Prof Gerald Schatten
University of Pittsburgh, USA
gschatten@pdc.magee.edu

Genome Editing Optimizations:

Implications for Pre- and Post-Implantation Genetics

Disclosure information: Nothing to declare
Brains, Genes, and Primates

null, Volume 86, Issue 3, 2015, 617–631

http://dx.doi.org/10.1016/j.neuron.2015.03.021

Juan Carlos Izpisua Belmonte, Edward M. Callaway, Sarah J. Caddick, Patricia Churchland, Guoping Feng, Gregg E. Homanics, Kuo-Fen Lee, David A. Leopold, Cory T. Miller, Jude F. Mitchell, Shoukhrat Mitalipov, Alysson R. Moutri...
EMERGING DISEASES

A race to explain Brazil’s spike in birth defects

Evidence points toward the fast-spreading Zika virus as the cause of microcephaly

By Gretchen Vogel

Brazilian fetal medicine specialist Manoel Sarno first noticed in July that something was seriously wrong. In just 2 weeks, he diagnosed four cases of microcephaly, a previously rare birth defect in which the baby’s head is too small because the brain fails to fully develop. Sarno, who works at the Federal University of Bahia in Salvador, Brazil, says he is not alone in his observations. “There are so many unexplained cases that you can’t ignore it,” he says.

Sarno is part of a growing international group of medical experts who are telling women not to get pregnant until more is known. The spectrum of emotions at maternity wards “would break anyone’s heart,” says Nikos Vasilakis, a virologist at the University of Texas Medical Branch in Galveston on assignment in Salvador to help improve Zika tests. In parents of afflicted infants, he says, “I have witnessed expressions of love but also rejection of the babies.”

Zika usually causes only mild symptoms, including fever and rashes; as many as 80% never even know they were infected. But in about 1.5% of cases, it can cause microcephaly, as well as other complications such as jaundice, red eyes and hearing loss. The illness can impair brain development in utero, leading to an abnormality in the skull in which the brain fails to grow properly. Zika babies are born with a head circumference at least two standard deviations below the mean for age, a condition known as microcephaly.

Many babies with microcephaly are born to mothers who have tested positive for Zika. But many of their mothers had been exposed to a flavivirus, the family to which Zika belongs.

Most of the Brazilian babies born with microcephaly don’t test positive for Zika, however, nor do their mothers. That may be because current tests for the virus are only conclusive for a short time after symptoms appear; tests on recovered patients can’t determine whether they were exposed before they fell ill.

The Zika epidemic is at least four years old, according to travel histories of the first babies born with microcephaly in Brazil. It is not clear how many mothers were actually infected. Some researchers estimate that about 10% of women are exposed to Zika without even knowing it. People can’t be tested for asymptomatic Zika infection. And after infection, the ability to transmit the virus wanes as pregnancy advances.

A few months after the first cases were diagnosed in Brazil, doctors in the eastern province of Bahia began noticing a peculiar pattern. For the next six months, the number of microcephaly cases spiked, then plummeted. Is it a coincidence, or could Zika be responsible for the spike? The researchers are now trying to answer that question.

Some researchers believe there have been many more cases of microcephaly in Brazil than have been confirmed by authorities, even for the 2014-2015 season. The failure to properly test for Zika and identify cases could be a contributing factor, they say. But another theory is that the Zika virus could have been imported into Brazil from other countries, where the virus is already spreading.

By the time the first babies were born with microcephaly, researchers knew very little about the virus. They did know that the Aedes aegypti mosquito, a carrier of the virus, is widespread in Brazil. They also knew that the virus is in the flavivirus family and causes similar Zika-like illness, including infections and fever. A number of flaviviruses, including yellow fever and dengue fever, are endemic to Brazil.

Many people believe that Zika will disappear in Brazil and the rest of the world as soon as the mosquitoes are eradicated. As if on cue, the National Institutes of Health announced last week that they were funding a study in Brazil to test a candidate vaccine for microcephaly.

Many of the researchers who first noticed the emerging disease in Brazil are now working on the project. Sarno is likely to be the first to hear the results. "We will do the tests," he says. "If we’re right, we could change the world for the better."

