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EPIGENETIC AND GENETIC ASPECTS OF THE PHENOTYPES OF PRADER-WILLI AND ANGELMAN SYNDROMES

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The study of the epigenetic and genetic mechanisms of genomic imprinting and single-parent dysomia, as the most common disorders in Prader-Willi syndrome and Angelman syndrome, contributes to the discovery of the pathogenesis of these genetic anomalies and provides a basis for searching for new methods for their prevention and epigenetic therapy. Monoallelic expression of genes in genomic imprinting develops due to different methylation of 15 chromosome DNA, which is specific for different sexes. Prader-Willi syndrome develops as a result of microdeletion at the *q11-q13* locus and the mutation of the candidate gene *SNRPN* of the paternal chromosomes. Angelman syndrome – as a result of microdeletion at the locus *q11-q13* and mutation of the candidate gene *UBE 3A* in the maternal chromosome. The father's single-parent dysomia becomes the cause of Angelman syndrome, and the mother's single-parent dysomia is the cause of Prader-Willi syndrome. It can be assumed that similar Prader-Willi syndrome and Angelman syndrome disorders of genomic imprinting can arise as a result of demethylation of female and male chromosomes with the help of methyltransferases.

Key words: genomic imprinting, single-parent dysomia, DNA methylation, Angelmann syndrome, Prader-Willi syndrome.

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ЕПІГЕНЕТИЧНІ ТА ГЕНЕТИЧНІ АСПЕКТИ ФЕНОТИПІВ СИНДРОМІВ ПРАДЕРА-ВІЛЛІ ТА АНГЕЛЬМАНА

Вивчення епігенетичних і генетичних механізмів геномного імпритинга і однобатьківської дисомії, як найбільш частих порушень при синдромі Прадера-Віллі та синдромі Ангельмана, сприяє розкриттю особливостей патогенезу цих генетичних аномалій і створюють основу для пошуку нових методів для їх профілактики та епігенетичної терапії. Моноалельна експресія генів у геномному імпритингу розвивається внаслідок різного специфічного для різної статі метилування ДНК 15 хромосоми. Синдром Прадера-Віллі розвивається внаслідок мікроделеції в локусі *q11-q13* і мутації кандидатного гена *SNRPN* батьківських хромосом. Синдром Ангельмана – в результаті мікроделеції в локусі *q11-q13* і мутації кандидатного гена *UBE 3A* в материнській хромосомі. Батьківська однобатьківська дисомія стає причиною Синдрому Ангельмана, а материнська однобатьківська дисомія – причиною синдрому Прадера-Віллі. Можна припустити, що подібні порушення геномного імпритинга при вищевказаних синдромах можуть виникати внаслідок деметилювання жіночих і чоловічих хромосом за допомогою метилтрансферази.

Ключові слова: геномний імпритинг, однобатьківська дисомія, метилювання ДНК, синдром Ангельмана, синдром Прадера-Віллі.

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Advances in medical genetics have made it possible to identify a new group of hereditary human diseases, namely, genomic imprinting disorders, to which Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are assigned. Hereditary genetic diseases of children caused by PWS and AS are manifested in the phenotype with various clinical symptoms and are rare genetic abnormalities with the incidence of 1:10,000 to 1:20,000 newborns. Genomic imprinting is referred to different gene activation, which depends on which of the parents (mother or father) the mutation was inherited [5, 7, 8]. The example can be the deletion of the long arm *q11-q13* of chromosome 15, containing 3 to 4 million base pairs. The genes of this locus, depending on the origin of the chromosome, are expressed in various tissues at different periods of ontogenesis. If the patient inherits this chromosomal aberration from the mother, then it will be clinically manifested by AS, if from the father – by PWS.

The purpose of the paper was to clarify and study possible epigenetic mechanisms of the Prader-Willi and Angelman syndromes development to fully reveal the features of these genetic abnormalities pathogenesis.

Materials and methods. On the basis of research material on epigenetic mechanisms of the development of Angelman syndrome and Prader-Willi syndrome, the molecular mechanisms of genomic imprinting disorders and uniparental disomy of chromosome 15 have been studied. This makes it possible to explain not only the differences in their phenotypes, but also the mechanism of individual

symptoms of the diseases, which can be explained by gender-specific methylation of cytosine bases, which is established during gametogenesis (spermatogenesis or ovogenesis) and deletes transcription of one of the parental alleles and such an allele is deleted or inactivated.

