Prenatal Diagnosis and Treatment

David A. Prentice, PhD
Family Research Council
Washington, DC, USA

CBHD Congressional Staff Briefing
March 22, 2012
Your destiny, from day one

The mammalian body plan starts being laid down from the moment of conception, it has emerged. Helen Pearson considers the implications of a surprising shift in embryological thinking.

Your world was shaped in the first 24 hours after conception. Where your head and feet would sprout, and which side would form your back and which your belly, were being defined in the minutes and hours after sperm and egg united.

Just five years ago, this statement would have been heresy. Mammalian embryos were instance, the egg inherits a molecule that is more concentrated at one end of the egg than the other, and thus defines the head–tail axis.

Heads or tails?

But mammalian embryos were considered to be a special case. First, they have a striking ability to compensate for damage. Split up Developmental Biology in Cambridge.

The first hint that the blastocyst was not the unassuming orb it appeared came in the 1980s. Two little-noticed studies from Jean Smith of Queen’s College in Flushing, New York, showed that the mouse blastocyst, rather than a being a symmetrical sphere, is slightly distorted and has recognizable
Amniocentesis

Amniotic cavity
Amniotic fluid

Cells from amniotic fluid

Cells are grown in culture for up to four weeks to obtain enough cells for biochemical tests and chromosome analysis.
Chorionic Villus Sampling

- **Chorionic villi**
- **Suction tube**
- **Ultrasonic scanner**
- **Suction tube**
- **Cells from chorionic villi**
- **Biochemical tests and chromosome analysis**
Pre-implantation Genetic Diagnosis (PGD)
First-Trimester or Second-Trimester Screening, or Both, for Down’s Syndrome


ABSTRACT

BACKGROUND
It is uncertain how best to screen pregnant women for the presence of fetal Down’s syndrome: to perform first-trimester screening, to perform second-trimester screening, or to use strategies incorporating measurements in both trimesters.

METHODS
From the Columbia University College of Physicians and Surgeons, New York (F.D.M., M.E.D.); the Royal College of Surgeons in Ireland, Dublin (F.D.M.); Brown University School of Medicine, Providence, R.I. (J.A.C., S.R.C., G.L.M.)
Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study

Rossa W K Chiu, professor,1 Ranjit Akrekar, clinical research fellow,1 Yama W L Zheng, student,2 Tak Y Leung, professor,3 Hao Sun, assistant professor,4 K C Allen Chan, associate professor,5 Fiona M F Lan, postdoctoral fellow,6 Attie T J Gs, professor,7 Elizabeth T Lau, department manager and honorary assistant professor;5 William W K To, consultant,8 Wing C Leung, consultant,9 Rebecca Y K Tang, consultant,9 Sidney K C Au-Yeung, consultant,9 Helena Lam, consultant,9 Yi Y Yung, obstetrician,10 Xueping Zhang, manager,12 John M G van Vught, professor,8 Ryoko Minekawa, postdoctoral fellow;7 Mary H Y Tang, consultant and honorary clinical associate professor;8 Jun Wang, professor,7 associate director;8 Cees B M Oudejes, associate professor,4 Tze K Lau, professor,2 Kypros H Nicolaides, professor,3 Y M Dennis Lo, professor10

Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, China
1Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong, China
2Queen Elizabeth Hospital, Department of Obstetrics and Gynaecology, University of Hong Kong, Hong Kong, China
3Tai hospital, Department of Obstetrics and Gynaecology, University of Hong Kong, Hong Kong, China
4Rotterdam University Medical Center, 10010 Nijmegen, Netherlands
5Kam Yuen Hospital, Department of Obstetrics and Gynaecology, University of Hong Kong, Hong Kong, China
6Tuen Mun Hospital, Hospital Authority, Hong Kong, China
7Eastern Hospital, Hospital Authority, Hong Kong, China
8Prince Margaret Hospital, Hospital Authority, Hong Kong, China
9Kam Medical Centre, Hong Kong
10Ku Chinese University of Hong Kong, Beijing Genomics Institute, Beijing Research Centre, Beijing, China
11BGI Institute of Stem Cell Research, Shenzhen, China
Correspondence: Y M Dennis Lo
dennislo@hku.hk

ABSTRACT

Objectives To validate the clinical efficacy and practical feasibility of massively parallel maternal plasma DNA sequencing to screen for fetal trisomy 21 among high risk pregnancies clinically indicated for amniocentesis or chorionic villus sampling.

Design Diagnostic accuracy validated against full karyotyping, using prospectively collected or archived maternal plasma samples.

Setting Prenatal diagnostic units in Hong Kong, United Kingdom, and the Netherlands.

