Research letters

Prenatal diagnosis of spinal muscular atrophy by genetic analysis of circulating fetal cells

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Spinal muscular atrophy (SMA) has a prevalence of one in 6000 births and a one in 40 heterozygote frequency. We aimed to develop a routine test for non-invasive prenatal diagnosis. We tested blood with ISET (isolation by size of epithelial tumour or trophoblastic cells) in 12 pregnant women whose babies were at risk of SMA. Using genetic analysis of fetal cells, we identified SMA in all nine isolated from the three mothers carrying an affected child. There was no mutation in any of the 26 fetal cells isolated from the nine women with an unaffected child. Our results show that non-invasive detection of genetic diseases by the analysis of maternal blood is feasible.

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Spinal muscular atrophy (SMA), a muscle denervation of neurogenic origin, is the second most common fatal autosomal recessive disorder after cystic fibrosis, with a prevalence of 1 in 6000 births and a 1 in 40 heterozygote frequency.[1] SMA results from mutations in the survival motor neuron (*SMN*) gene, located on chromosome 5q13. A telomeric (*SMN1*) and a duplicated centromeric (*SMN2*) gene have been identified, which can be discriminated by single-base changes in exons 7 and 8.[1] Homozygous deletion of the *SMN1* gene is recorded in about 96% of patients with SMA, and 4% of cases are related to heterozygous *SMN1* deletion in combination with *SMN1* mutation (or conversion of *SMN1* sequence to *SMN2*) on the other allele.[1]

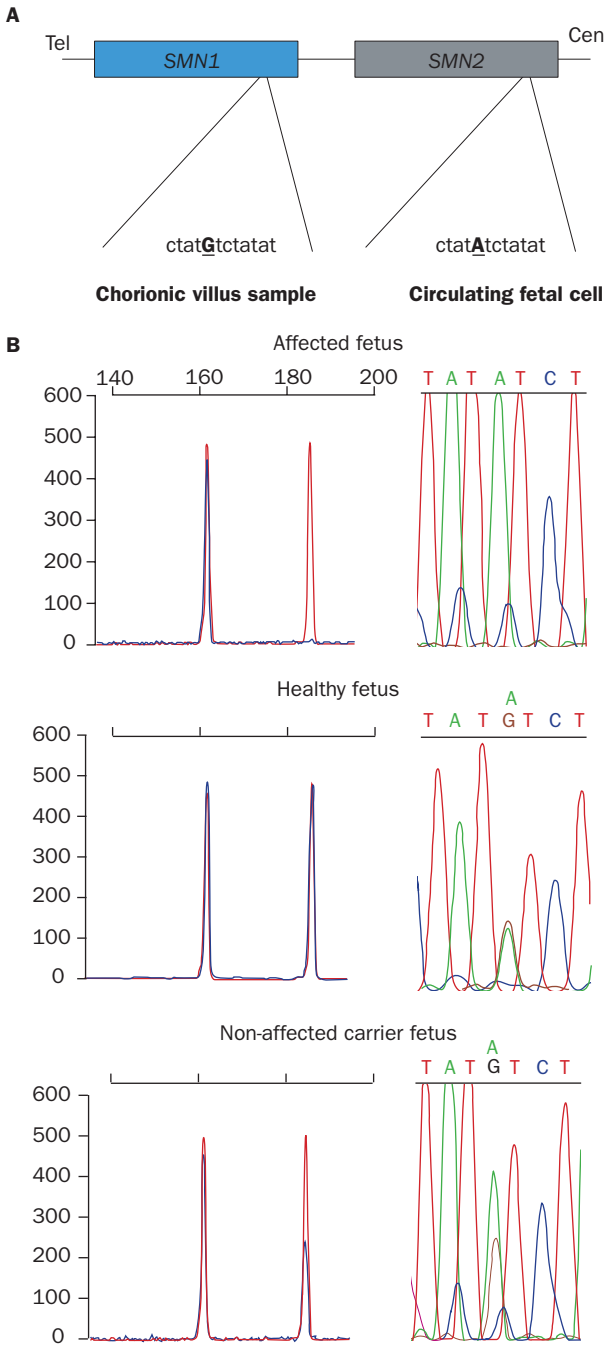
The development of non-invasive tests for prenatal diagnosis is an important goal in medical genetics.[2] However, no routine application of a safe prenatal test has been developed in clinical practice. Circulating fetal cells in maternal blood include lymphoid and erythroid cells, myeloid precursors, and epithelial (trophoblastic) cells. Trophoblastic cells are shed in the maternal blood very early in pregnancy, but unlike lymphoid and myeloid fetal cells, do not persist for years after delivery.[2] We have reported on a promising approach to prenatal diagnosis. ISET (isolation by size of epithelial tumour or trophoblastic cells) isolates cells from maternal blood in accordance with size.[3] Cells are individually microdissected and genotyped to identify those with fetal DNA. Because at least five PCR assays can be done on DNA from one cell, gene mutations can be reliably detected in a fetal and pure genome.

We analysed peripheral blood from 12 pregnant women, who, with the child's father, were all heterozygous for *SMN1* deletion, and were requesting prenatal diagnosis of SMA because a previous child had had SMA. All women gave informed consent and the study was approved by the local ethics committee.

