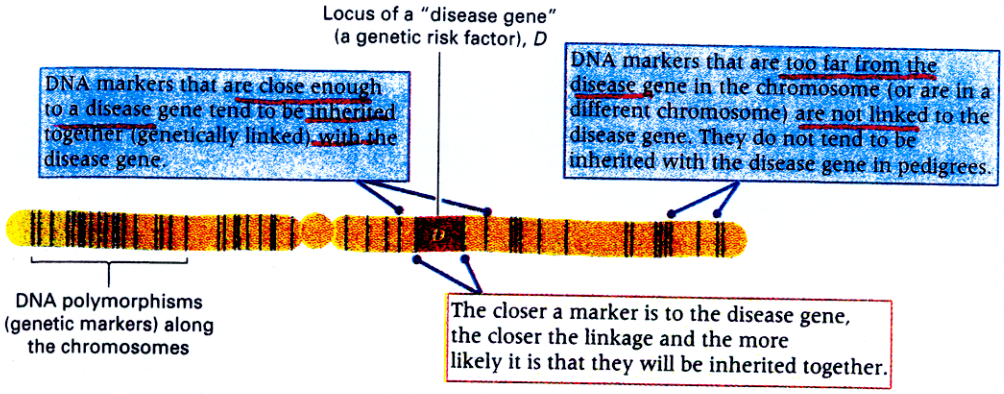
Figure 22.7 Detection of the sickle-cell hemoglobin mutation by Southern blot analysis of genomic DNAs cut with restriction enzyme *Mst*II.

Marker is IN Gene! ∴ always linked with Phenotype!

∴ 1 fragment at this locus is a marker for the normal s allele.

Markers Tightly Linked to Disease Gene CAN BE USED TO IDENTIFY what the Disease Gene is

Positional Cloning in absence of known Protein / Gene Product



How Find Gene?

Figure 2.29 Concepts in genetic localization of genetic risk factors for disease. Polymorphic DNA markers (indicated by the vertical lines) that are close to a genetic risk factor (*D*) in the chromosome tend to be inherited together with the disease itself. The genomic location of the risk factor is determined by examining the known genomic locations of the DNA polymorphisms that are linked with it.

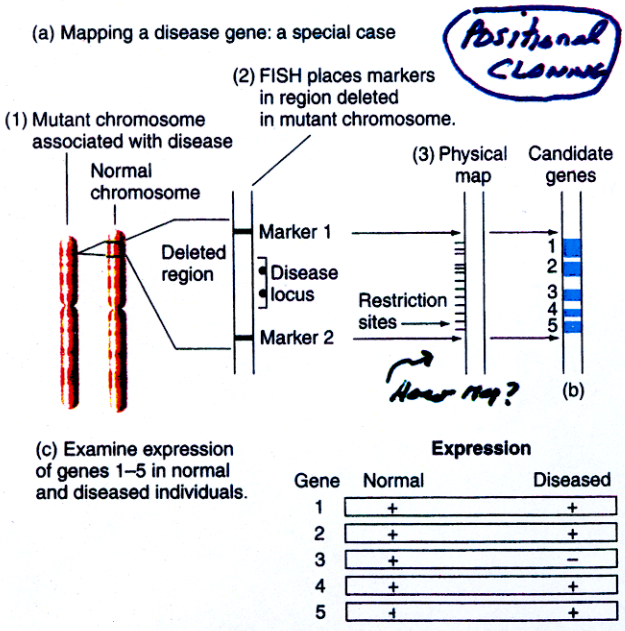


Figure 10.11 Positional cloning: From phenotype to gene. (a) Correlating the expression of a phenotype with one small segment of the genome. (1) Some diseases, such as Duchenne muscular dystrophy, are caused by a deletion. It is sometimes possible to observe directly the absence or shortening of a band in a chromosome from an affected individual as compared to the same chromosome from a healthy individual. Even when it is not possible to observe the deletion directly, the FISH protocol can detect it. (2) A marker in the deleted region will hybridize to the chromosome from the healthy individual, but not to the same chromosome from the diseased individual. Markers associated with the disease can be used in linkage analyses of families carrying mutant disease alleles that are not deletions. (3) When linkage analysis shows that a specific chromosomal region contains the disease locus, researchers can subject the marked region to physical analysis. (b) Investigators next analyze the region between recombination sites that define the smallest area within which the disease locus can lie for the presence of candidate genes (as described later in this chapter). (c) They then compare the structure and expression of each candidate gene in many diseased and nondiseased individuals. A correlation between a mutant structure or expression for a particular candidate gene and the disease phenotype can provide evidence that a particular gene is responsible for the disease phenotype. Proof of the association, however, requires further functional studies, which we describe later in this chapter.