Participants 753 pregnant women at high risk for fetal trisomy 21 who underwent definitive diagnosis by full karyotyping, of whom 86 had a fetus with trisomy 21.

Intervention Multiplexed massively parallel sequencing of DNA molecules in maternal plasma according to two protocols with different levels of sample throughput: 2-plex and 8-plex sequencing.

Main outcome measures Proportion of DNA molecules that originated from chromosome 21. A fetus was diagnosed when the score for the proportion of chromosome 21 DNA molecules was >3. Diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were calculated for trisomy 21 detection.

Results Results were available from 753 pregnancies with the 8-plex sequencing protocol and from 314 pregnancies with the 2-plex protocol. The performance of the 8-plex protocol was superior to that of the 2-plex protocol. With the 2-plex protocol, trisomy 21 fetuses were detected at 1.0% sensitivity and 99.9% specificity, which resulted in a positive predictive value of 96.6% and a negative predictive value of 100%. The 8-plex protocol detected 79.3% of the trisomy 21 fetuses and 98.4% specificity, giving a positive predictive value of 95.0% and a negative predictive value of 96.9%.

Conclusion Multiplexed maternal plasma DNA sequencing analysis could be used to rule out fetal trisomy 21 among high risk pregnancies. If referrals for amniocentesis or chorionic villus sampling were based on the sequencing test results, about 98% of the invasive diagnostic procedures could be avoided.

INTRODUCTION

Trisomy 21, Down's syndrome, occurs in 1 in 800 live births.1 Prenatal diagnosis of trisomy 21 requires invasive sampling of fetal genetic material through amniocentesis or chorionic villus sampling. However, these tests carry a risk of miscarriage of about 1%2 and they are therefore reserved for pregnancies considered to be at high risk of fetal trisomy 21. The traditional method of identifying the high risk group has been increased maternal age, but screening by this method would require invasive testing in about 9% of pregnant women and identify only 30% of affected fetuses.3 In the past 20 years maternal age has been combined with ultrasonographic examination of the fetus and biochemical measurement of various proteins or hormones in the maternal circulation to improve identification of high risk pregnancies. This combined approach of screening can now identify more than 90% of affected fetuses, but there is still a need for invasive testing in 3–5% of the population.4

Cell-free DNA from the fetus has been found in the plasma of pregnant women, and this has been used successfully for non-invasive determination of the fetal sex and fetal RhD genotype in RhD negative women.5,6 The basis of these tests is the detection of fetal-specific DNA sequences in maternal plasma.7 The same approach of searching for fetal-specific nucleic acids, such as DNA methylation and mRNA
Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21

Elisavet A Papageorgiou, Alex Karagrigoriou, Evdokia Tsaliki, Voula Velissariou, Nigel P Carter, & Philippos C Patsalis

The trials performed worldwide toward noninvasive prenatal diagnosis (NIPD) of Down’s syndrome (or trisomy 21) have shown the commercial and medical potential of NIPD compared to the currently used invasive prenatal diagnostic procedures. Extensive investigation of methylation differences between the mother and the fetus has led to the identification of differentially methylated regions (DMRs). In this study, we present a strategy using the methylated DNA immunoprecipitation (MeDIP) methodology in combination with real-time quantitative PCR (qPCR) to achieve fetal chromosome dosage assessment, which can be performed noninvasively through the analysis of fetal-specific DMRs. We achieved noninvasive prenatal detection of trisomy 21 by determining the methylation ratio of normal and trisomy 21 cases for each tested fetal-specific DMR present in maternal peripheral blood, followed by further statistical analysis. The application of this fetal-specific methylation ratio approach provided correct diagnosis of 14 trisomy 21 and 26 normal cases.

clade B member 5 (SERPINB5) has been the first gene identified to be fetal-specific hypomethylated in maternal plasma. A number of additional studies have focused on single-gene promoter regions or CpG islands on chromosome 21 (refs. 11–13).

Current approaches developed using fiaDNA for NIPD are subject to a number of limitations. The two main applications are the use of methylation-sensitive restriction enzymes and the use of sodium bisulfite. In the first application, the requirement for DMRs containing a restriction site limits the number of loci suitable for testing, whereas in the second application the bisulfite conversion leads to DNA degradation. To overcome these limitations, we have recently used a newly developed technique called MeDIP to investigate the DNA methylation pattern of chromosomes 13, 18, 21, X and Y. The MeDIP methodology uses an antibody specific for 5-methylcytidine to capture methylated sites and therefore enrich for fetal-specific methylated DNA.

We have selected a subset of DMRs on chromosome 21 and have applied the MeDIP methodology in combination with real-time qPCR in normal and trisomy 21 cases. To provide chromosome dosage infor-
Are Political Orientations Genetically Transmitted?