ISET was done on 4 mL of peripheral blood (6 mL for mother 7) taken at 11 weeks of pregnancy, before chorionic villus sampling (CVS). The analysis, which takes 3 days, was done by workers who were unaware of results of the quantitative fluorescent PCR analysis, and in parallel with the quantitative fluorescent PCR analysis of CVS,[4] which was done in a different laboratory, and took 2 days. ISET isolated cells were individually microdissected, DNA was preamplified and then amplified with microsatellite primers that we knew from a previous test on parental DNA would be informative.[3] Single cell genomes, shown to be fetal by the presence of maternal and paternal alleles, were amplified with primers that encompassed the *SMN1* exons 7 and 8 sequence. PCR (40 cycles, 94°C 30 s, 60°C 45 s, 72°C 45 s) was done in 20 μL containing 2 μL of the preamplified product, 20 pmol of each primer (forward: TTGTGGGATTGTAGGCATGA, reverse: CTGGCA GACTTACTCTTAA) and 1 U of Taq polymerase (Perkin-Elmer Cetus, Emeryville, CA, USA). Nested PCR (40 cycles, 94°C 30 s, 55°C 45 s, 72°C 45 s) was done with 0·1 μL of the first PCR product, and 20 pmol of internal primers (forward: GCCTAATAATTGTTTTCTTTGG, reverse: CCTTCCTTCTTTTTGATTTTGTTT). We purified the PCR product using MicroSpin S-400R columns (Amersham Biosciences, Buckinghamshire, UK) and did sequencing with Big Dye terminator sequencing kit (Applied Biosystem, Foster City, CA, USA).

Since *SMN1* exon 7 differs from *SMN2* exon 7 by one nucleotide (codon 280: G in *SMN1* and A in *SMN2*) (figure), the loss of G/A heterozygosity in the exon 7 sequence obtained from a fetal cell shows the homozygous absence of *SMN1* gene (figure). The figure shows the prenatal diagnosis of SMA made in parallel on DNA from CVS and from single circulating fetal cells in an affected fetus (homozygous absence of *SMN1* allele), an unaffected fetus (homozygous presence of *SMN1* allele), and a non-affected carrier fetus (heterozygous presence of *SMN1* allele). For CVS analysis, *SMN2* (left peak, 164-bp fragment) and *SMN1* (right peak, 180-bp fragment) exon 7 sequences are simultaneously amplified and discriminated by DraI digestion.[4] Sequence analyses are not quantitative and thus cannot show the heterozygous deletion of one *SMN1* allele, but can discriminate between affected and non-affected fetuses.

Microsatellite genotyping was done in 69 epithelial cells and fetal DNA was detected in 35 (50%) of them. *SMN1* homozygous gene deletion was repeatedly identified in all nine fetal cells isolated from the three mothers shown to be carrying an affected fetus by CVS analysis,



Non-invasive prenatal diagnosis of SMA

A: Sequence divergence in *SMN1* and *SMN2* exon 7. B: Prenatal testing for SMA made in parallel in three fetuses by invasive (CVS) and non-invasive (circulating fetal cells) methods. The red and blue traces correspond to the quantification reference (presence of two copies of both *SMN1* and *SMN2*) and the tested DNA, respectively. Arbitrary units on the *y* axis indicate the intensity of the fluorescent signal.

whereas absence of homozygous *SMN1* gene deletion was consistently seen in the 26 fetal cells isolated from the nine mothers with non-affected fetuses (table). By contrast with other work,[5] our results provide evidence that SMA can be diagnosed non-invasively in the fetus.

We have shown the feasibility and repeatability of a non-invasive prenatal diagnosis of SMA through the genetic analysis of fetal cells circulating in maternal blood. Although large studies are needed to define the diagnostic sensitivity and specificity of this approach,

	Family history (affected children/parity)	Ongoing pregnancy		
		Microdissected cells (n=69)	Fetal cells (n=35)	Fetal cells with *SMN1* mutation (n=9)
Mothers with affected fetus				
1	1/1	9	5	5
2	1/3	4	2	2
3	1/2	5	2	2
Mothers with normal fetus				
4	1/3	4	2	0
5	1/2	8	4	0
6	1/3	4	2	0
7	1/2	10	4	0
8	1/2	7	4	0
9	1/5	7	4	0
10	1/2	5	2	0
11	1/2	2	2	0
12	1/3	4	2	0

Prenatal diagnosis confirmed on aborted fetuses of mothers 1 and 3 by genotyping, and by genotyping and clinical examination of all other babies.

Detection of *SMN1* mutation in fetal cells isolated from maternal blood

the enrichment of fetal cells, and single-cell micro-dissection, produces consistent results with a very small amount of peripheral blood.

Our results should encourage the development of safe prenatal tests in clinical practice.

Contributors

C Béroud and M Karliova contributed equally to molecular tests on circulating fetal cells, discussions about the manuscript, and interpretation of results. J P Bonnefont and A Munnich did molecular tests on CVS samples; J P Bonnefont also contributed to writing of the manuscript. A Benachi, Y Dumez, and B Lacour collected and treated blood samples. P Paterlini-Bréchot designed the study and developed the testing method.

Conflict of interest statement

None declared.

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