Dejailson Arruda holds his daughter Luiza, born with microcephaly, at their house in Pemambuco state, Brazil.
From: Therapeutic applications of CRISPR RNA-guided genome editing
Brief Funct Genomics | © The Author 2016. Published by Oxford University Press. All rights reserved. For permissions, please email: journals.permissions@oup.com

Phages kill Staph A, Pneumonia, Salmonella, etc.

HIV/AIDS free Cells KO CCR5 or CXCR4; HPV, EBV, Hep B etc

DMD, CF, SCD, SCID, etc
One particularly controversial application of this powerful gene editing technology is the possibility of driving certain species to extinction – such as the most lethal animal on Earth, the malaria-causing *Anopheles gambiae* mosquito. This is, as far as scientists can tell, actually possible, and some serious players like the Bill and Melinda Gates Foundation are already investing in the project. (The BMGF funds The Conversation Africa.)

Yang’s genetically modified mushrooms were deemed exempt from current USDA regulation.
In 2014, researchers at the University of Texas showed that CRISPR could correct mutations associated with muscular dystrophy in isolated fertilized mouse eggs which, after being reimplanted, then grew into healthy mice. By February of this year, a team here at the University of Washington published results of a CRISPR-based gene replacement therapy which largely repaired the effects of Duchenne muscular dystrophy in adult mice. These mice showed significantly improved muscle strength – approaching normal levels – four months after receiving treatment.

A large team out of Harvard and MIT just debuted a CRISPR-based technology that enables precise detection of pathogens like Zika and dengue virus at extremely low cost – an estimated $0.61 per sample.
Designer Babies?
Unexpected mutations after CRISPR–Cas9 editing in vivo

To the Editor: CRISPR–Cas9 editing shows promise for correcting disease-causing mutations. For example, in a recent study we used CRISPR-Cas9 for sight restoration in blind FVB/NJ mouse by correcting a mutation in the Pde6b gene. However, concerns persist regarding secondary mutations in regions not targeted by the single guide RNA (sgRNA). Algorithms generate likely off-target sites for a given gRNA, but these algorithms may miss mutations. Whole-genome sequencing (WGS) has been used to assess the presence of small insertions and deletions (indels) but not to probe for single-nucleotide variants (SNVs) in a whole organism. We performed WGS on a CRISPR-Cas9-edited mouse to identify all off-target mutations and found an unexpectedly high number of SNVs compared with the widely accepted assumption that CRISPR causes mostly indels at regions homologous to the sgRNA.

We tested four sgRNAs in cells then chose the sgRNA with the highest activity for in vivo targeting. DNA was isolated from two CRISPR-edited mice (F03 and F05) and one uncorrected control. CRISPR-Cas9-treated mice were sequenced at an average depth of 50× and the control was sequenced at 30×. Variant calls were confirmed by at least 25× sequencing coverage (Supplementary Tables 1 and 2). Multiple variant-calling software pipelines identified indels and SNVs (Fig. 1 and Supplementary Methods).

In the CRISPR-treated mice, targeted alleles were repaired. Off-target mutations were identified as those present in the CRISPR-treated animals but absent in the uncorrected control. All pipelines showed that F03 harbored 164 indels and 1,736 SNVs (63 and 885 of these, respectively, associated with known genes). F05 harbored 128 indels and 1,696 SNVs (51 and 865 of these, respectively, associated with known genes) (Fig. 1). The same 117 indels and 1,397 SNVs were detected in both of the CRISPR-treated mice, which indicated nonrandom targeting. SNVs appeared to slightly favor transitions over transversions (Supplementary Fig. 1). The mutation rate detected in CRISPR–Cas9-treated mice was substantially higher than that generated by spontaneous germline mutations (3 to 4 indels and 90 to 100 SNVs per gene per generation). 1,4