JOHN R. ALFORD  Rice University
CAROLYN L. FUNK  Virginia Commonwealth University
JOHN R. HIBBING  University of Nebraska

We test the possibility that political attitudes and behaviors are the result of both environmental and genetic factors. Employing standard methodological approaches in behavioral genetics—specifically, comparisons of the differential correlations of the attitudes of monozygotic twins and dizygotic twins—we analyze data drawn from a large sample of twins in the United States, supplemented with findings from twins in Australia. The results indicate that genetics plays an important role in shaping political attitudes and ideologies but a more modest role in forming party identification; as such, they call for finer distinctions in theorizing about the sources of political attitudes. We conclude by urging political scientists to incorporate genetic influences, specifically interactions between genetic heritability and social environment, into models of political attitude formation.

Why do people think and act politically in the manner they do? Despite the foundational nature of this question, answers are unfortunately incomplete and unnecessarily tentative, largely because political scientists do not take seriously the possibility of nonenvironmental influences. The sug-

the world and over the decades is difficult for behavioralists to explain. But if there is a genetic component to political ideologies, if the constraints on belief systems come not just from intellectualization or indoctrination but from something deeper, the concept of ideology takes on greater meaning and the commonality of ide-
Is there a ‘liberal gene’?

A new study claims to have found a link between biology and political orientation.

By Alexandra Nikolchev

November 3, 2010

The identification of a “liberal gene,” described as such in the study’s press release, suggests that our political orientation may be hardwired into our brains—an uncomfortable idea for those who believe in free will. But it’s not that simple, says the study’s head author, James Fowler, a professor in the School of Medicine and the Division of Social Sciences at the University of California, San Diego. He said the study’s significance is not derived from finding a “liberal gene” per se, but rather from establishing a correlation between biology and political ideology.

Appearing in the latest edition of The Journal of Politics published by Cambridge University Press, research from the University of California, San Diego, and Harvard University focused on about 2,000 subjects from The National
Prenatally and Postnatally Diagnosed Conditions Awareness Act

Kennedy-Brownback

Signed into law Oct 8, 2008
“Soon it will be a sin for parents to have a child that carries the heavy burden of genetic disease. We are entering a world where we have to consider the quality of our children.”

Dr. Robert Edwards, embryologist, 1999
**Embryonic Stem Cells**

from Embryos created by Fertilization or by Cloning (Somatic Cell Nuclear Transfer)

- Egg cell
- Sperm cell
- Fertilization
- Fertilization finished
- Fertilized embryo
- Single-cell Embryo
- Embryonic Stem Cells

- Egg cell
- Remove or inactivate egg nuclear material
- Insert donor nuclear material
- Isolate donor nuclear material
- Cloning finished
- Cloned embryo
- Embryonic Stem Cells

**Induced Pluripotent Stem Cells (iPS cells)**

from Normal Cells that are Reprogrammed to behave like Embryonic Stem Cells

- Add genes ± chemicals
- Target cell
- Cells behave like embryonic stem cells

**Adult Stem Cells**

Stem Cells normally found in body tissues from birth onward, as well as umbilical cord, etc.

- Umbilical Cord Blood, Placenta, Amniotic Fluid, and other tissues
- Bone Marrow
- Adult Stem Cells
Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases

Lin Ye\textsuperscript{a}, Judy C. Chang\textsuperscript{a}, Chin Lin\textsuperscript{a}, Xiaofang Sun\textsuperscript{b}, Jingwei Yu\textsuperscript{c}, and Yuet Wai Kan\textsuperscript{a, c, d, 1}

Departments of \textsuperscript{a}Medicine and \textsuperscript{b}Laboratory Medicine, \textsuperscript{2}Institute for Human Genetics and Cardiovascular Research Institute, University of California, San Francisco, CA 94143-0793; and \textsuperscript{b}Guangzhou Key Laboratory of Reproductive and Genetics, Institute of Gynecology and Obstetrics, The Third Affiliated Hospital of Guangzhou Medical College, Guangzhou 510150, China

Contributed by Yuet Wai Kan, April 28, 2009 (sent for review April 16, 2009)

