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A Problem in Genetic Counseling

Our individual genome is considered to be immutable, but mutations sometimes appear. Identifying them can be a complex challenge.

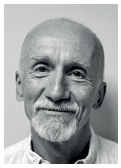
Janusz G. Zimowski

Department of Genetics and Genomics, National Medical Institute of the Ministry of the Interior and Administration in Warsaw, Poland

We very often hear that the set of genes we received from our parents is given to us once and for all, that it remains unchanging throughout our lives. While this is true in principle, there are nevertheless certain deviations from it.

The first exception is the impact of the wider external environment on our genome – despite the various repair systems we have in place. This includes the factors that cause mutations (UV light, oxidative agents, many chemicals, etc.), which can, in individual cells of our body, lead to changes in the nucleotide sequence of DNA.

The changes may be insignificant and never make themselves known to us, but they can also have serious consequences for cellular function and lead to pathogenic processes. This results in somatic mutations – mutations that arise in the cells that build our body. The body tries to get rid of such damaged cells, for which it uses the mechanism of programmed



Janusz G. Zimowski, PhD, DSc

has been an Assistant Professor at the Institute of Biochemistry, University of Warsaw and an Assistant Professor at the Department of Genetics, Institute of Psychology and Neurology in Warsaw. He specializes in the genetics of rare diseases, including DMD/BMD, SMA and prion diseases.

janusz.zimowski@cskmswia.gov.pl

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cell death – a process known as apoptosis. Sometimes, however, a genetically altered cell gets out of control and becomes a cancer cell, which begins to live a life independent of the rest of the cells in that organism.

Mutations

Another exception to the invariability of our genome happens when information about what an organism has encountered during its life gets "inscribed" into its genome. This is done by chemically modifying DNA by methylating certain sections rich in C and G nucleotides (so-called CpG islands). This process is mostly reversible and serves to regulate the function of many genes. But it, too, can escape from the organism's control and lead to pathogenic changes. As long as such changes do not affect reproductive cells, they are not passed on to offspring and can only affect the health of the individual organism in whose genome they appear.

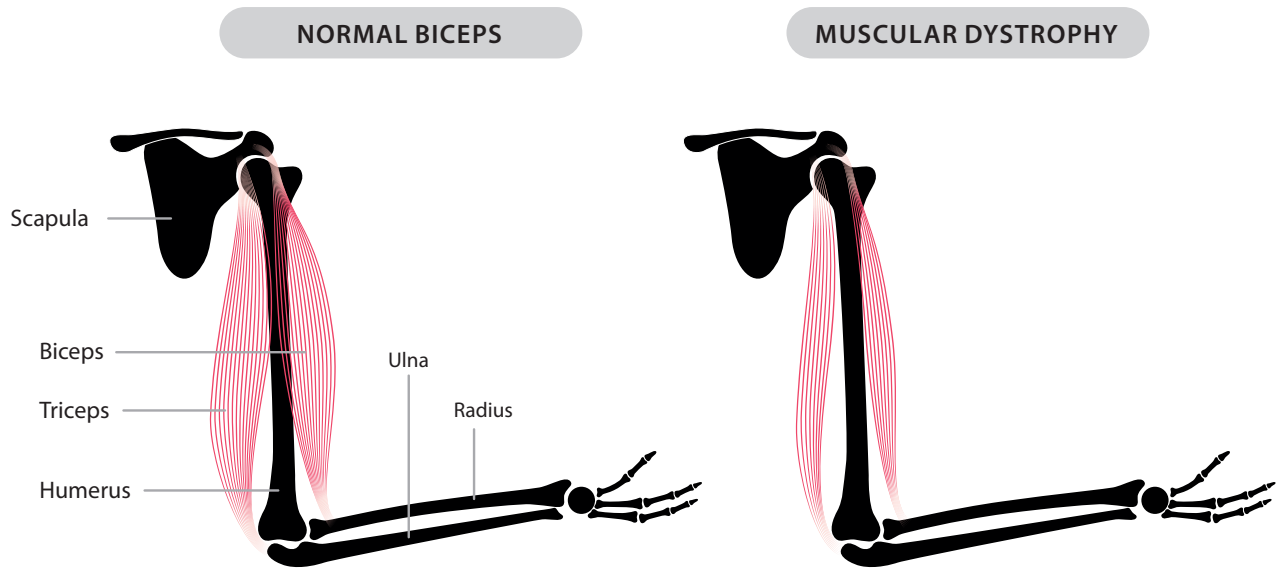
> A distinctive kind of mutation can occur during embryogenesis, when rapidly dividing cells differentiate to give rise to different types of tissue.

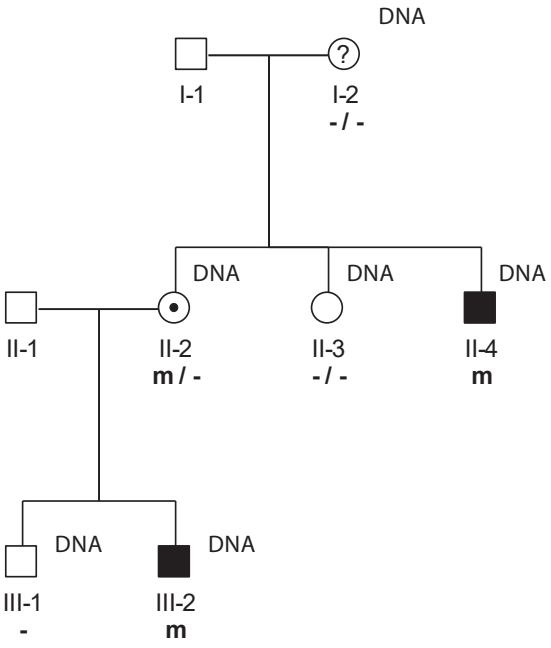
The situation is different with genetic changes that occur during the formation of reproductive cells. The process of cell division is highly imperfect and can generate numerous errors, creating certain defective reproductive cells alongside normal ones. If a reproductive cell containing a genetic change takes part in fertilization, it gives life to a new organism with this change in its genome. Some such changes will occur in non-coding sequences which are irrelevant in the processes of regulating the operation of the genome and so they will not manifest themselves in how the organism functions. Others, however, may turn out to be relevant in combination with ancestrally inherited (previously occurring) mutations and so give a phenotypic effect (disease), while some others will give a pathogenic effect.

