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Are there fetal stem cells in the maternal brain?☆

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Abstract

Fetal cells can enter maternal blood during pregnancy but whether they can also cross the blood-brain barrier to enter the maternal brain remains poorly understood. Previous results suggest that fetal cells are summoned to repair damage to the mother's brain. If this is confirmed, it would open up new and safer avenues of treatment for brain damage caused by strokes and neural diseases. In this study, we aimed to investigate whether a baby's stem cells can enter the maternal brain during pregnancy. Deceased patients who had at least one male offspring and no history of abortion and blood transfusion were included in this study. DNA was extracted from brain tissue samples of deceased women using standard phenol-chloroform extraction and ethanol precipitation methods. Genomic DNA was screened by quantitative fluorescent-polymerase chain reaction amplification together with short tandem repeat markers specific to the Y chromosome, and 13, 18, 21 and X. Any foreign DNA residues that could be used to interpret the presence of fetal stem cells in the maternal brain were monitored. Results indicated that fetal stem cells can not cross the blood-brain barrier to enter the maternal brain.

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Key Words

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Research Highlights

- (1) Fetal cells are known to enter maternal blood circulation during pregnancy.
- (2) Whether fetal cells in the maternal blood circulation can cross the blood-brain barrier to enter the maternal brain remains poorly understood.
- (3) We tested this possibility using 13 women who had one male infant in their lifetime.
- (4) We used quantitative fluorescent-PCR amplification peaks of 27 short tandem repeat markers on five chromosomes (chromosome 13, 18, 21, X and Y).
- (5) There were no additional (residual) peaks except the main peaks for all DNA samples from the two different brain regions (hippocampal dentate gyrus and the subventricular zone) of each woman.

INTRODUCTION

A study in mice has shown that fetal stem cells can colonize in the brains of pregnant dams^[1]. If this finding occurs in humans, the medical implications could be profound. Previous results suggest that fetal cells are summoned to repair damage to the mother's brain^[1-2]. If this is confirmed, it would open up new and safer avenues of treatment for brain damage caused by some diseases such as stroke and Alzheimer's disease. Although the benefits of this finding cannot be put into practice currently, there are good reasons for thinking that fetal stem cells may one day act as a brain repair kit. Can fetal stem cells cross the blood-brain barrier of the mother in the conception period?

Neural stem cells exist not only in the developing mammalian nervous system but also in the adult nervous system of all mammalian organisms, including humans, and can also be derived from more primitive embryonic stem cells. Researchers are reporting the first successful use of fetal stem cells to grow neurons in stroke-damaged brain areas in rats^[3-4]. Potential uses of stem cells in repair include transplantation to repair missing cells and the activation of endogenous cells to provide "self-repair". If these cells can replace cells responsible for speech and movement functions and also those destroyed by stroke damage, this procedure may one day help people recover after they suffer a stroke^[3-4]. There is evidence that when fetal stem cells were injected close to areas of induced stroke in rat brains, the stem cells could migrate to the location of the stroke damage and turn into the appropriate type of neurons^[5-6]. In another study, it has been concluded that fetal cells did not persist postpartum^[7]. However, recent studies have shown that neural stem cells are present in the subventricular zone lining the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus in the adult mouse, rat, primate, and human brain^[8-12] (Figure 1). The idea that fetal stem cells cross the blood-brain barrier and convert into neural stem cells or act like neural stem cells is plausible. Dawe *et al*^[5] concluded that this can be tested by looking for cells containing a Y chromosome in postmortem brain tissue from mothers who had previously had boys^[6].

In this important observation, interphase Y body fluorescence and the subsequent disappearance of male fetal cells were used to determine the frequency of male fetal cells in maternal blood. Investigators who used PCR amplification of Y chromosome-specific

sequences to identify fetal cells in maternal blood noted "false positive" results; *i.e.*, detection of male DNA in female fetus^[13-14]. Although, laboratory contamination can be considered as a reason of such a false positive^[15], it appears that, at least in some cases, the male DNA is a real finding, possibly originating from a prior pregnancy^[16]. Here, we tested this hypothesis by monitoring for the presence of stem cells originating from the fetus in the mother's brain during the gestational period.