USING LINKED MARKERS TO IDENTIFY A DISEASE GENE

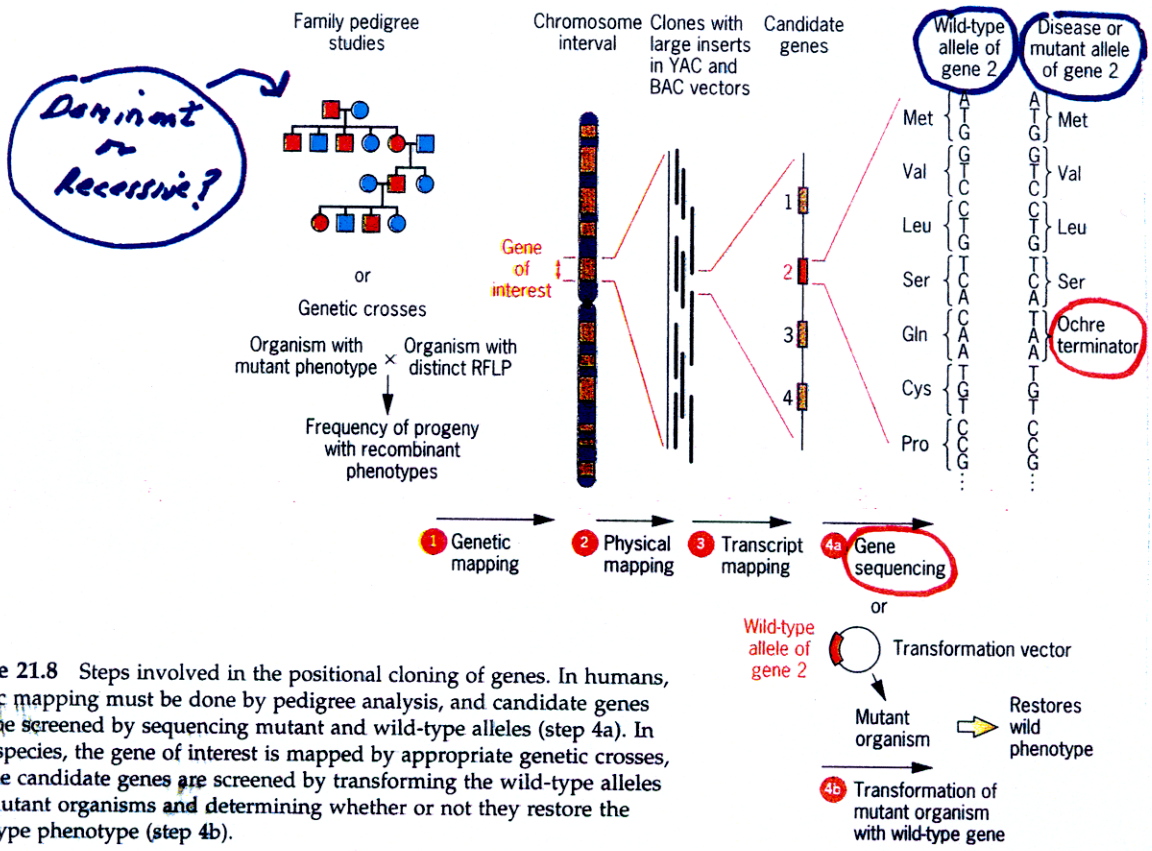


Figure 21.8 Steps involved in the positional cloning of genes. In humans, genetic mapping must be done by pedigree analysis, and candidate genes must be screened by sequencing mutant and wild-type alleles (step 4a). In other species, the gene of interest is mapped by appropriate genetic crosses, and the candidate genes are screened by transforming the wild-type alleles into mutant organisms and determining whether or not they restore the wild-type phenotype (step 4b).

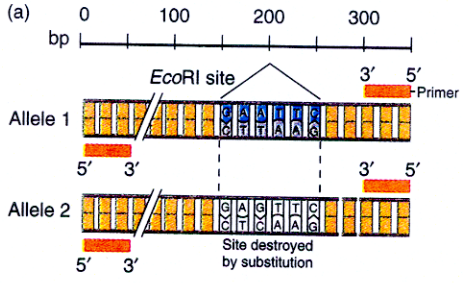
How Find the Gene Responsible for the Disease?

RFLPs CAN BE detected USING PCR

SNPs cause RFLPs!

NO NEED FOR BLOT!
ONLY NEED TINY AMOUNT OF DNA

1. AMPLIFY Gene Region Containing the Polymorphism
2. Digest with Relevant Restriction Enzyme
3. Visualize fragments directly on Gel!



why different polarities? for primers?

→ 2 fragments (A2)

→ 1 fragment (A1)

PCR → Then Restriction Enzyme Digestion

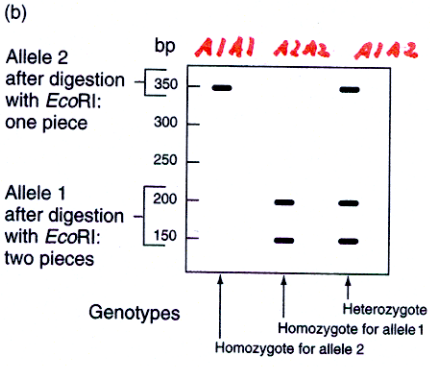


Figure 9.7 Restriction site polymorphisms can be detected most efficiently with PCR-based protocols. (a) PCR amplification of two alleles of a DNA locus with a restriction site polymorphism. Allele 1 has an *Eco*RI site that is eliminated in allele 2. The PCR products amplified from both alleles are identical in size. (b) Exposure of these PCR products to *Eco*RI causes cleavage of the allele 1 product but not the allele 2 product. Gel electrophoresis and ethidium bromide staining distinguish the three genotypes possible with the two alleles at this locus.