The innovation of reprogramming somatic cells to induced pluripotent stem cells provides a possible new approach to treat β-thalassemia and other genetic diseases such as sickle cell anemia. Induced pluripotent stem (iPS) cells can be made from these patients' somatic cells and the mutation in the β-globin gene corrected by gene targeting, and the cells differentiated into hematopoietic cells to be returned to the patient. In this study, we reprogrammed the skin fibroblasts of a patient with homozygous \( \beta^0 \) thalassemia into iPS cells, and showed that the iPS cells could be differentiated into hematopoietic cells that synthesized hemoglobin. Prenatal diagnosis and selective abortion have been effective in decreasing the number of β-thalassemia births in some countries that have instituted carrier screening and genetic counseling. To make use of the cells from the amniotic fluid or chorionic villus sampling that are used for prenatal diagnosis, we also showed that the iPS cells could be reprogrammed into iPS cells. This raises the possibility of providing a new option following prenatal diagnosis of a fetus affected by a severe illness. Currently, the parents would choose either to terminate the pregnancy or continue it and take care of the sick child after birth. The cells for prenatal diagnosis can be converted into iPS cells for treatment in the perinatal period. Early treatment has the advantage of requiring much fewer cells than adult treatment, and can also prevent organ damage in those diseases in which damage can begin in utero or at an early age.

Cure for β-thalassemia can be achieved by bone marrow or cord blood transplantsations if histocompatible donors are available (2, 3). However, because these patients' families are usually small, donors are not commonly found. Furthermore, graft versus host diseases of variable severity commonly follow. Prevention of new births with homozygous β-thalassemia has been achieved with prenatal diagnosis. In countries such as Italy, Greece, and Cyprus, carrier screening, genetic counseling, prenatal diagnosis, and selective abortion of the homozygous fetuses have effectively decreased the number of births with homozygous β-thalassemia.

An experimental approach to treatment is gene therapy of β-thalassemia and sickle cell anemia. Using lentiviral vectors expressing the β-globin gene to transduce hematopoietic cells ex vivo, several groups of investigators have successfully treated mouse models of β-thalassemia or sickle cell anemia (4–6). A human trial is said to be proceeding in Europe (7).

The reprogramming (8–10) of somatic cells into induced pluripotent stem (iPS) cells opens a new approach of treating β-thalassemia. iPS cells have been made from a variety of tissues, including skin fibroblasts, hepatocytes, stomach, testicular, and neural cells (11–14). In addition, iPS cells have also been made from the somatic cells of patients with a number of diseases including adenosine deaminase deficiency, Gaucher disease, muscular dystrophies, Parkinson disease, Huntington disease.
Figure 1. Global Distribution of Hematopoietic Stem Cell Transplantations (HSCTs) in 2006

Regions are colored by World Health Organization regional office code (see text) (http://www.who.int/about/regions/en/). Transplant rates indicate the number of first HSCTs per 10 million inhabitants in 2006 and are allogeneic and autologous by continental region.
Nate Liao was successfully treated for a fatal genetic skin disease with donor adult stem cells.
Kaitlyn McNamara had a new functional bladder constructed from her own adult stem cells.
Adult Stem Cells Help Create Synthetic Windpipe, Save Cancer Patient

by David Prentice
July 8, 2011

A cancer patient has received the first synthetic windpipe transplant. The new windpipe was created using the patient’s own adult stem cells which were seeded onto a synthetic scaffold to grow the new tissue. According to his doctors, the patient—36-year-old Andemariam Teklesenbet Beyene, a father of two—no longer has cancer, will be released from the hospital today, and is expected to have a normal life expectancy.

Professor Paolo Macchiarini, of Karolinska University Hospital and Karolinska Institute, led the team that performed the transplant operation on 9 June 2011 at Karolinska University Hospital in Huddinge, Stockholm. Professor Macchiarini also led the international team that developed the artificial windpipe, which included Professor Alexander Seifalian from the University College London, UK, who designed and built the nanocomposite tracheal scaffold, and Harvard Bioscience, a Boston, USA company that produced a specifically designed bioreactor used to seed the scaffold with the patient’s own adult stem cells from bone marrow.

According to the Karolinska institute:

“Because the cells used to regenerate the trachea were the patient’s own, there has been no rejection of the transplant and the patient is not taking (anti-rejection) drugs.”

Creating a new windpipe using the patient’s own adult stem cells and a synthetic scaffold is a tremendous breakthrough, allowing production of tubular organs for transplant within a short period of time. As Prof. Macchiarini noted:
Angela Irizarry, 4 years old, grew a new heart vessel using an implanted biodegradable scaffold seeded with her own bone marrow adult stem cells. Treatment for hypoplastic left heart syndrome, a potentially lethal defect.

