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New Knowledge on the Causes of Human Birth Defects: Impact on Society

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Introduction

Two newly discovered causes of human birth defects will be discussed here. First, it has been found that *de novo* heterozygous mutations are a common cause of congenital heart disease, implicating hundreds of genes. Second, Zika virus presents us with a new epidemic that causes defects in fetal brain development by killing neuron progenitor cells in the cerebral cortex. The possibilities of new technologies for control of our ancient enemy *Aedes aegypti*, the mosquito vector of Zika, will be discussed. Finally, the consequences of these diseases for society and the dignity of the human person will be addressed.

Heart malformations caused by *de novo* loss of single genes

Congenital heart disease is the most frequent birth defect in humans affecting 0.8% of live births. Richard Lifton of Yale University invented the exome sequencing method by which one can readily sequence with the new high-throughput methods all human protein-coding regions, which are contained in less than 2% of our DNA. His team sequenced trios of hundreds of probands and their two parents. Unexpectedly, 10% of cardiac malformations had *de novo* loss-of-function point mutations that were deleterious to protein function causing premature termination, genetic code frameshifts, or splicing defects (1). These mutations were *de novo*, as they were absent from both parents. Since the patients were heterozygous, the mutations originated in the germ cells of one of the two parents.

While surgery can now repair heart defects very effectively, clinically some of these children fail to thrive presenting autism, slower growth and shorter life spans. By comparing the number of mutations detected only once to the number of genes mutated twice independently in the patients analyzed, the authors could calculate that an astounding 400 genes may be haploinsufficient for heart development in humans (1). Similarly, hundreds of potential heterozygous gene targets were found in children with autism (2, 3).

Some of the mutations were in histone methylation enzymes. Histones are proteins that form nucleosomes around which the DNA wraps around in order to be packaged and condensed. Histone-modifying enzymes cause epigenetic marks in every gene enhancer and promoter in the genome. Humans have at least 400,000 tissue-specific enhancers which are marked by a modification called H3K4Me (mono-methylation in Lysine 4 of Histone 3). Multiple mutated genes in this methylation pathway were identified (1). This means that specific phenotypes in heterozygous mutations can arise from very basic biochemical processes that affect every single cell in the body.

The basis of Mendelian genetics is that the loss of one gene (called an allele) is recessive and has no phenotype. These new findings imply that humans have hundreds, likely thousands, of genes that can cause disease if present in only one copy.

Microcephaly epidemic caused by Zika virus

Zika virus is an RNA virus of the Yellow fever family (called Flavivirus, for golden-yellow in Latin). Other members of this family include Dengue and Chikungunya viruses. Zika virus was first isolated from a sentinel monkey in the Zika forest in Uganda in 1947. In 2007 it infected humans in Micronesia, and in 2013 and 2014 epidemics took place in French Polynesia (4). In July 2015 an outbreak of the rare birth defect microcephaly appeared in the Brazilian Northeast, one of the poorest regions in the continent.

Zika virus infection presents itself with fever, skin rash, painful joints and muscles, conjunctivitis and sometimes blood in semen. The latter sign led to the early discovery that Zika virus can be transmitted sexually. With low frequency it can also induce Guillain-Barré syndrome, a paralysis caused by an autoimmune attack of peripheral nerves.

Zika virus causes microcephaly by infecting cerebral cortex neuron progenitor cells called radial glia and causing their death (5, 6). The human cortex adds neurons throughout embryonic development. One month after fertilization we have 10,000 brain cells, at two months 100,000, at 5 months 10,000,000, and at birth

an estimated ten billion neurons. Additional cortical neurons are added during the first year. Consequently, infection at any time of pregnancy can produce grave defects. Most organ systems are formed between 4-8 weeks of embryonic development and teratogenic insults during the following fetal growth period produce relatively mild defects. The continuous birth of neurons in the cerebral cortex generates a uniquely prolonged target. Infection during late pregnancy may not cause microcephaly, but can still cause epilepsy and learning disabilities. Brain calcifications, a thin cerebral cortex, and enlarged brain ventricular cavities are radiologic signs of Zika virus viral infection.

Fighting our ancient enemy *Aedes aegypti*

Zika virus is transmitted by the Yellow fever mosquito *Aedes aegypti*. *Aedes*, which means odious in Greek, learned how to cohabit with humans 10,000 years ago in Africa by breeding in water storage containers inside dwellings (7). This species can be readily distinguished by the shape of a lyre on the back of its thorax. In Kenya, domestic and sylvatic (forest) *Aedes aegypti* mosquitoes coexist and interbreed in the same areas. By sequencing genes expressed in antennae, where their sense of smell resides, it was found that the key difference between both types of mosquitoes is the level of expression of an odorant receptor that senses a human odor called sulcatone (8). The domestic *Aedes aegypti,* but not the sylvatic form, strongly prefers biting humans rather than animals such as guinea pigs. The female of this mosquito has the odious habit of resting inside the shade of homes and biting multiple times per day. It usually bites during daytime, making bed nets ineffective. This behavior makes *Aedes aegypti* a very efficient vector to spread viruses from one human to another.

Aedes aegypti breeds near or inside homes in water barrels, flowerpots, plant pot bases, humid gutters, ditches, and discarded car tires and containers. The mosquito was imported into the Americas by the slave trade and even today redistributed by the transport of used car tires.