USING RFLPs TO detect a mutant factor VIII Gene

A PCR Approach

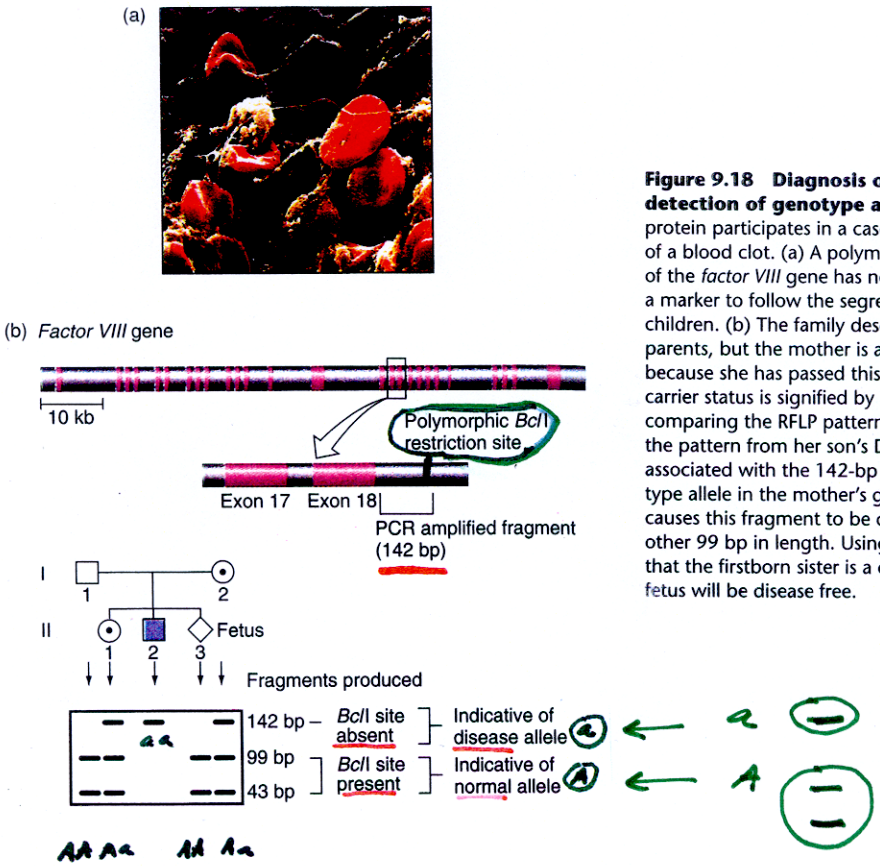
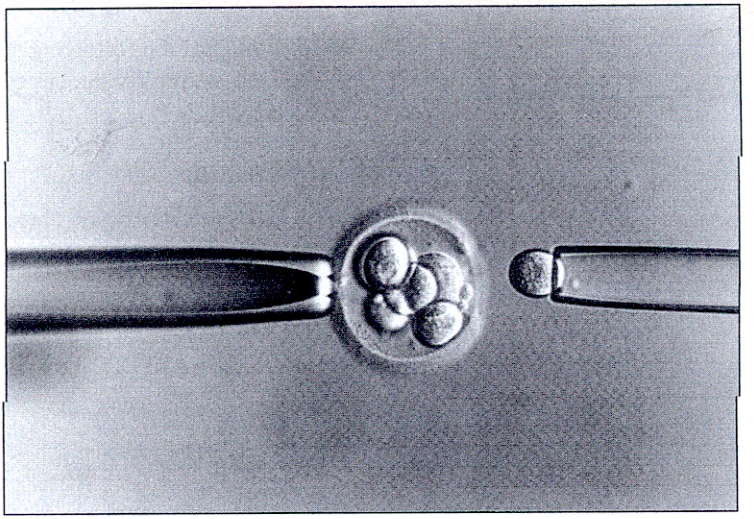


Figure 9.18 Diagnosis of hemophilia through the indirect detection of genotype at the factor VIII locus. The factor VIII protein participates in a cascade of reactions that result in formation of a blood clot. (a) A polymorphic *BclI* restriction site within intron 18 of the *factor VIII* gene has no effect on gene function but can provide a marker to follow the segregation of the gene from parents to children. (b) The family described by the pedigree has two healthy parents, but the mother is an obligate carrier of the disease mutation because she has passed this X-linked disease on to her son; her carrier status is signified by a circle with a dot in the middle. By comparing the RFLP pattern obtained from the mother's DNA with the pattern from her son's DNA, you can see that the disease allele is associated with the 142-bp *BclI* restriction fragment, and the wild-type allele in the mother's genome contains a *BclI* restriction site that causes this fragment to be cut into two pieces, one 43 bp and the other 99 bp in length. Using this information, you can determine that the firstborn sister is a carrier like her mother, while the male fetus will be disease free.