A Pilot Study Investigating the Clinical Use of Tissue Engineered Vascular Grafts in Congenital Heart Surgery, C. Breuer, ClinicalTrials.gov Identifier: NCT01034007
Prenatal Treatment

• Open fetal surgery
• Laser treatment
• Prenatal stem cell and gene therapy
A Randomized Trial of Prenatal versus Postnatal Repair of Myelomeningocele

N. Scott Adzick, M.D., Elizabeth A. Thorn, Ph.D., Catherine Y. Spong, M.D., John W. Brock III, M.D., Pamela K. Burrows, M.S., Mark P. Johnson, M.D., Lori J. Howell, R.N., M.S., Jody A. Farrell, R.N., M.S.N., Mary E. Dabrowiak, R.N., M.S.N., Leslie N. Sutton, M.D., Nalin Gupta, M.D., Ph.D., Noel B. Tulipan, M.D., Mary E. D’Alton, M.D., and Diana L. Farmer, M.D., for the MOMS Investigators

BACKGROUND

Prenatal repair of myelomeningocele, the most common form of spina bifida, may result in better neurologic function than repair deferred until after delivery. We compared outcomes of in utero repair with standard postnatal repair.

METHODS

We randomly assigned eligible women to undergo either prenatal surgery before 26 weeks of gestation or standard postnatal repair. One primary outcome was a composite of fetal or neonatal death or the need for placement of a cerebrospinal fluid shunt by the age of 12 months. Another primary outcome at 30 months was a composite of mental development and motor function.

RESULTS

The trial was stopped for efficacy of prenatal surgery after the recruitment of 183 of a planned 260 patients. This report is based on results in 158 patients whose children were evaluated at 12 months. The first primary outcome occurred in 68% of the infants in the prenatal-surgery group and in 98% of those in the postnatal-surgery group (relative risk, 0.70; 97.7% confidence interval [CI], 0.58 to 0.84; P<0.001). Actual rates of shunt placement were 40% in the prenatal-surgery group and 82% in the postnatal-surgery group (relative risk, 0.48; 97.7% CI, 0.36 to 0.64; P<0.001). Prenatal surgery also resulted in improvement in the composite score for mental development and motor function at 30 months (P=0.007) and in improvement in several secondary outcomes, including hindbrain herniation by 12 months and ambulation by 30 months. However, prenatal surgery was associated with an increased risk of preterm delivery and uterine dehiscence at delivery.

CONCLUSIONS

Prenatal surgery for myelomeningocele reduced the need for shunting and improved motor outcomes at 30 months but was associated with maternal and fetal risks. (Funded by the National Institutes of Health; ClinicalTrials.gov number, NCT00060606.)
Successful prenatal mannose treatment for congenital disorder of glycosylation-Ia in mice

Anette Schneider¹, Christian Thiel¹, Jan Rindermann², Charles DeRossi³, Diana Popovici³, Georg F Hoffmann¹, Hermann-Josef Gröne³ & Christian Körner¹

Congenital disorder of glycosylation-Ia (CDG-Ia, also known as PMM2-CDG) is caused by mutations in the gene that encodes phosphomannomutase 2 (PMM2, EC 5.4.2.8) leading to a multisystemic disease with severe psychomotor and mental retardation. In a hypomorphic Pmm2 mouse model, we were

In contrast to Pmm2¹¹¹ mice, Pmm2¹¹ mice were viable, fertile and comparable in size and proportion to their wild-type siblings. They developed normally without any major phenotype up to adulthood. Histological examination of organs in 3- to 6-month-old mice and isoelectric focusing of serum transferrin (a glycoprotein used as marker in CDG diagnostics) did not yield any pathological findings (data not shown). Pmm activity (measured by an assay that does not differentiate between PMM1 and PMM2) in mouse embryonic fibroblasts (MEFs) cultivated from 9.5-d.p.c. Pmm2¹¹¹ embryos was 42% (0.75 ± 0.19 pmol min⁻¹ per mg protein) of that of wild-type siblings (1.79 ± 0.38 pmol min⁻¹ per mg protein, n = 7, P < 0.002 (Student’s t test)). Likewise, we found that Pmm activity was 38% of wild-type activity (0.22 ± 0.01 pmol min⁻¹ per mg protein in primary fibroblasts established from skin biopsies of 6-month-old homozygous mice compared to 0.58 ± 0.11 pmol
Stem cell and genetic therapies for the fetus

Jessica L. Roybal a, Matthew T. Santore a,b, Alan W. Flake a,b,*

a Children’s Center for Fetal Research, Children’s Hospital of Philadelphia, PA, USA
b University of Pennsylvania School of Medicine, Philadelphia, PA, USA

SUMMARY

Advances in prenatal diagnosis have led to the prenatal management of a variety of congenital diseases. Although prenatal stem cell and gene therapy await clinical application, they offer tremendous potential for the treatment of many genetic disorders. Normal developmental events in the fetus offer unique biologic advantages for the engraftment of hematopoietic stem cells and efficient gene transfer that are not present after birth. Although barriers to hematopoietic stem cell engraftment exist, progress has been made and preclinical studies are now underway for strategies based on prenatal tolerance induction to facilitate postnatal cellular transplantation. Similarly, in-utero gene therapy shows experimental promise for a host of diseases and proof-in-principle has been demonstrated in murine models, but ethical and safety issues still need to be addressed. Here we review the current status and future potential of prenatal cellular and genetic therapy.
Brief Report

