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Congenital imprinting disorders: Application of multilocus and high throughput methods to decipher new pathomechanisms and improve their management☆

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ABSTRACT

Imprinting disorders (IDs) are a group of congenital diseases affecting growth, development and metabolism. They are caused by changes in the allele-specific regulation ("epigenetic mutation") or in the genomic sequence ("genetic mutation") of imprinted genes. Currently molecular tests in ID patients are generally restricted to single loci classically associated with the disease, but this approach limits diagnostic yield, because of the molecular and clinical heterogeneity between IDs. From the technical point of view, these limitations are aggravated by the lack of standardization in testing methodology, in the DNA sequences tested, and in clinical inclusion criteria prompting testing.

However, an increasing number of new studies show that these problems can be addressed by the use of new tests targeting multiple loci and/or a total exome and genome analysis.

The rapid development of efficient and high-throughput molecular techniques and their applications in research and diagnostics in the last decade have led to an impressive increase of knowledge on IDs and their basic pathomechanisms. In combination with the improvement of data recording and documentation, the diagnostic strategies are increasingly based on standardized protocols, and thereby provide the backbone for directed counseling, more personalized management, and new therapeutic approaches.

1. Introduction

Imprinting disorders (IDs) are a group of congenital diseases affecting growth, development and metabolism characterised by similar molecular alterations (Table 1).

They are caused by changes in the allele-specific regulation ("epigenetic mutation") or in the genomic sequence ("genetic mutation") of imprinted genes and regions, respectively (Fig. 1). In contrast to the majority of biallelically expressed genes, imprinted genes are expressed monoallelically in a parent-of-origin specific manner - i.e. either from the maternal or the paternal allele (for review: [1]). At the molecular level, the expression of genes within imprinted regions is influenced by specific patterns of DNA methylation, changes in chromatin structure, and post-translational histone modifications, collectively designated as epigenetic regulation (for review: [2,3]). So far, more than 90 human genes have been confirmed to be imprinted, but there are probably more based on bioinformatics predictions (for review: http://www.geneimprint.com/site/home, [www.geneimprint.com/site/genes-by-species] last check: 19.04.2015). The normal imprinting marks are inherited from the parental gametes and are then maintained in the majority of somatic cells and tissues of an individual. Their programming is subject to an imprinting cycle during life which leads to a reprogramming at each generation (for review: [4,5]). Methylation of the mammalian genome is comprehensively remodeled in early development. However, imprinting marks are exempted from developmental reprogramming; instead, they are erased in the germ-line and re-established according to the

☆ Monogenic orphan diseases in man.

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Table 1
The known congenital disorders associated with disturbances at imprinted loci, their frequencies, and the associated molecular and clinical findings. (NR not yet reported, IUGR intrauterine growth retardation, PNGR postnatal growth retardation, PTH parathormone; hypom. hypomethylation; hyperm. hypermethylation) (*absolute numbers for the frequencies of the molecular subtypes are taken from representative studies or reviews; ** in case of AS and PWS these frequencies are well established from huge cohorts and therefore not documented by specific references).