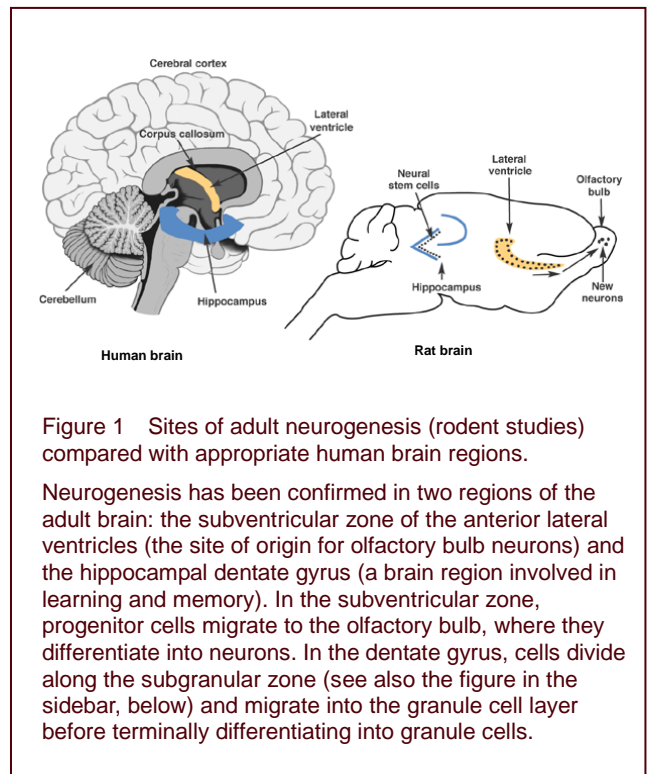


Figure 1 Sites of adult neurogenesis (rodent studies) compared with appropriate human brain regions.

Neurogenesis has been confirmed in two regions of the adult brain: the subventricular zone of the anterior lateral ventricles (the site of origin for olfactory bulb neurons) and the hippocampal dentate gyrus (a brain region involved in learning and memory). In the subventricular zone, progenitor cells migrate to the olfactory bulb, where they differentiate into neurons. In the dentate gyrus, cells divide along the subgranular zone (see also the figure in the sidebar, below) and migrate into the granule cell layer before terminally differentiating into granule cells.

RESULTS

Review of prior pregnancy histories of the 13 women indicated that each had had one male infant prior to sampling for the current study (Table 1). Both of two women had two pregnancies and four elective terminations, respectively, prior to the current pregnancy with a female fetus.

At the end of examination of quantitative fluorescent-PCR amplification peaks of 27 short tandem repeat markers on five chromosomes (chromosome 13, 18, 21, X and Y), which were the common primers used in quantitative fluorescent-PCR examinations, analysis showed that there were no additional (residual) peaks except the main peaks for all DNA samples from the two different brain regions from each woman (Figure 2).

Table 1 Life histories of study participants

Case no.	Age (year)	Consanguinity situation of the parents	Number of children			Cause of death	Medical conditions	Drug consumption
			M	F	T			
1	36	–	3	1	4	Suicide	–	–
2	64	–	2	3	5	Road accident	Diabetes, myocardial infarct	+
3	57	–	1	?	?	Cardiac, pathology	Paralysis	+
4	73	–	1	?	?	Suspicious	Diabetes, hypertension	+
5	21	–	1	–	1	Homicide	–	–
6	69	–	4	3	7	Homicide	–	–
7	64	+	4	4	8	Suicide	Psychiatric diseases	+
8	41	–	1	–	1	Homicide	–	–
9	76	?	4	3	7	Homicide	–	–
10	79	?	1	?	?	Suicide	–	–
11	79	–	4	3	7	Burn out	–	–
12	36	–	2	2	4	Suicide	Depression	+
13	?	?	?	?	?	?	?	?

M: Male; F: female; T: total number of children; ?: there is no exact information; +: positive; –: negative.

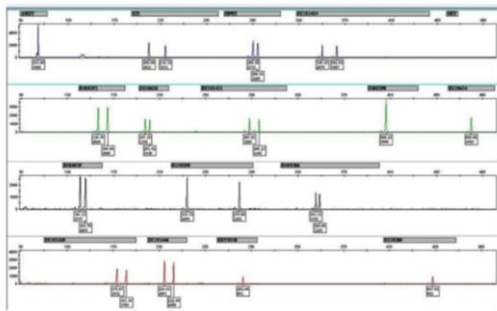


Figure 2 Partial electrophoretogram of quantitative fluorescent-PCR amplification of short tandem repeat markers in the maternal brain.