THE DIRECT DETECTION OF GENOTYPE

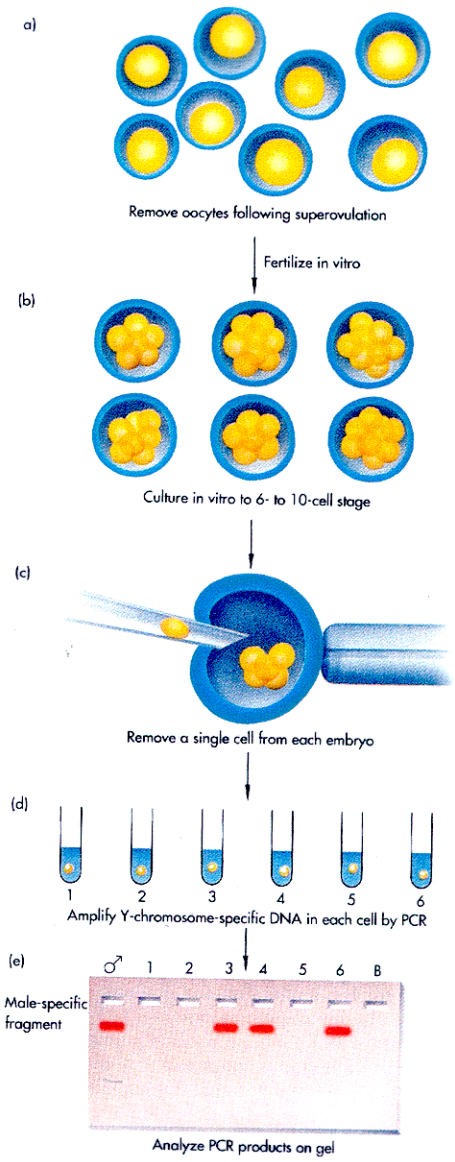


Plucking one cell from an 8-cell stage human embryo for direct determination of genotype.

PCR CAN BE USED TO DETECT DISEASE GENES PRIOR TO IMPLANTATION

PREIMPLANTATION Genetic DIAGNOSIS

PGD



..... IN ADDITION TO AMNIOCENTESIS & Chorionic Villi Sampling

Figure 10.12
Chorionic villus sampling, a procedure used for early prenatal diagnosis of genetic defects.

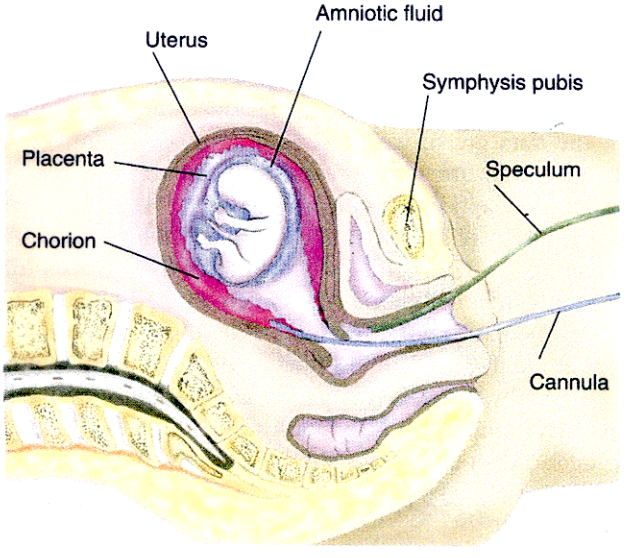
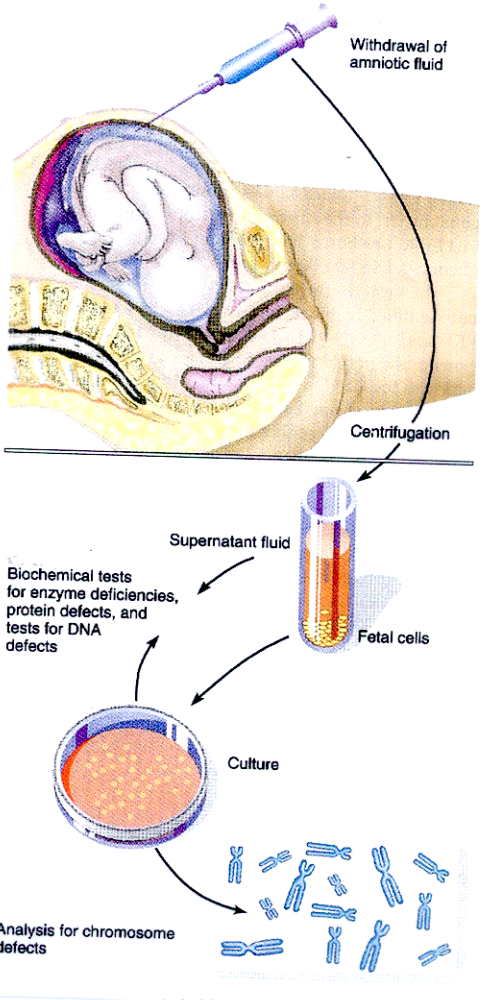


Figure 10.11
Amniocentesis, a procedure used for prenatal diagnosis of genetic defects.