Results of the study and their discussion. PWS was first described in 1956 in Switzerland. The incidence of this pathology is 1:12000-15000 live births. 70 % of PWS cases result from deletion on the parental chromosome, in other cases it is caused by maternal disomy of chromosome 15. PWS is the most common genetic cause of obesity in newborns (1: 15-20000). At birth, babies are sedentary, have prominent hypotension, tendon reflexes are reduced, as well as sucking and swallowing, which makes it difficult to feed the baby. Up to 2-5 years of age, patients have severe hyperphagia and a number of metabolic disorders. Obesity in children develops after 6 months of age, as patients are constantly hungry. In addition, the phenotypic signs of PWS are: neonatal muscle hypotension, growth retardation and psychomotor development delay, hypogonadism, sleep apnea, anthropometric peculiarities (acromicria, narrow temporal region of the skull, dolichocephaly), low insulin resistance compared to children with exogenous constitutional obesity. Metabolic syndrome in PWS is a risk factor for atherosclerosis development. Mortality of PWS patients often results from the consequences of complications of obesity, type 2 diabetes, respiratory failure, cardiovascular diseases [1].

AS was first described in 1965. Harry Angelman presented the case histories of three patients. Taking into account the peculiarities of their behavior and phenotype, the researcher named this syndrome “puppet children”. It received its final name in 1982. The main diagnostic signs of AS are: microbrachycephaly, large lower jaw, wide mouth with protruding tongue, macrostomy, wide-spaced teeth, hypopigmentation (40 %), psychomotor retardation, ataxia, hypotension, hyperreflexia and hyperkinesia, fits of uncontrollable laughing, hand-clapping, as well as a specific facial expression that resembles a puppet [7]. Typical are asthenic body type, “birdy” nose, micrognathia. Sick children start to walk late and their gait resembles movements of a puppet.

In addition to the deletion of *15q11-q13* on the maternal chromosome, the development of AS is caused by uniparental disomy of chromosome 15. The following mechanisms of AS occurrence are described: 1) *de novo* deletion at the *15q11-q13* locus (70-80% of all cases), 2) parental disomy, 3) imprinting centre defect, 4) mutation of the maternal copy of the gene that encodes ubiquitin protein ligase (*UB3A* gene) [7].

In the critical segment of chromosome 15, oppositely imprinted candidate PWS and AS loci, *SNRPN* and *UBE3A*, were found. The *UBE3A* gene is localized on the long arm (*q*) of chromosome 15 at locus *11.2* (Fig. 1). It contains 101,779 base pairs [8, 11].