TREATMENT OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY BY IN UTERO TRANSPANTATION OF PATERNAL BONE MARROW

ALAN W. FLAKE, M.D.,
MARIA-GRAZIA RONCAROLO, M.D., PH.D.,
JENNIFER M. PUCK, M.D.,
GRAZA ALMEIDA-PORADA, M.D., PH.D.,
MARK L. EVANS, M.D., MARK P. JOHNSON, M.D.,
ESTABAN M. ABELA, M.D., DUANE D. HARRISON, M.D.,
AND ESMAIL D. ZANJANI, PH.D.

SEVERE combined immunodeficiency is a congenital syndrome due to various genetic abnormalities that cause susceptibility to infection, failure to thrive, lymphoid hypoplasia, very low levels of T lymphocytes, and hypogammaglobulinemia. Untreated, the disorder is usually fatal within the first year of life. We report the successful treatment of a fetus with the X-linked variant of severe combined immunodeficiency by the in utero transplantation of paternal bone marrow that was enriched with hematopoietic cell progenitors.

CASE REPORT

The patient, 11 months old at this writing, is the second son of a 28-year-old woman known to carry a mutation found in X-linked severe combined immunodeficiency. Her first son died at seven months of age of severe combined immunodeficiency, confirmed by autopsy and molecular analysis. Studies of his DNA identified a splice-donor-site mutation in the gene for the common chain of the interleukin-2 receptor (IL2RG) in complementary DNA at position 868(+5) in intron 6.

The woman became pregnant again. Analysis of DNA obtained at 12 weeks' gestation by chorionic-villus sampling showed that the fetus was an affected male. After extensive nondirective counseling the family decided in favor of prenatal treatment.

Bone marrow was harvested under general anesthesia from the 30-year-old father of the fetus. After enrichment of the bone marrow with CD34+ cells (hematopoietic cell progenitors), the fetus received three transplants of 14.8 million, 2.0 million, and 1.8 million cells (114 million, 8.9 million, and 6.2 million cells per kilogram of estimated fetal weight), respectively, by percutaneous, ultrasound-guided, intraperitoneal injection at 16, 17.5, and 18.5 weeks' gestation. At delivery by cesarean section, the infant appeared normal except for a mild macular rash. A biopsy of the rash revealed no evidence of graft-versus-host disease, such as infiltrating lymphocytes, apoptotic keratinocytes, or vacuolar changes of the basal epithelium. The rash resolved with a seven-day course of methylprednisolone at a dose of 1 mg per kilogram of body weight per day intramuscularly.

METHODS

Ethical Considerations

A decision to continue the pregnancy independently of the option of in utero transplantation was made by the parents after consultation with specialists in genetics and pediatric immunology. Both parents subsequently gave informed consent for the in utero transplantation procedures. The protocol and consent forms were reviewed and approved by the Human Investigation Committee of Wayne State University.

Prenatal Genetic Evaluation

Identification of the IL2RG mutation and analysis of DNA extracted from the biopsy of chorionic villi were performed according to published techniques.12

Donor-Cell Processing

Paternal bone marrow was obtained by aspiration from the posterior iliac crest and placed in RPMI-1640 medium with preservative-free heparin. The mononuclear cells were separated and divided into three aliquots; the first was processed immediately, and the other two were cryopreserved.

After separation by Ficoll-Hypaque density-gradient centrifugation, mononuclear cells were incubated with biotinylated monoclonal antibody against CD34 in RPMI with 0.1 percent human serum albumin. The cells were washed and passed through a Captiva avidin-biotin immunosorption column (CellPro, Seattle). The CD34+ cells that bound to the column were removed by gentle agitation. Incubation with the antibody was repeated, and the cells were passed through a second Captiva column. After each step of enrichment, aliquots were taken for phenotypic assessment, assays to monitor loss of progenitor cells, and bacterial and fungal cultures.

Injection Procedure

Injections were performed transabdominally with a 22-gauge 3.5-in. (9-cm) spinal needle under real-time ultrasound guidance. The maximal volume injected was 1 ml.