DNA → Genotype

PRE-IMPLANTATION GENETIC TESTING

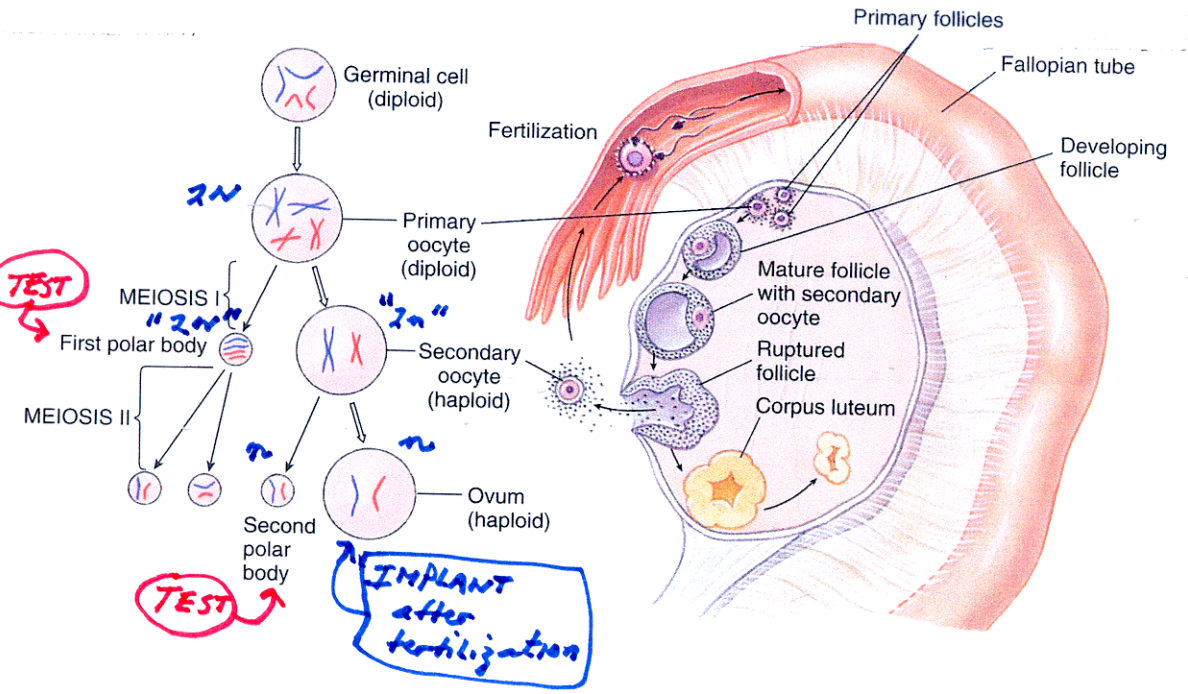


FIGURE 59.19
The meiotic events of oogenesis in humans. A primary oocyte is diploid. At the completion of the first meiotic division, one division product is eliminated as a polar body, while the other, the secondary oocyte, is released during ovulation. The secondary oocyte does not complete the second meiotic division until after fertilization; that division yields a second polar body and a single haploid egg, or ovum. Fusion of the haploid egg with a haploid sperm during fertilization produces a diploid zygote.

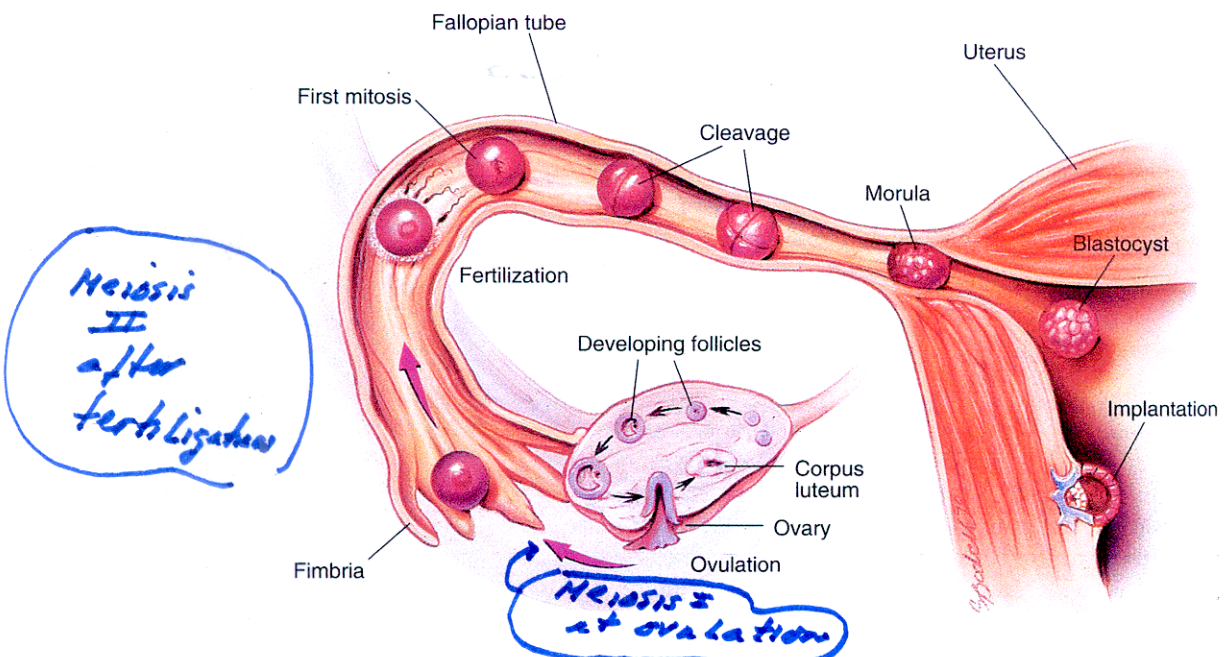


FIGURE 59.20
The journey of an egg. Produced within a follicle and released at ovulation, an egg is swept into a fallopian tube and carried along by waves of ciliary motion in the tube walls. Sperm journeying upward from the vagina fertilize the egg within the fallopian tube. The resulting zygote undergoes several mitotic divisions while still in the tube, so that by the time it enters the uterus, it is a hollow sphere of cells called a blastocyst. The blastocyst implants within the wall of the uterus, where it continues its development. (The egg and its subsequent stages have been enlarged for clarification.)