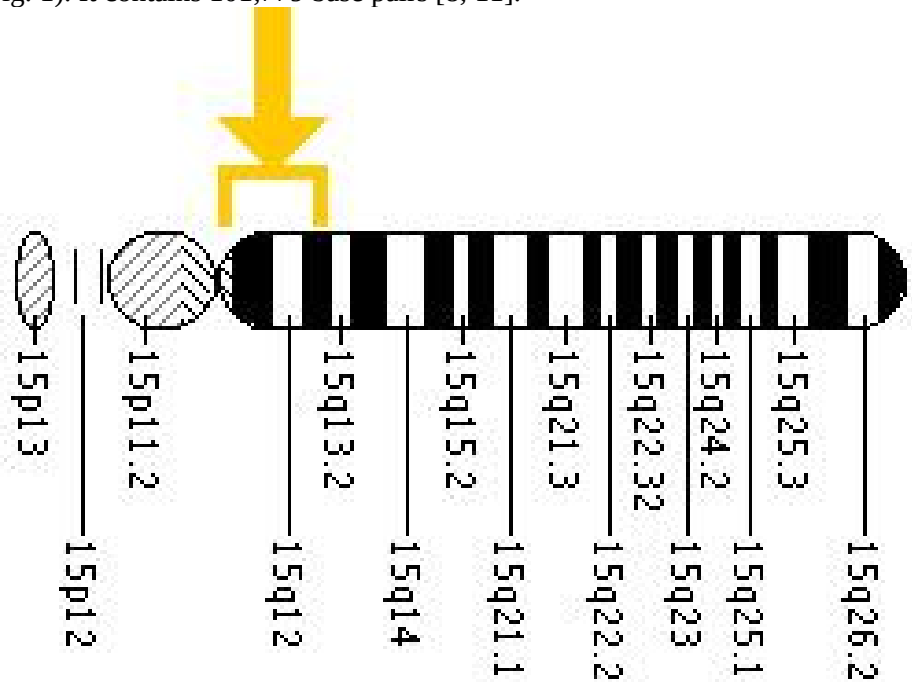

Fig. 1. Locus of the *UBE3A* gene on chromosome 15

Mutations in chromosome 15, leading to Angelman syndrome are shown in fig. 2 [11].

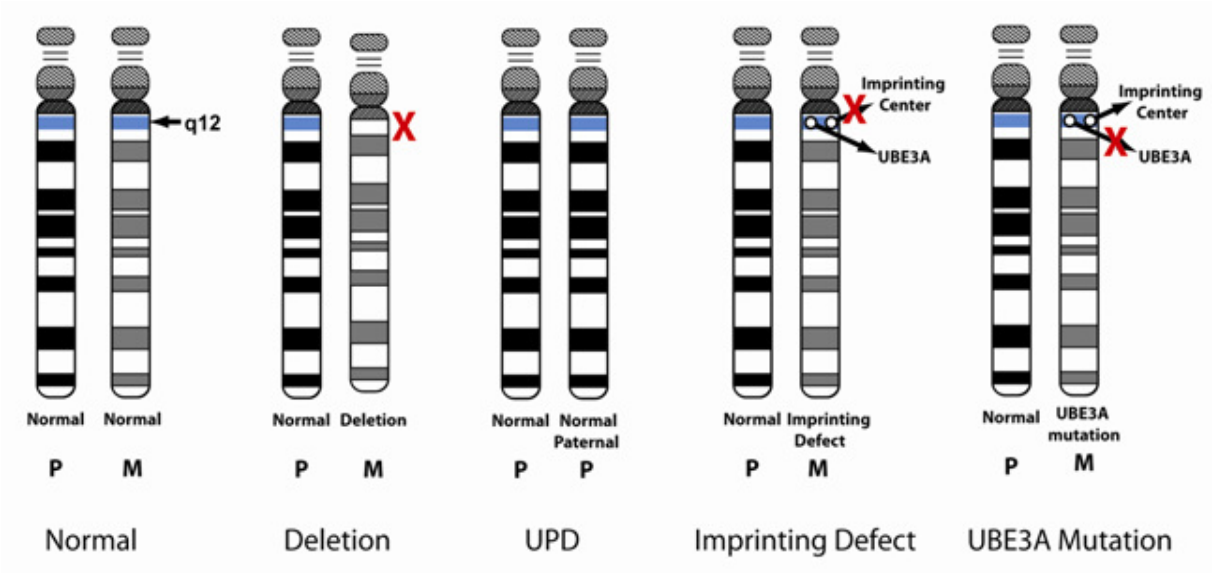

Fig. 2. Types of chromosome 15 mutations in Angelman syndrome

Figure 3 shows the incidence of genetic classes of Angelman syndrome [4].