Detection of Donor-Cell Engraftment

...
In-utero transplantation of parental CD34 haematopoietic progenitor cells in a patient with X-linked severe combined immunodeficiency (SCIDXI)

Georg S Wengler, Arnalda Lanfranchi, Tiziana Frusca, Rosanna Verardi, Arabella Neva, Duilio Brugnoni, Silvia Giliani, Maurilia Fiorini, Patrizia Mella, Fabiola Guandalini, Evelina Mazzolari, Sergio Pecorelli, Luigi D Notarangelo, Fulvio Porta, Alberto G Ugazio

Summary

Background X-linked severe combined immunodeficiency (SCIDXI) is an inherited immune defect which leads to death in infancy from severe infections. The defect is caused by mutations of the IL-2RG gene that encodes for the common γ chain shared by several cytokine receptors. The disease is characterised by lack of T and NK cells with normal numbers of B cells. SCIDXI can be cured by bone marrow transplantation (BMT) or prevented by abortion after prenatal diagnosis.

Methods A male fetus was diagnosed as having SCIDXI by molecular, immunophenotypic, and functional analyses. The fetus was injected intrapartumone under ultrasound guidance with CD34 haematopoietic progenitor cells purified from paternal bone marrow and T-cell depleted by E rosetting. Chimerism analysis was by HLA-DQα typing and ζ-chain staining on cord blood.

Findings A healthy 36 kg boy was delivered by caesarean section at 38 weeks of gestation with no clinical or laboratory signs of graft-versus-host disease. Engraftment of donor-derived CD2 cells was found at birth. At 3.5 months of age the infant is well and his T-cell counts and function are normal.

Interpretation In-utero transplantation of haematopoietic progenitor cells allowed immune reconstitution of a fetus with SCIDXI and may be an alternative to elective abortion. Our report should encourage applications of this method to other inherited disorders curable by BMT.

Introduction

X-linked severe combined immunodeficiency (SCIDXI) is the predominant form of SCID, in which there is a lack of T and NK cells with a normal or increased number of B lymphocytes. SCIDXI is caused by mutations of the IL-2RG gene that encodes for the common γ chain shared by several interleukin receptors.

The disease is fatal unless cured by bone-marrow transplantation (BMT). The best results are achieved with HLA-identical donors (97%), whereas HLA haploidentical family donors result in lower success rates (52%). Prevention of the disease is based on genetic counselling, prenatal diagnosis by mutation analysis of chonic villi, or immunological evaluation of fetal blood. After diagnosis of SCIDXI in a fetus, the only alternative to the birth of an affected infant is elective abortion.

An alternative to postnatal BMT has been suggested: to do BMT in utero, when the haematopoietic system is still developing and might be overcome by donor haematopoietic stem cells. The immune system is immature early in pregnancy and might allow donor cells to be tolerated. Rhesus monkey fetal liver cells and T-cell depleted bone marrow have been transplanted successfully into fetal rhesus monkeys at 40 to 60 days gestation (length of gestation is 165 days) with long-term engraftment. Xenogenic HLA barriers can also be overcome without graft-versus-host disease (GvHD) if human CD34 cells are transplanted into fetal sheep in utero at 50 days of gestation (length of gestation is 150 days). Genetic defects in mice such as moderate anaemia and severe combined immunodeficiency SCID have been successfully treated by in-utero transplantation of haematopoietic stem cells.

Lancet 1996; 348: 1484-87
Moving neurons back into place

Geraldine Kerjan & Joseph G Gleeson

Mental retardation and epilepsy can result from the aberrant migration of neurons during development. An experimental treatment in prenatal mice restores normal patterns of migration and eases symptoms (pages 84–90).

Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder

Jean-Bernard Manent¹, Yu Wang¹, YoonJeung Chang¹, Murugan Paramasivam¹ & Joseph J LoTurco¹

Disorders of neuronal migration can lead to malformations of the cerebral neocortex that greatly increase the risk of seizures. It remains untested whether malformations caused by disorders in neuronal migration can be reduced by reactivating cellular migration and whether such repair can decrease seizure risk. Here we show, in a rat model of subcortical band heterotopia (SBH) generated by in utero RNA interference of the Dcx gene, that aberrantly positioned neurons can be stimulated to migrate by reexpressing Dcx after birth. Restarting migration in this way both reduces neocortical malformations and restores neuronal patterning. We further find that the capacity to reduce SBH continues into early postnatal development. Moreover, intervention after birth reduces the convulsant-induced seizure threshold to a level similar to that in malformation-free controls. These results suggest that disorders of neuronal migration may be eventually treatable by reengaging developmental programs both to reduce the size of cortical malformations and to reduce seizure risk.
In Utero Hematopoietic Stem-Cell Transplantation —
A Match for Mom

Ornella Parolini, Ph.D.

Once researchers recognized that adult stem cells can generate multiple cell types and contribute to tissue homeostasis, it became conceivable to exploit this potential to treat genetic or acquired disorders characterized by tissue degeneration or organ dysfunction. The concept of regenerative medicine was thus born, with the general aim of transplanting donor stem cells to replace or repair defective cells of the host.