RFLPs can also be used to detect disease genes in embryo cells

www.nytimes.com
The New York Times ON THE WEB

February 27, 2002

Baby Spared Mother's Fate by Genetic Tests as Embryo

By DENISE GRADY

A 30-year-old woman who is very likely to develop a rare form of Alzheimer's disease before she turns 40 has had a baby girl who will be spared that fate because she was genetically screened as an embryo before being implanted in her mother's womb, doctors are reporting.

The case is a medical milestone, the first use of genetic testing to prevent an early onset form of Alzheimer's disease. But some find it ethically disturbing because within a few years the mother will probably become unable to take care of her daughter, who will witness her deterioration and death.

The 30-year-old woman, who carried a rare gene making it almost certain she will develop Alzheimer's, wanted to have a child but hoped to avoid passing on the bad gene. So she sought preimplantation diagnosis. In that procedure, embryos are created in the laboratory from the mother's eggs and the father's sperm and are tested genetically. Only healthy embryos are implanted in the mother's uterus.

The case is being described today in The Journal of the American Medical Association by Dr. Yury Verlinsky and his associates from two private clinics in Chicago.

In a commentary article accompanying Dr. Verlinsky's report, Dr. Roberta Springer Loewy and Dr. Dena Towner wrote that the mother "most likely will not be able to care for or even recognize her child in a few years." Dr. Towner is director of the prenatal diagnosis center at the University of California at Davis, and Dr. Loewy is a bioethicist there.

In an interview, Dr. Loewy said that it was laudable for the mother to try to protect her child from illness but that the child's physical health was not her only responsibility.

"I'm not trying to pounce on this poor woman," Dr. Loewy said, but she added that she thought it would be traumatic for the child to watch the mother's slow decline and that if this same woman "wanted to adopt, our society essentially holds the position that, gee, no, we wouldn't let her do this, wouldn't let her subject a child to this. But because it's coming from her own loins, this is something we shouldn't have a say about?"

r. William Thies, vice president of medical and scientific affairs for the Alzheimer's Association, said: "It's a discussion being ıeld in absence of any data. Does this end up being harmful to the child? Nobody knows."

Dr. Loewy also said that society as a whole should consider whether this kind of treatment was the best use of limited medical resources.

In a case like that of Dr. Verlinsky's patient, each effort to begin a pregnancy costs more than $12,000. Insurance coverage varies.

Dr. Verlinsky said he had no qualms about helping the woman and her husband. "It's totally up to the patient," he said.

As for the child's losing her mother, he said that many children were brought up by single parents, and that this family would be no different. Dr. Verlinsky said, too, that the couple had not made their decision in "a moment of emotion," but had had weeks and months to mull it over, because the medical procedures they went through in order to conceive take time.

Asked whether the couple would have gone ahead and had children anyway if preimplantation diagnosis was not available, Dr. Verlinsky said he did not know.

The couple were not available for interviews, he said.

The new report does not apply to most families with Alzheimer's disease, because the form the woman has accounts for less than 1 percent of all cases. The type of testing the woman had is not done for the more common types of Alzheimer's disease, which develop much later in life, and in which the role of particular genes is far less clear.

Dr. Thies said, "It's important for people to recognize that this does not represent a strategy that will have much of a public health impact."

The woman described by Dr. Verlinsky and his colleagues carries a rare genetic mutation that makes it almost certain she will develop Alzheimer's disease in her 30's. Her sister, who also has the mutation, developed Alzheimer's symptoms at age 38. The gene is involved in the formation of the tangled protein deposits, known as amyloid plaques, found in the brain in people with Alzheimer's disease. People who carry certain mutations in the gene nearly always develop dementia.

The sister with Alzheimer's declined so much mentally that she had to be moved to an assisted-living center. A brother also had the mutation and began suffering memory problems at 35. Their father died at 42, with psychological and memory problems.

People who do not want the expense and trouble of preimplantation diagnosis can become pregnant naturally and then have the ·tus tested via amniocentesis or another procedure, chorionic villus sampling. But if the fetus has a genetic disease, the parents .ıust decide whether to end the pregnancy. Preimplantation testing is particularly appealing to people who want to avoid abortion.

The technique has been used to prevent many diseases, including hemophilia, sickle cell anemia, muscular dystrophy, Tay-Sachs disease, cystic fibrosis and Huntington's disease. It should not be used just to pick embryos of a particular sex, according to an opinion issued this month by a professional group, the American Society for Reproductive Medicine.

http://www.nytimes.com/2002/02/27/health/27BABY.html?todaysheadlines=&pagewanted=print

♀ od → Alzheimer Gene

PRELIMINARY COMMUNICATION

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

Yury Verlinsky, PhD
Svetlana Rechitsky, PhD
Oleg Verlinsky, MS
Christina Masciangelo, MS
Kevin Lederer, MD
Anver Kuliev, MD, PhD

Context Indications for preimplantation genetic diagnosis (PGD) have recently been expanded to include disorders with genetic predisposition to allow only embryos free of predisposing genes to be preselected for transfer back to patients, with no potential for pregnancy termination.