Disorder	Chromosomal region	Molecular disturbance	Frequencies (n=)	Multilocus defects	Clinical features
Transient Neonatal Diabetes mellitus (TNDM)	6q24	UPD(6)pat dup(6q)*PLAGL1*hypom. *ZFP57*mutations	41% 29% 30% 50% (n = 163) [34]	50%	IUGR, transient diabetes, hyperglycemia without ketoacidosis, macroglossia, omphalocele
Silver-Russell syndrome (SRS)	7	UPD(7)mat	7-10% (n = 109) [13]	1 case	IUGR/PNGR, rel. macrocephaly, hemihypotrophy, triangular face, feeding difficulties
	11p15.5	UPD(11)mat dup(11p15)mat ICR1 hyp. *CDKN1C* mutations *IGF2* mutations	n = 1 1-2% >38% n = 1 n = 1 (n = 109) [13]	– – ~10% – –	
Beckwith-Wiedemann syndrome (BWS)		UPD(11)pat Genomewide paternal UPD dup(11p15)pat ICR1 hyperm. ICR2 hypom. *CDKN1C* mutations	20% ~10%? ~90% 1-2% 4% 50% 5% [n = 40) [13]	– – – 25% –	pre- and postnatal overgrowth, organomegaly, macroglossia, omphalocele, neonatal hypoglycemia, hemihypertrophy, increased tumor risk
Temple syndrome (UPD(14)mat)	14q32	UPD(14)mat del(14q32)pat *MEG3* hypom.	78.4% 9.8% 11.7% (n = 51) [21]	– – NR	IUGR, PNGR, Hypotonia, feeding difficulties in infancy, truncal obesity, scoliosis, precocious puberty
Kagami-Ogata syndrome (UPD(14)pat)		UPD(14)pat del(14q32)mat MEG3 hyperm.	65.4% 19.2% 15.4% (n = 34) [42]	– – NR	IUGR, polyhydramnion, abdominal and thoracal wall defects, bell-shaped thorax, coat-hanger ribs
Angelman syndrome (AS)	15q11q13	UPD(15)pat del(15q11q13)mat aberrant methyl. *UBE3A* mutations	1-2% 75% ~3% 5-10%**	– – – –	mental retardation, microcephaly, no speech, unmotivated laughing, ataxia, seizures
Prader–Willi syndrome (PWS)		UPD(15)mat del(15q 11q13)pat aberrant methyl.	25-30% 70-75% ~1%**	– – 1 case	PNGR, mental retardation, neonatal hypotonia, hypogenitalism, hypopigmentation, obesity/ hyperphagia
Precocious puberty	15q	MKRN3 mutations	Unknown	5 families	Precocious puberty (girls: 5.75 years, boys: 8.10 years)
Pseudohyperparathyreoidism	20q13	UPD(20)pat; aberrant methyl.	Unknown	– 12.5%	Resistance to PTH and other hormones; Albright hereditary osteodystrophy; Subcutaneous ossifications Feeding behavior anomalies; Abnormal growth
UPD(20)mat	20	UPD(20)mat	Unknown	9 cases	IUGR, PNGR, failure to thrive

sex of the contributing parent for the next generation. Many genes regulated by genomic imprinting are found in clusters, i.e. imprinted loci often comprise multiple genes under coordinated control. A prominent example is the chromosomal region 11p15.5 which harbors genes encoding several growth promoting and inhibiting factors. It spans around 1 Megabase (Mb) and maintains two separate imprinting control regions (ICRs): the telomeric imprinting control region 1 (ICR1; H19 differentially methylated region - DMR) is methylated on the paternal allele, whereas the centromeric ICR2 (KvDMR1; KCNQ1OT1 DMR) is maternally methylated. In addition to its central physiological role in human growth and development it has been postulated that the 11p15.5 region is a central element of a network of imprinted genes [6,7].

2. Imprinting disorders (IDs): (epi)genetic aetiology and phenotypes

ID patients carry molecular disturbances which result in an unbalanced expression of imprinted genes. So far, four different types of alterations have been reported (Fig. 1), (i) uniparental disomy (UPD), (ii) deletions or duplications of the imprinted region, (iii) aberrant methylation marks (called epimutations), and (iv) point mutations in (imprinted) genes (see below). It is assumed that these (epi)mutations cause unbalanced expression of imprinted genes and thereby the clinical features of IDs, but functional proof of this is lacking in the majority of IDs.

In several IDs, two additional molecular features may be present: (a) mosaic distribution of epimutations and UPD, i.e. not all cells carry the disturbance causative of disease; and (b) the occurrence of multilocus imprinting defects (MLID) in a proportion of patients with epimutations (Table 1).

For the majority of the known IDs and their molecular defects, the pathophysiological mechanisms resulting in the specific phenotypes are unknown. So far only three genes have been shown to be directly associated with clinical phenotypes (Table 1): *CDKN1C* in Beckwith-Wiedemann syndrome and Silver-Russell-Syndrome (BWS, SRS), *UBE3A* in Angelman syndrome (AS) and *MKRN3* in central precocious puberty [8]. These genes are themselves imprinted; as a result, the inheritance of mutations in *UBE3A* and *CDKN1C* is autosomal dominant but its penetrance depends on the

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