All cases show peak areas representing short tandem repeat markers belonging to chromosome X. Chromosome Y-representing peak areas were not detected. For interpretation of the electrophoretogram piece, please see the Materials and Methods section.

DISCUSSION

At the end of the 20th century, stem cells were identified in the adult mouse/rat brain and later in the brain of other mammals including humans^[17]. Neural stem cells can also be derived from more primitive fetal stem cells. Fetal stem cells, which develop into every tissue and organ in the body, can give rise to different types of brain and other neural cells. It has been shown that fetal stem cells transplanted into the brain of mice can replace dysfunctional brain cells. These new cells can migrate to various brain regions where they differentiate into apparently normal cells. Many studies have demonstrated that fetal cells concentrate in clinically

affected tissues, giving rise to the hypothesis that they may contribute to tissue repair and regeneration. However, the identity of these pregnancy-associated progenitor cells is not known^[18-19]. During pregnancy, a small number of fetal stem cells stray across the placenta and into the mother's bloodstream, persist in maternal circulation after pregnancy, and engraft many maternal tissues, including bone marrow, the spleen and liver in both mice and humans^[1, 20-24]. At the same time, they can survive for decades in tissues such as skin, the liver and spleen, where they have been shown to repair damage^[1]. In humans, such cells have been found to persist in maternal blood as long as 27 years postpartum. However, it remains unclear whether fetal cells that are capable of crossing the placental barrier to enter maternal blood during pregnancy can also cross the blood-brain barrier to enter the maternal brain. Bianchi^[25] showed conclusively that fetal cells cross the blood-brain barrier. Dawe *et al*^[5] showed that once the stowaways enter the mouse brain, they can mature into different cell types. A recent study has also demonstrated the presence of allogeneic fetal cells in maternal tissue during and long after pregnancy^[3].

It is known that hormonal changes during pregnancy can influence neurogenesis^[26], suggesting that pregnancy may make certain niches in the brain a more receptive environment for fetal cells. Adult neurogenesis is limited to the well-characterized subgranular layer of the hippocampal dentate gyrus and the forebrain subependymal zone of the mammalian brain^[8-9]. It is now accepted that neurogenesis occurs in two brain regions in adult mammals, namely the hippocampus and olfactory bulb^[27]. The problem with the localization of

stem cells in a mature brain has not yet been resolved and actively discussed. In the hippocampus, neurons are formed in the subgranular zone of the dentate gyrus and gliogenesis in all periventricular sections of the brain and spinal cord during the whole animal or human lifespan^[28]. Sanai *et al*^[29] reported no evidence of chains of migrating neuroblasts in the subventricular zone.

We investigated whether any fetal cell migrates to the maternal brain during pregnancy. Genomic DNA for the Y, 13, 18, 21 and X chromosome-specific sequences showed that fetal stem cells were not present in the maternal brain. We thought that they are not likely to be found within a primitive stem cell population in the mother. However, we cannot say that fetal stem cells do not cross the blood-brain barrier of the mother during the gestational period. Some investigators have already reported that fetal stem cells can cross the blood-brain barrier following studies using animal models to monitor fetal cell microchimerism^[9-10]. In addition, some scholars^[3, 30] used quantitative PCR to assess the number of male cells in multiple tissues from normal mice undergoing their first pregnancy. Negative results of this study may be interpreted with the following explanation. It is possible to think that fetal cell migration occurs in only a fraction of pregnancies. It is also possible that the maternal immune system has a capacity to eliminate these cells without eliminating the fetus. Moreover, we only studied regions of the mothers' brains that had neuroregenerative potential, namely the hippocampal dentate gyrus and the subventricular zone, so we could not confirm whether other parts of the brain have fetal cells.

In conclusion, our negative results suggest that there may be differences in terms of the traffic across the placental barrier, the mothers' immune systems and the degree of histocompatibility between the fetal cells and the mothers' cells. Therefore, further studies are required to evaluate whether fetal stem cells can cross the blood-brain barrier.

MATERIALS AND METHODS

Design

A genomic study.

Time and setting

This study was performed at the Department of Medical Biology and Forensic Medicine, Faculty of Medicine, Çukurova University, Turkey between April 2009 and

March 2010.

Materials

This study was permitted by the Ministry of Forensic Medicine in Adana, Turkey. Review of the prior pregnancy and life histories of the 13 deceased women are summarized in Table 1. Patients were selected for this study on the basis of the following criteria: pregnancy with at least one male offspring, and no history of abortion and blood transfusion. All deceased women who had no sign of putridity were included in this study without looking to the cause of death. Informed consents were taken from executors of the cases.