Objective To perform PGD for early-onset Alzheimer disease (AD), determined by nearly completely penetrant autosomal dominant mutation in the amyloid precursor protein (*APP*) gene.

Design Analysis undertaken in 1999-2000 of DNA for the V717L mutation (valine to leucine substitution at codon 717) in the *APP* gene in the first and second polar bodies, obtained by sequential sampling of oocytes following in vitro fertilization, to preselect and transfer back to the patient only the embryos that resulted from mutation-free oocytes.

Setting An in vitro fertilization center in Chicago, Ill.

Patients A 30-year-old AD-asymptomatic woman with a V717L mutation that was identified by predictive testing of a family with a history of early-onset AD.

Main Outcome Measures Results of mutation analysis; pregnancy outcome.

Results Four of 15 embryos tested for maternal mutation in 2 PGD cycles, originating from V717L mutation–free oocytes, were preselected for embryo transfer, yielding a clinical pregnancy and birth of a healthy child free of predisposing gene mutation according to chorionic villus sampling and testing of the neonate's blood.

Conclusion This is the first known PGD procedure for inherited early-onset AD resulting in a clinical pregnancy and birth of a child free of inherited predisposition to early-onset AD.

JAMA. 2002;287:1018-1021 www.jama.com

According to the most recent review,[1] 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 preexisting single-gene disorders.[2,3] 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,[4] presenilin 2 on chromosome 1,[5] and amyloid precursor protein (*APP*) on chromosome 21,[6] 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

See also p 1038.

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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).[7] 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.[7] 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,[7] although predictive testing was done only in the sisters, who appeared to be free of the *APP* gene mutation (FIGURE 1).

Two PGD cycles were performed, involving 2 standard in vitro fertilization cycles, coupled with micromanipulation procedures, including removal of polar body 1 (PB1) 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 PB1 and PB2, which were removed sequentially following maturation and fertilization of oocytes.[8] A multiplex nested polymerase chain reaction (PCR) was performed,[9] involving the mutation testing simultaneously with the linked polymorphic marker, representing the short tandem repeat in intron 1 ($[GA]_n \ldots [GT]_n$).[10]

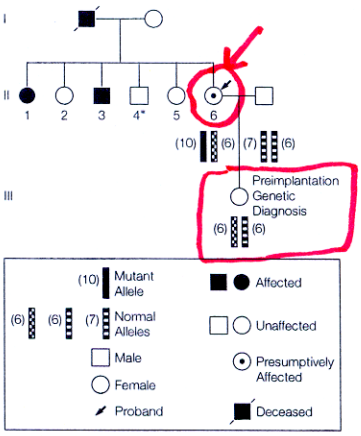
The first-round amplification cocktail for the multiplex nested PCR system contained outer primers for both the *APP* gene and linked marker, whereas the second-round PCR used inner primers for each gene. We designed the outer primers APP-1 (5′-GTGTTCTTTGCAGAAGATG-3′) and APP-102 (5′-CATGGAAGCACACTGATTC-3′) for performing the first-round amplification and the inner primers APP-101 (5′-GTTCAAACAAAGGTGCAATC-3′) and APP-103 (5′-TCTTAGCAAAAAGCTAAGCC-3′) for the second round of PCR. As shown in FIGURE 2, second-round PCR produces a 115–base pair (bp) product, undigested by *Mnl*I 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 $(GA)_n \ldots (GT)_n$ in intron 1, we designed the outer primers In1-1 (5′-CCTTATTTCAAATTCCCTAC-3′) and In1-2 (5′-GATTGGAGGTTAAGTTTCTG-3′) for the first round and the inner primers In1-3 (5′-CAGCATCTGTCACTCAAG-3′) and In1-4 (5′-AATATTTGTTACATTCCTCTC-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.

Why don't it?

Figure 1. Pedigree of Family With Early-Onset Alzheimer Disease (AD)



Numbers in parentheses indicate number of repeats. Preimplantation genetic diagnosis for asymptomatic carrier (II: 6) of the mutant gene linked to the 10 repeats (10) (normal gene [N] is linked to 6 repeats [6]), resulting in the birth of an unaffected child (III). Paternal genotype is also shown, with the normal alleles (N) linked to 6 repeats (6) and 7 repeats (7). Haplotype analysis shows that the child inherited normal maternal allele (N) linked to the 6 repeats (6). The patient's sister (II: 1), brother (II: 3), and father (I) were affected by early-onset AD. No predictive testing was performed in her asymptomatic brother, indicated by an asterisk.