A separate type of genetic changes involves mutations that occur during embryogenesis, when rapidly dividing cells differentiate to form cell clones that give rise to individual tissues. When a change in the DNA nucleotide sequence occurs in a cell that is one of the first clone cells leading to the formation of the tissue of, for example, a gonad, the change will be present only among the cells of the resulting organ. The remaining cells of the organism will be normal in the genetic sense and the organism will develop without any visible change. The individual carrier of such a change will not be aware that there are mutated cells in his/her body. This genetic change may only become apparent in the next generation – provided that a mutated reproductive cell is involved in fertilization.

Genetic diseases

One of the many genetically determined diseases known today is called Duchenne/Becker muscular dystrophy (DMD/BMD). It is inherited in a sex-linked recessive manner, meaning that boys are affected, while women remain healthy. In its acute form (DMD), the disease manifests itself and is diagnosed before the end of the third year of life, although some





Example family affected by Duchenne/Becker muscular dystrophy. A square symbolizes a male individual, a circle a female. Blackened symbols denote affected individuals, whereas a black dot inside the symbol indicates a confirmed carrier of the mutation. The question mark indicates a woman who tested negative for carrier status according to a molecular study, but whose two children nevertheless have a disease-causing mutation in their genome, signifying hidden carrier status due to germinal mosaicism

discrete symptoms may be noticed earlier. It causes progressive and irreversible atrophy of the skeletal muscles, leading to immobility at age 10 and inevitable death in the third decade of the patient's life. The disease is caused by changes in the nucleotide sequence of a gene located on the X chromosome, responsible for encoding information on the synthesis of a protein called dystrophin. This results in the complete absence of a protein necessary for the proper functioning of muscle cells. The continued stable incidence of the disease (about 1 in 3,500 live-born boys), despite the childless deaths of affected males, attests to the systematic natural emergence of mutations of this type. In addition, the extremely severe course of the disease and the lack of any therapy able to effectively address the cause of the disease gives rise to high diagnostic interest in women who are or may be carriers – especially those in families affected by DMD/BMD.

Molecular diagnosis of whether a woman is a DMD/BMD carrier involves looking for mutations in the dystrophin gene in her genome. Obviously, since women have two X chromosomes (whereas men have one X and one Y chromosome), they have two copies of this gene – in DMD/BMD carriers one of them is damaged, the other normal. The protein produced from the undamaged copy of the gene ensures that the female carriers themselves have properly functioning muscle cells. Unfortunately, however, a woman carrying DMD/BMD has a high, 50% risk of giving birth to a son suffering from the disease.

A woman in a family affected by Duchenne/Becker muscular dystrophy – in which, for example, her brother and son have the condition – is certainly a carrier of the disease, having inherited the mutation from her mother. On the other hand, the first appearance of the disease in a particular family (with no information about any other sick relatives in the mother's family) raises the question of whether the mother of the affected son is the carrier of a hidden mutation in her genome and is therefore at considerable risk of giving birth to another child who has the condition, or whether this is a case caused by a newly occurring mutation and so the risk of another diseased son is very low, equal to the population risk. Taking into account the stable incidence of DMD/BMD and the childless death of affected individuals, the theoretical probability of the mother of an affected son being a carrier is 66 percent. This means that some 33 percent of mothers of affected boys are not actually carriers of the disease. A more conclusive answer to the question can therefore only be provided by molecular testing.

Diagnostics

The analysis begins by identifying the mutation in the affected boy, in order to confirm the clinical diagnosis made by the doctor. This is followed by carrier testing in women who are related to him. Carrier testing as well as molecular testing of the patient is performed using DNA isolated from blood cells, which are assumed to be representative of all cells in the tested individual's body. Finding mutations in the patient and his mother in such a test gives unambiguous information: the mother certainly has the status of a carrier. The situation is different, however, if no mutation is identified. This is usually interpreted as ruling out carrier status of the mother, but such a conclusion may be subject to error due to the use of genetic material not derived from the germ cells for DNA testing. Women in whom no mutation of the dystrophin gene is detected by molecular testing, but who nevertheless give birth to another child with a mutation causing DMD/BMD, have so-called germinal mosaicism (or germline mosaicism), which means they have clones of mutated cells intermingled with their normal cells. We can refer to such women as having "hidden carrier" status. It is estimated that the incidence of germinal mosaicism among women who have been ruled out as carriers of Duchenne/Becker muscular dystrophy is above 10 percent.

The phenomenon of germinal mosaicism poses a significant problem in genetic counseling, making it impossible to confidently rule out carrier status. For this reason, women who have given birth to a son affected by Duchenne/Becker muscular dystrophy and in whom no disease-causing mutation has been detected in molecular testing of their genome should always be informed that they are nevertheless at increased risk of giving birth to a son affected by DMD/BMD or a daughter who is a carrier of the disease. ■

Further reading:

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