As additional controls, each of the variants was compared with the FVB/NJ genome in the mouse dbSNP database (138), and each of the SNVs was also compared with all 36 strains in the Mouse Genome Project (139). None of the CRISPR-generated off-target mutations were found in any of these strains, which further confirmed that these WGS-identified SNVs were the result of CRISPR-Cas9 off-targeting. All pipelines identified 6 and 3 indels and 60 and 51 SNVs in F03 and F05 mice, respectively, in exonic regions only (Fig. 1); 5 indels and 24 SNVs caused nonsynonymous mutations in protein-coding sequences (Supplementary Tables 3 and 4). Of these, all five indels and one SNV (introducing a premature stop codon) were expected to be deleterious. Several muta-ted protein-coding genes were associated with a human and/or mouse phenotype (Supplementary Tables 3 and 4). Of the 29 coding-sequence variants, 7 variants were mutated identically in both mice. 24 CRISPR-associat-ed variants were selected, and all were confirmed by Sanger sequencing (Supplementary Fig. 2 and Supplementary Methods). Among the top fifty sequences predicted for off-targeting, none were mutated. Additionally, there was poor sequence homology between the sgRNA and sequences near the actual off-target coding and noncoding variants (Supplementary Fig. 4). Our results suggest current in vivo modeling cannot predict bona fide off-target sites.

Together, these results indicate that at least certain sgRNAs may target too low independently of their target in vivo. The unpredictable generation of these variants is of concern. The impact of the numerous mutations occurring in noncoding RNAs or other regulatory intragenic regions could be detrimental to key cellu-lar processes (Supplementary Fig. 4 and Supplementary Table 5). Although our CRISPR-treated mice did not display obvious extracellular phenotypes, it is possible the mice may reveal phenotypes in time, when they are challenged or bred to homozygosity. The present study demonstrates WGS analysis of both indels and SNVs as the most thorough method for identifying off-target mutations and shows a significantly higher number of potentially deleterious CRISPR-Cas9-induced mutations than have been previously reported. 1,5 It is not clear whether improved sgRNA
"These predictive algorithms seem to do a good job when CRISPR is performed in cells or tissues in a dish, but whole genome sequencing has not been employed to look for all off-target effects in living animals," says Alexander Bassuk, MD, PhD. In the new study, the researchers sequenced the entire genome of mice that had undergone CRISPR gene editing in the team's previous study and looked for all mutations, including those that only altered a single nucleotide.

The researchers determined that CRISPR had successfully corrected a gene that causes blindness, but Kellie Schaefer, a PhD student in the lab of Vinit Mahajan, MD, PhD, associate professor of ophthalmology at Stanford University, and co-author of the study, found that the genomes of two independent gene therapy recipients had sustained more than 1,500 single-nucleotide mutations and more than 100 larger deletions and insertions. None of these DNA mutations were predicted by computer algorithms that are widely used by researchers to look for off-target effects.

"Researchers who aren't using whole genome sequencing to find off-target effects may be missing potentially important mutations."

"Even a single nucleotide change can have a huge impact."

The researchers didn't notice anything obviously wrong with their animals. "We're still upbeat about CRISPR," says Dr. Mahajan. "We're physicians, and we know that every new therapy has some potential side effects—but we need to be aware of what they are."
**a**

- **Oocyte**
  - Maternal DNA
  - Sperm
  - Paternal DNA
  - Mutation
  - Cas9
  - Guide RNA
  - Inefficient gene editing
  - Mosaicism in later-stage embryo
  - Cell with non-repaired gene
  - Cell with repaired gene

**b**

- **Metaphase-II-stage oocyte**
  - Maternal DNA
  - Paternal DNA
  - MYBPC3
  - Efficient gene editing
  - Uniform later-stage embryo
Gene correction in S-phase-injected human embryos

NHEJ and HDR

Nonhomologous end joining (NHEJ) vs. Homology-directed repair (HDR)

Nuclease-induced double-strand break

Deletions

Insertions

Variable length indels

Precise insertion or modification

Donor template
Gene correction in M-phase-injected human embryos

Preimplantation development of CRISPR–Cas9-injected embryos

paternal
maternal

Inter-homologue HDR
(non-crossover)

Inter-homologue HDR
(crossover)

NHEJ
(long deletion)

no PCR product

DNA / beta tubulin
BF

Interphase
Interphase
NEBD
Prometaphase

Metaphase
Telophase
G1
Interphase
Prometaphase
Metaphase

0-30min
100 min
3.5h
day 1
day 1
day 1

Concerns...