RESULTS

In the first in vitro fertilization cycle, 8 oocytes were available for testing, of which 2 were tested by both PB1 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 PB1 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

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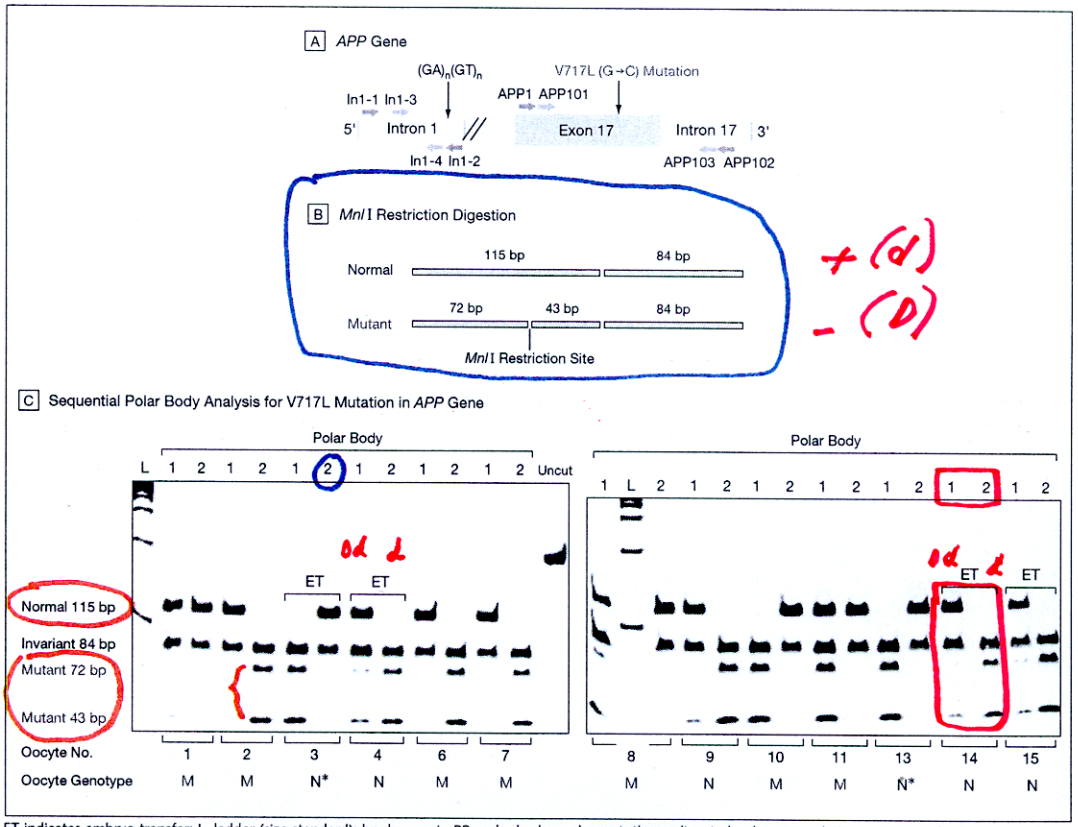
in this figure, oocytes 4, 9, 14, and 15 were clearly normal because both mutant and normal genes were present in their PB1, with the mutant gene further extruded with the corresponding PB2, leaving only the normal gene in the resulting oocyte. In addition, oocytes 3 and 13 were also normal because their 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 PBI 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 heterozygous PB2 and mutant PB2. However, only 3 of these embryos developed into the cleavage stage and

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



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 heterozygous 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).

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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.

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Drafting of the manuscript: Kuliev.
Critical revision of the manuscript for important intellectual content: Y. Verlinsky, Rechitsky, O. Verlinsky, Masciangelo, Lederer.
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Administrative, technical, or material support: O. Verlinsky, Masciangelo, Lederer.
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EMBRYO SPLITTING CAN LEAD TO QUADRUPLETS IN PRIMATES

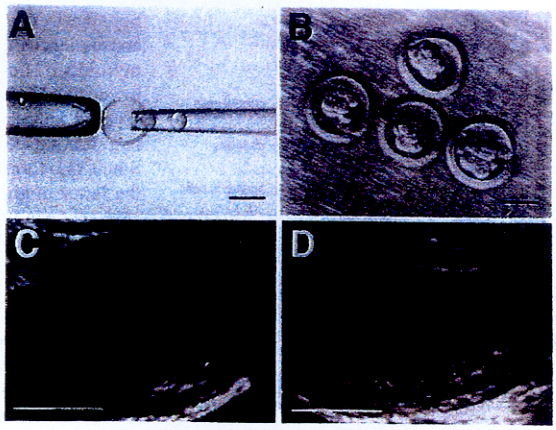

Fig. 1. Embryo splitting and development of nonhuman primates after embryo transfer. A zona-free eight-cell stage rhesus embryo, fertilized in vitro, is dissociated into eight individual blastomeres by mechanical disruption in Ca^{2+}- and Mg^{2+}-free medium. Two dissociated blastomeres are transferred into each of four empty zonae (**A**), thereby creating the four quadruplet embryos, each with two of the eight original cells (**B**). Split embryos are scored daily for development and structural normalcy, and embryos showing signs of compaction are selected for transfer 1 to 3 days after splitting. Endocrine profiles are traced daily and implantation is confirmed by ultrasound on day 31 after transfer. A miscarried pregnancy in which the fetus is absent though the placenta appears normal (**C**), and the quadruplet pregnancy with normal fetal development (**D**) that resulted in the birth of Tetra (Fig. 2) resulted from the transfer of two quadruplet embryos each to two surrogates. Bar in (A) and (B), 120 μm; in (C) and (D), 5 cm.

Fig. 2. Tetra, a nonhuman primate quadruplet cloned from an eight-cell embryo by splitting.

Humans? ASO/ABBI Test → good embryo → split into 3 (2 cells each)
implant 1 → daughter → 40 yo → implant a second (gives birth to her daughter/sister!! Twin!!!

USE OF ASOs or

Allele-Specific Oligonucleotides

to Detect SNPs and

Genes