Unfortunately, in the case of an HLA mismatch between donor and recipient, transplantation is hampered by the risks of immunologic recognition and rejection of the graft. However, a recent article by Nijagal and colleagues revived the discussion of the potential advantages of transplanting stem cells into the fetus early in gestation. Because in utero stem-cell transplantation can be carried out when the immune system is immature, it provides the theoretical opportunity to induce fetal tolerance of the foreign cells and thereby avoid rejection and the need for immunosuppressive therapy. For these reasons, this potential clinical approach is attractive.

rejection of cells allogeneic to both mother and fetus. Using mouse models, these researchers tested their hypothesis in a series of experiments to evaluate the role of the maternal immune response in limiting engraftment. First, they found that in utero transplantation of fetal HSCs elicited an increase in trafficking of maternal T cells to the fetal blood. To test the hypothesis that maternal cells play a pivotal role in the fetal engraftment of allogeneic cells, they transferred allogeneic fetal HSCs into fetuses of mothers with experimentally induced B-cell or T-cell deficiency (Fig. 1). Levels of engraftment were significantly higher in fetuses of mothers with T-cell deficiency than in fetuses of wild-type mothers or of mothers with B-cell deficiency. Finally, when in utero stem-cell transplantation was performed with HSCs matched to the mother, similar levels of engraftment were observed in fetal recipients of syngeneic and allogeneic fetal grafts.

The evidence put forward is clear and striking, although further research is warranted to
Review

Amniotic Fluid as a Rich Source of Mesenchymal Stromal Cells for Transplantation Therapy

Ivana Antonucci,* Liborio Stuppia,* Yuji Kaneko,† Seongjin Yu,† Naoki Tajiri,† Eunkyung C. Bae,† Sonia H. Chheda,† Nathan L. Weinbren,† and Cesar V. Borlongan†

*Biomedical Science, Chieti University and Stem TeCh Group, Aging Research Center (CESI), Chieti, Italy
†Department of Neurosurgery and Brain Repair, University of South Florida College of Medicine, Tampa, FL, USA

Stem cells isolated from amniotic fluid are known to be able to differentiate into different cells types, thus being considered as a powerful tool for cellular therapy of different human diseases. In the last 4 years, amniotic fluid-derived stem (AFS) cells have been shown to express embryonic and adult stem cell markers. These cells can be considered an intermediate stage between embryonic stem cells and adult stem cells. AFS cells can give rise to adipogenic, osteogenic, myogenic, endothelial, neurogenic, and hepatic lineages, inclusive of all embryonic germ layers. AFS cells have a high renewal capacity and can be expanded for over 250 doublings without any detectable loss of chromosomal telomere length. Taken together, all these data provide evidence that amniotic fluid represents a new and very promising source of stem cells for research, as well as clinical applications. Certainly stem cells from amniotic fluid will be useful both for a customized cell supply for newly born children and for banking cells to be used for therapeutic cell transplantation in immunologically matched recipients. Further investigations are also warranted to fully explore the amniotic cells’ potential for adult human disorders.
Isolation of amniotic stem cell lines with potential for therapy

Paolo De Coppi1,3, Georg Bartsch, Jr1,3, M Minhaj Siddiqui1, Tao Xu1, Cesar C Santos1, Laura Perin1, Gustavo Mostoslavsky2, Angéline C Serre2, Evan Y Snyder2, James J Yoo1, Mark E Furth1, Shay Soker1 & Anthony Atala1

Stem cells capable of differentiating to multiple lineages may be valuable for therapy. We report the isolation of human and rodent amniotic fluid–derived stem (AFS) cells that express embryonic and adult stem cell markers. Undifferentiated AFS cells expand extensively without feeders, double in 36 h and are not tumorigenic. Lines maintained for over 250 population doublings retained long telomeres and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. Examples of differentiated cells derived from human AFS cells and displaying specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue-engineered bone.
Prenatally Fabricated Autologous Human Living Heart Valves Based on Amniotic Fluid –Derived Progenitor Cells as Single Cell Source
Dörthe Schmidt, Josef Achermann, Bernhard Odermatt, Christian Breymann, Anita Mol, Michele Genoni, Gregor Zund and Simon P. Hoerstrup

Circulation 2007, 116:I-64-I-70

Living Autologous Heart Valves Engineered From Human Prenatally Harvested Progenitors
Dörthe Schmidt, Anita Mol, Christian Breymann, Josef Achermann, Bernhard Odermatt, Matthias Gössi, Stefan Neuenschwander, René Prêtre, Michele Genoni, Gregor Zund and Simon P. Hoerstrup

Circulation 2006, 114:I-125-I-131