NHEJ (16 of 58) vs HDR
Efficiency
Mosaicism
Off-target Edit

41 of 42 embryos relied on maternal template – Unique and challenging, almost miraculous, especially since pronuclear union delay during ICSI
Removal or Repair of Defective Gene? Developmental Anomalies?
In ‘Enormous Success,’ Scientists Tie 52 Genes to Human Intelligence

Carl Zimmer

May 22, 2017

In a significant advance in the study of mental ability, a team of European and American scientists announced on Monday that they had identified 52 genes linked to intelligence in nearly 80,000 people.

These genes do not determine intelligence, however. Their combined influence is minuscule, the researchers said, suggesting that thousands more are likely to be involved and still await discovery. Just as important, intelligence is profoundly shaped by the environment.

Still, the findings could make it possible to begin new experiments into the biological basis of reasoning and problem-solving, experts said. They could even help researchers determine which interventions would be most effective for children struggling to learn.
<table>
<thead>
<tr>
<th>Life Chances</th>
<th>High Risk</th>
<th>Uphill Battle</th>
<th>Keeping Up</th>
<th>Out Ahead</th>
<th>Yours to Lose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training Style</td>
<td>Slow, simple, hands-on</td>
<td>Very explicit, hands-on</td>
<td>Mastery learning, hands-on</td>
<td>Gathers, infers own information</td>
<td>College format</td>
</tr>
<tr>
<td>Career Potential</td>
<td>Assembler, food service, nurse’s aide</td>
<td>Clerk, teller, police officer, machinist, sales</td>
<td>Manager, teacher, accountant</td>
<td>Attorney, chemist, executive</td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>Population Percentages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total population distribution</td>
<td>5</td>
<td>20</td>
<td>50</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Out of labor force more than 1 month out of year (men)</td>
<td>22</td>
<td>19</td>
<td>15</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Unemployed more than 1 month out of year (men)</td>
<td>12</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Divorced in 5 years</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Had illegitimate children (women)</td>
<td>32</td>
<td>17</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Lives in poverty</td>
<td>30</td>
<td>16</td>
<td>6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ever incarcerated (men)</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Chronic welfare</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Spermatogonial stem cell transplantation into Rhesus testes regenerates spermatogenesis producing functional sperm. Hermann, B…Mitalipov, S; Schatten, G; Orwig, K. Cell Stem Cell
Spermatogonial stem cell transplantation into Rhesus testes regenerates spermatogenesis producing functional sperm.

*CELL STEM CELL* 11, 715–726

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H</strong></td>
<td>Oocyte Donor 1 26618</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>Oocyte Donor 2 28510</td>
</tr>
<tr>
<td><strong>J</strong></td>
<td>M092 Transplant Donor</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>M027 Transplant recipient</td>
</tr>
<tr>
<td><strong>L</strong></td>
<td>Embryo 1 (5-cell) (dam 28510)</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td>Embryo 49 (monula 2.5) (dam 25168)</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>Embryo 51 (monula 2.5) (dam 25168)</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>Embryo 63 (monula 2.5) (dam 25168)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marker</th>
<th>DXS2506</th>
<th>D16S823</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H</strong></td>
<td>262/262</td>
<td>353/361</td>
</tr>
<tr>
<td><strong>I</strong></td>
<td>268/270</td>
<td>341/357</td>
</tr>
<tr>
<td><strong>J</strong></td>
<td>286/Y</td>
<td>337/341</td>
</tr>
<tr>
<td><strong>K</strong></td>
<td>270/Y</td>
<td>317/365</td>
</tr>
<tr>
<td><strong>L</strong></td>
<td>270/Y</td>
<td>337/341</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td>262/286</td>
<td>337/361</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>262/286</td>
<td>341/361</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>282/Y</td>
<td>337/363</td>
</tr>
</tbody>
</table>
Are we too brave?