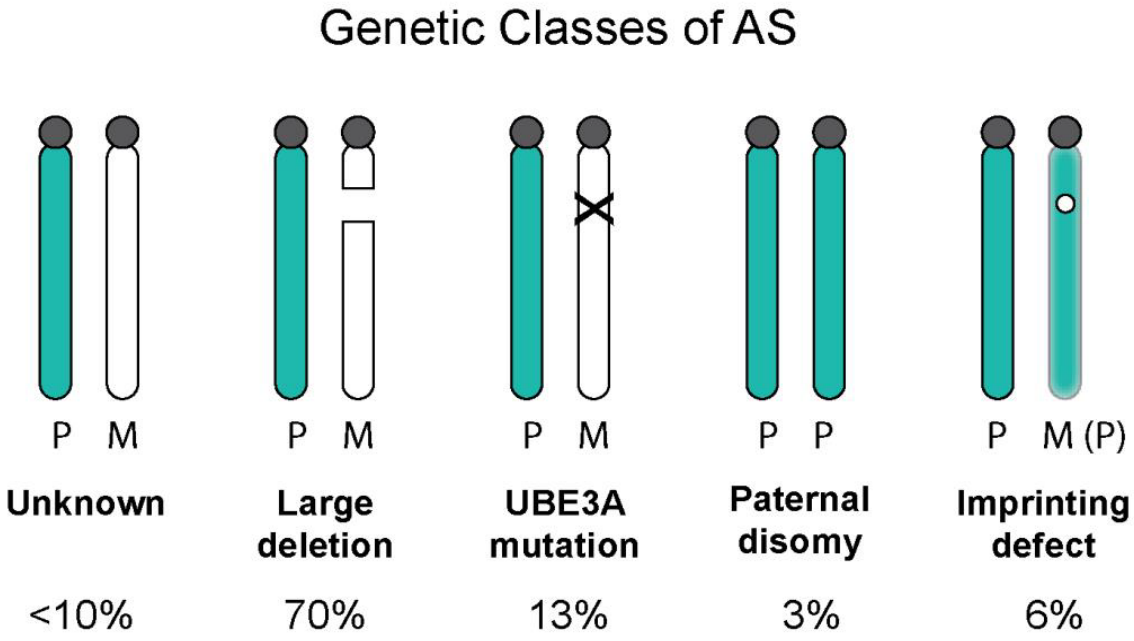

Fig. 3. The incidence of genetic classes of Angelman syndrome

Table 1 presents the differential diagnosis of genetic defects in Prader-Willi syndrome and Angelman syndrome [9].

Table 1

Differential diagnosis of genetic manifestations of Prader-Willi and Angelman syndromes

Genetic manifestations	PWS	AS
deletion of *15q11-q13* on paternal chromosome	+	-
deletion of *15q11-q13* on maternal chromosome	-	+
UBE3A point mutation	-	+
imprinting centre defect	-	+
maternal uniparental disomy	+	-
paternal uniparental disomy	-	+

The *SNRPN* gene encodes a small nuclear ribonucleoprotein polypeptide N, mutations of which have been found in PWS. Closer to the centromere from the locus of this gene, the imprinting center is located, which includes 5 & apos;-exones of the *SNRPN* gene, where alternative splicing occurs. Epigenetic mutations (epimutations) in these exons cause AS through the ability to erase the imprint of the previous generation, that is, in the ontogeny, the ability to rewrite the imprint of imprinting in the

direction from male to female is lost, and during spermatogenesis – from female to male. Distally from the *SNRPN* gene, there are genes that encode non-translating RNA, which can be involved in the control of imprinting. The *UBE 3A* gene, which encodes the ubiquitin ligase protein, is expressed from the maternal chromosome in the brain, and in other organs biallelic. Mutations at this imprinting target-locus have been found in familial cases.

In the case of sporadic mutation in the imprinting center, the risk for the disease in the family is less than 1 %. However, in the case of mutation in the imprinting center on the maternal chromosome 15 proband, which was inherited from the father, the risk of having a child with AS increases to 50 %. It is possible to differentiate the described molecular forms of the disease in proband using molecular-genetic methods [2].

In addition to karyotyping, which makes it possible to determine deletions on chromosome 15, a diagnostic test for PWS and AS is carried out to determine the methylation status of genes that have been imprinted. In PWS, regardless of the cause of its occurrence (parental deletion, maternal uniparental disomy, or mutation of the imprinting center), only methylated alleles of the loci *D15S63, ZNF127* and the promoter portion of the *SNRPN* gene are present. Cases of AS (except for point mutations in the candidate gene *UBE3A*) are characterized by the presence of only unmethylated alleles of these loci [7].

Thus, classical mutations do not always play a crucial role in the inheritance of monogenic human diseases. Epigenetic transgenerational monoallelic inheritance and the phenomenon of genomic imprinting are characteristic of Angelman and Prader-Willi syndromes. It is important to carry out the methyl-specific polymerase chain reaction for screening DNA diagnostics of these syndromes. The search for other portions in the human genome that affect the inheritance of chromosomal diseases is promising. The study of the epigenetic and genetic mechanisms of genomic imprinting and uniparental disomy (UPD), as the most frequent disorders in PWS and AS, contributes to the disclosure of the features of pathogenesis of these genetic abnormalities and creates the basis for the search for novel methods for their prevention and epigenetic therapy.