Substandard housing and lack of sanitation are a major problem in the underdeveloped world, explaining the sudden epidemic that has swept through Recife, Natal, Rio de Janeiro, Colombia, Venezuela, Puerto Rico and Cuba. During the Spanish-American war the US army lost more soldiers to Yellow fever than to fighting in Cuba. Dr. Walter Reed, serving as an officer, discovered that *Aedes aegypti* was the disease vector of a virus present in human serum after filtering through porcelain filters. Soldiers that consented to be bitten by infected mosquitoes died in this quest, but as a consequence of these studies most homes in the United States now have insect screens in their windows (and air conditioning in warm regions). Thus, diseases like Dengue fever that are very prevalent in poor countries of Latin America and the Caribbean have not affected the United States as much, despite the presence of *Aedes*.

In addition to sanitation, new mosquito control mechanisms are needed. Mosquitoes have killed more humans than any other animal if one includes malaria, which is transmitted by *Anopheles* (7). DDT use is now prohibited. Release of irradiated sterile males (or transgenic debilitated males) is not very effective because continued release in large numbers is required to decrease the population.

***Wolbachia* and CRISPR/Cas9 population-drive approaches**

A very promising approach has been pioneered by Scott O'Neill of Melbourne, Australia, using *Wolbachia* bacteria. These intracellular bacteria infect 40% of all insects, but not mosquitoes. *Wolbachia* has spread through fruit flies of the genus *Drosophila* worldwide. *Wolbachia* is driven through insect populations by a phenomenon called cytoplasmic incompatibility. If an uninfected female mates with a *Wolbachia*-infected male, most of the resulting eggs become infected and die due to lack of immunity. However, once an infected female survives, she can mate productively with both infected and uninfected males. This results in the rapid spread (or drive) of the bacterium throughout the insect population. *Wolbachia* colonizes salivary glands, oocytes, gut and other organs without affecting lifespan (9). *Wolbachia* infection has an important beneficial effect for the insect, because it stimulates innate immunity preventing replication of RNA viruses. Consequently, *Wolbachia*-infected mosquitoes are unable to spread Dengue and Zika viruses in their saliva (9, 10).

A strain called wMel obtained from *Drosophila melanogaster* was cultured for two years inside a mosquito cell line and then microinjected into *Aedes aegypti* eggs (9). Once *Aedes* infected with *Wolbachia* is released it spreads rapidly throughout the population driven by the cytoplasmic incompatibility mechanism. The population levels of *Aedes aegypti* are maintained but are no longer able to transmit disease. Infected mosquitoes of both sexes can be released into the wild because females will not transmit the viruses. This appears to be a very promising approach and small-scale releases into cities have already been carried out in Cairns and Rio de Janeiro, and much larger ones are planned in Brazil.

Another gene-drive approach which is still in its infancy, and so far has only been tested in the malaria mosquito, is based on the new CRISPR/Cas9 gene editing technology. Ethan Bier at the University of California San Diego has devised methods by which DNA segments containing both the Cas9 nuclease and a guide

RNA flanked by segments of DNA on either side of the cleavage site will insert at desired targets in the germ line of *Drosophila* and *Anopheles*. Once inserted, it is enough to have the gene in one chromosome, since it will edit a wild-type chromosome at the same exact site, converting a heterozygote into a homozygote and rapidly spreading through the population (11). Genes that provide immunity to the malaria parasite have been inserted into this gene-drive cassette in *Anopheles*. These tests are purely experimental at the time of writing (November 2016) and these fruit flies or mosquitoes have not been released into the wild as they are expected to spread rapidly. This gene-drive method will probably be impractical in its present form because resistance will eventually arise by insertions and deletions that are no longer recognized by the guide RNA. However, it serves to illustrate the awesome power of the emerging gene editing technology.

Fortunately, a Zika virus vaccine is expected to be simple to prepare. Several approaches have already been shown to be effective in monkeys. Yellow fever was defeated by a vaccine. The only Nobel Prize awarded for a vaccine was that of Max Theiler in 1951, who pioneered the use of chicken eggs to grow Yellow fever virus.

Conclusion

High-throughput DNA sequencing will make it possible to identify a large number of babies with disease caused by loss-of-function mutations in a single gene allele. The Zika virus epidemic will result in many persons with brain defects. It seems to me that the sanctity of human life will have to be defended vigorously from a global secular culture of death openly promoting euthanasia, if we are to avoid the specter of eugenics and infanticide.

In the encyclical *Evangelium Vitae* (12) Saint John Paul wrote: “Every individual, precisely by reason of the mystery of the Word of God who was made flesh (cf. Jn 1:14), is entrusted to the maternal care of the Church. Therefore every threat to human dignity and life must necessarily be felt in the Church's very heart; it cannot but affect her at the core of her faith in the Redemptive Incarnation of the Son of God, and engage her in her mission of proclaiming the Gospel of life in all the world and to every creature (cf. Mk 16:15). Today this proclamation is especially pressing because of the extraordinary increase and gravity of threats to the life of individuals and peoples, especially where life is weak and defenceless. In addition to the ancient scourges of poverty, hunger, endemic diseases, violence and war, new threats are emerging on an alarmingly vast scale.” *Verbum sapientiae*, wise words indeed. The protection of the weak is as necessary now as ever.

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