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REVIEW

Prenatal testing for imprinting disorders: A laboratory perspective

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Abstract

Imprinting Disorders (ImpDis) are a group of congenital syndromes associated with up to four different types of molecular disturbances affecting the monoallelic and parent-of-origin specific expression of genomically imprinted genes. Though each ImpDis is characterized by aberrations at a distinct genetic site and a specific set of postnatal clinical signs, there is a broad overlap between several of them. In particular, the prenatal features of ImpDis are non-specific. Therefore, the decision on the appropriate molecular testing strategy is difficult. A further molecular characteristic of ImpDis is (epi)genetic mosaicism, which makes prenatal testing for ImpDis challenging. Accordingly, sampling and diagnostic workup has to consider the methodological limitations. Furthermore, the prediction of the clinical outcome of a pregnancy can be difficult. False-negative results can occur, and therefore fetal imaging should be the diagnostic tool on which decisions on the management of the pregnancy should be based. In summary, the decision for molecular prenatal testing for ImpDis should be based on close exchanges between clinicians, geneticists, and the families before the initiation of the test. These discussions should weigh the chances and challenges of the prenatal test, with focus on the need of the family.

Key points

What is already known about this topic?

- Imprinting Disorders (ImpDis) are caused by molecular disturbances affecting the balanced monoallelic and parent-of-origin specific expression of genomically imprinted genes.
- Prenatal testing for ImpDis is challenging due to molecular heterogeneity and mosaicism, with a risk for false-negative results.

What does this review add?

- A comprehensive overview of the ImpDis in prenatal setting and the diagnostic challenges.
- It should be considered to focus prenatal testing for ImpDis on indications with an increased risk.

Jasmin Beygo and Silvia Russo contributed equally.

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1 | INTRODUCTION

Imprinting Disorders (ImpDis) are a molecularly defined group of congenital syndromes caused by aberrant genomic imprinting and have a prevalence of less than 1 out of 10,000 newborns[1,2] (Table 1). They are associated with molecular disturbances of differentially methylated regions (DMRs), which regulate the monoallelic parent-of-origin specific expression of imprinted genes. Accordingly, the clinical phenotype of its carrier depends on the sex of the parent contributing the affected allele.

Postnatally, each ImpDis is characterized by a specific set of clinical features and associated with a distinct chromosomal region, but the non-specificity of some of the major findings (growth and metabolic disturbances, development delay, neurological signs, dysmorphisms) results in a clinical overlap (e.g. Ref. 3). In fact, some of these features can already occur prenatally but are mainly non-specific as well (Dufke et al., in the same issue). This clinical overlap is reflected by a molecular overlap, and therefore the decision on the appropriate molecular testing strategy might be difficult.[4]

2 | MOLECULAR ALTERATIONS IN IMPDIS

Four different types of molecular changes can affect DMRs; they either have an impact on the DNA itself or the methylation pattern of the DMR (Figure 1). Genomic alterations comprise uniparental disomies (UPDs), copy number variants (CNVs), and single nucleotide variants (SNVs), whereas imprinting defects (epimutations, ImpDef) are characterized by aberrant imprinting marks without changes of the DNA sequence at the DMR.

A further molecular characteristic of ImpDis is mosaicism, the occurrence of two different (epi)genetic constitutions in the same body. Mosaicism is commonly observed in case of ImpDef, with the exception of Prader-Willi syndrome (PWS) and to a certain extent of Angelman syndrome (AS).[5] Additionally, it is frequent among patients with paternal UPD of chromosome 11 (upd(11)pat) in Beckwith-Wiedemann syndrome (BWS).

2.1 | Uniparental disomy

UPD is defined as the exceptional inheritance of the two chromosomes of a pair from the same parent[6] and is characteristic for several ImpDis. UPD has been reported for nearly all human chromosomes, and it becomes clinically relevant in case an imprinted region is involved (UPD of chromosomes 6, 7, 11, 14, 15, 20) or an autosomal-recessive pathogenic variant is reduced to homozygosity (e.g. Ref. 7).

UPD can either affect the whole chromosome or parts of it (segmental UPD).[8] In the majority of whole chromosome UPDs, it is the result of trisomic rescue of a meiotically derived trisomic zygote (Figure 2A), but other mechanisms have been suggested as well (for review[9]).

While the major predisposition factor for UPD formation via trisomic rescue is maternal age,[10] (familial) structural chromosomal variants (SVs) are another relevant predisposing factor. In particular, Robertsonian translocations (RT, SVs of chromosomes 13–15, 21, 22) as the most frequent SVs in humans are prone to nondisjunctional events and can thus result in UPD (Figure 2B–D). While UPDs of chromosomes 13, 21 and 22 are not associated with ImpDis, carriers of UPDs of chromosomes 14 and 15 depict distinct clinical syndromes (Table 1). It has been estimated that the overall risk of a UPD for chromosomes 14 or 15 in a fetus carrying an inherited RT is about 0.6%–0.8%,[11] but a recent study and own data (Table 2) indicate that the risk of UPD for these chromosomes is lower (0.06%,[12]). UPD might also occur in case of non-RT and in carriers of de novo small supernumerary marker chromosomes.[8,13,14]