Methods

Sample selection

In the included cases, autopsy was performed within 24 hours and the dead bodies were kept at 4°C during the period of procedures accomplished in the Ministry of Forensic Medicine in Adana. The brain was dissected and coronal cross-sections were taken with 1 cm intervals. Brain tissue samples (approximately 1 cm³) were taken from hippocampal dentate gyrus and the subventricular zone. Neurogenesis occurs primarily in the hippocampal dentate gyrus and the subventricular zone of the adult mammalian brain, as in rodents and non-human primates (Figure 1)^[12]. In the dentate gyrus, newly generated neuronal cells in the subgranular zone migrate to the granule cell layer^[8, 31-33]. Newly generated neuronal cells in the subventricular zone migrate to the olfactory bulb, through the rostro-migratory stream, where they differentiate into olfactory bulb interneurons^[34-36]. All tissue samples were put into labeled tubes containing 70% ethanol. Male babies' stem cells were detected by quantitative fluorescent-PCR amplification of Y, 13, 18, 21 and X chromosome-specific sequences.

Quantitative fluorescent-PCR analysis

Quantitative fluorescent-PCR analysis was performed based on the amplification of chromosome-specific DNA sequences with short tandem repeats polymorphic in length between subjects. By means of fluorescent primers, the amplified segments could be visualized and quantified as peak areas on automated DNA scanners. Normal heterozygous subjects were expected to show two peak areas (peaks ratio 1:1) for each chromosome analyzed, while trisomies were visualized either as an extra peak (triallelic subjects) or as a 2:1 ratio peak between the two areas^[37]. Quantitative fluorescent-PCR amplifications were performed for the determination of any foreign DNA residues originating from the cells that

could migrate from the babies to the brains of the mothers during the gestational period. DNA was extracted from brain tissue samples of deceased women using standard phenol-chloroform extraction and ethanol precipitation methods. Quality and quantity of DNA samples were evaluated by Implen Nanophotometer™ (Implen GmbH, Munich, Germany). Quantitative fluorescent-PCR amplifications were performed using the Aneufast™ (Molgentix SL, Barcelona) trisomy detection kit, which includes fluorescently labeled primers for 27 predefined short tandem repeat marker sites on chromosomes 13, 18, 21, X and Y, and primer pairs for AMXY and SRY regions (AMXY, X22, HPRT, SRY, DXYS218, D135631, D135634, D135258, D135305, D18S391, D18S390, D18S635, D18S386, D21S1411, D211435 and D21S1446). The kit also contained dNTPs and Hot Start Taq DNA polymerase in an optimized reaction buffer. DNA (2 µL, 10–50 ng) and PCR-grade water (3 µL) were added to each of the reaction tubes containing 10 µL of the master mix. After initial denaturation at 95°C for 15 minutes, amplification was achieved by 28 cycles of 95°C for 40 seconds, 58°C for 80 seconds and 72°C for 40 seconds, and final extension was 30 minutes at 60°C. Quantitative fluorescent-PCR products (1.5 µL from each mix) were added to 40 µL Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA) containing 0.3 µL of GeneScan™-500 LIZ™ (Applied Biosystems) size standard. After denaturation at 95°C for 3 minutes, the mixture was cooled to 4°C and then capillary electrophoresis was carried out on an ABI 3130 Genetic Analyzer using POP7 polymer. Results were analyzed using GeneMapper 4.0 software (Applied Biosystems). By means of quantitative fluorescent-PCR together with short tandem repeat markers, amplifications were achieved from trace amounts of foreign DNA and distinguished from the amplification of maternal DNA samples.

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Author contributions: Osman Demirhan, Necmi Çekin, Deniz Taştemir, Erdal Tunç, Demet Meral and Bülent Demirebek designed this study. Deniz Taştemir, Erdal Tunç and Ali İrfan Güzel performed the research. Osman Demirhan, Deniz Taştemir, Ali İrfan Güzel, and Demet Meral analyzed the experimental data. Osman Demirhan, Necmi Çekin, Erdal Tunç, and Bülent Demirebek were responsible for writing the paper. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Ethical approval: This study was approved by the Ethics Committee, Faculty of Medicine, University of Çukurova, Turkey.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

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