Studies of the molecular mechanisms of genomic imprinting disorder and UPD on chromosome 15 in PWS and AS make it possible to explain not only the differences in their phenotypes, but also the mechanism of individual symptoms of the diseases. The diverse clinical symptoms of PWS and UPD can be explained by the cytosine base methylation that is specific for individuals of different gender, which is established during gametogenesis (spermato- or ovogenesis) and deletes the transcription of one of the parental alleles, while this allele is deleted or inactivated [6]. Thus, as a result of methylation of imprinted genes, chromosome 15 can express genes of only one of the alleles – paternal or maternal. Normally, maternal genes are active and paternal genes are inactive. This selective suppression of imprinted genes occurs due to methylation during passage through the germ cells. Selective methylation of regulatory portions of genes during spermatogenesis or ovogenesis plays the key role in the genomic imprinting. When methylation is impaired in PWS children, paternal genes are expressed, and in AS children, maternal genes are expressed.

UPD occurs due to the “erroneous” inheritance of two homologous chromosomes 15 from one of the parents by the child. Parental disomy can occur as a result of nondisjunction of 15 pair of chromosomes in meiosis during the formation of male and female germ cells, as well as during mitosis at the early stages of embryonic development. Male or female mutant alleles of imprinted genes can cause different clinical picture of diseases in PWS and AS.

For a long time, it remained unclear how, in microdeletions in the same portion of *q11-q13* chromosome 15, some children develop PWS, while others develop AS. On the basis of cytogenetic studies, it was found that when a sick child inherits microdeletions from the mother, AS develops, and with the paternal origin of microdeletions, PWS. As it turned out, paternal genes were inactivated in patients with PWS on the *q11-q13* locus. In all cases of AS, inactivation of genes of maternal origin is found.

On the proximal portion of chromosome 15, oppositely imprinted candidate genes *SNRPN* (encodes the small nuclear nucleoprotein polypeptide N) and *UBE 3A* (encodes ubiquitin ligase protein *3A*) have been found. Mutations in these genes are observed in PWS and AS, respectively. The loss of small nucleolar RNA (*SNRPN* gene), as shown in the experiments on mice, is possibly responsible for neonatal mortality. The mechanism of the development of hypogonadism remains unclear. In this case, the pathology of the hypothalamus is assumed.

Mental retardation, tremor, ataxia (the symptoms characteristic of AS) can be explained as a consequence of the deficiency of the *UBE 3A* gene in Purkinje cells (pyriform monocytes of the

cerebellum) and hippocampal neurons. At the same time, UPD is suggested to be the cause of intrauterine growth retardation.

Mutations in the imprinting center lead to imprinting “errors”, i.e. the ability to erase the imprinting of previous generations is lost [2]. And if in the spermatogenesis of the father, no replacement of the maternal imprinting by the paternal one on its maternal chromosome occur, then in the next generations a condition, similar to uniparental maternal disomy appear, which will be accompanied by the PWS phenotype. “Maternal” epigenotype disorder on the paternal chromosomes in oogenesis will lead to the development of AS.

Demethylation of paternal and maternal chromosomes and the conversion of 5-methylcytosine to cytosine by enzyme systems can also depend on environmental factors [3, 10].

It can be hypothesized that disorders of genomic imprinting, similar to PWS and AS syndromes, may arise as a result of demethylation on maternal and paternal chromosomes with the formation of cytosine from methylcytosine. Methyltransferases can contribute to epimutation 5mC → C with the formation of unmethylated cytosine.

Conclusion

Studies on the molecular mechanisms of genomic imprinting disorder and uniparental disomy on chromosome 15 in PWS and AS make it possible to explain not only the differences in their phenotypes, but also the mechanism of individual symptoms of the diseases. The most common causes of PWS and AS are genomic imprinting disorder and UPD. In PWS, due to deletion, the paternal imprinted genes of chromosome 15 will be active, whereas in AS, the maternal ones. The paternal UPD is the cause of AS, and the maternal UPD is the cause of PWS. Apparently, genomic imprinting disorders similar to PWS and AS can occur due to demethylation of the maternal and paternal chromosomes by methyltransferases.

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