The formation mechanism of UPD implies the loss of a homologous chromosome in the trisomic zygote, and in this process mosaicism can occur (Figure 2). This mosaicism refers to the distribution of UPD itself, but it can also comprise mosaicism for the primary chromosomal aberration (e.g. trisomy). The latter has been discussed as an explanation for clinical features in patients with upd(16)mat or upd(6)mat.[15,16] However, at least upd(16)mat is currently discussed as an ImpDis.[17]

2.2 | SNVs and CNVs

SNVs and CNVs affecting imprinted genes or regions can either be inherited or occur de novo. As several ImpDis represent molecular "mirror" syndromes (e.g. PWS and AS in 15q11q13, BWS and Silver-Russell syndrome [SRS] in 11p15.5, Temple syndrome [TS14] and Kagami-Ogata syndrome [KOS14] in 14q32, pseudohypoparathyroidism [PHP] and Mulchandani–Bhoj–Conlin syndrome 20q13), different phenotypes can be expected in case of maternal or paternal origin of a CNV (e.g.Refs 18,19). In case of CNVs, the clinical phenotype might additionally be modified by the size and gene content of the variant.

In case of SNVs in imprinted genes, pathogenic variants only cause a clinical picture linked to one specific parental sex, whereas the inheritance from the other parent does not cause an aberrant phenotype. Thus, healthy carriers of pathogenic variants in *IGF2* (SRS), *CDKN1C* (SRS, BWS), *UBE3A* (AS), *MAGEL2* (Schaaf-Yang syndrome, SHYNG) and *GNAS* (PHP) can be identified, as they carry the variant on the silenced allele.

2.3 | Imprinting defects

ImpDef (also called epimutations) represents the type of variant which is most characteristic for ImpDis. They comprise aberrant methylation marks without an obvious causative genomic alteration at the DMR itself. The proper setting of imprinting marks at DMRs underlies a complex regulation, summarized as imprinting cycle of life that comprises the erasure of epigenetic signatures in the primordial

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TABLE 1 Overview on the currently known Imprinting Disorders and the frequencies of the molecular subtypes.

Imprinting disorder OMIM	Chromosome	Molecular defects	Frequencies
Transient neonatal diabetes mellitus (TNDM) 601410	6q24	upd(6)pat	41%
		dup(6q24)pat	29%
		PLAGL1:alt-TSS-DMR: LOM	30%
Silver-Russell syndrome (SRS) 180860	7	upd(7)mat	5.8%
	11p15.15	upd(11p15)mat	Single cases
		del(11p15)pat	Single cases
		dup(11p15)mat	2.3%
		H19/IGF2:IG:DMR: LOM	28.3%
		CDKN1C, IGF2, HMGA2, PLAG1: SNVs, CNVs	Several cases
Birk-Barel syndrome (BBS) 612292	8q24.3	*KCNK9*:SNVs	ND
Beckwith–Wiedemann syndrome (BWS) 130650	11p15.5	upd(11p15)pat	20%
		dup(11p15)pat	<1%
		H19/IGF2:IG-DMR: GOM	5%–10%
		KCNQ1OT1:TSS-DMR: LOM	50%
		CDKN1C:SNVs: Sporadic, familial	5%, 40%
Temple syndrome (TS14) 616222	14q32	upd(14)mat	54.0%
		del(14q32)pat	12.2%
		MEG3/DLK1:IG-DMR: LOM	33.8%
Kagami–Ogata syndrome (KOS14) 608149	14q32	upd(14)pat	51.5%
		del(14q32)mat	21.9%
		MEG3/DLK1:IG-DMR: GOM	26.6%
(Familial) Central precocious puberty (CPPB)	14q32	*DLK1*:SNVs, SVs	ND
Prader–Willi syndrome (PWS) 176270	15q11q13	del(15q11q13)pat	70%–75%
		upd(15)mat	25%–30%
		SNURF:TSS-DMR: GOM	1%
Angelman syndrome (AS) 105830	15q11q13	del(15q11q13)mat	75%
		upd(15)pat	1%–2%
		SNURF:TSS-DMR: LOM	1%
		UBE3A: SNVs, CNVs	10%
Central precocious puberty 2 (CPPB2) 615356	15q11.2	*MKRN3:* SNVs, SVs	ND
Schaaf–Yang syndrome (SHFYNG) 615547	15q11.2	*MAGEL2*:SNVs	ND
upd(16)mat	16	upd(16)mat	100%
Pseudohypoparathyroidism			
PHP1B (iPPSD3) 603233	20q13	upd(20q13)pat	2.7%
		Broad LOI (all GNAS DMRs)	38%
		Broad LOI (all GNAS DMRs)	Rare
		GNAS A/B:TSS-DMR: LOM	13.5%
		GNAS A/B:TSS-DMR: LOM	Rare
PHP1A/PPHP/POH (iPPSD2) 103580/612463		*GNAS:* LoF SNVs and CNVs	37.7%
Mulchandani–Bhoj–Conlin syndrome (MBCS) 617352	20	upd(20)mat	ND

Note: For abbreviations see text.

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Normal status

UPD

CNV, e.g. deletion

SNV

Imprinting Defect

	UPD	CNV	SNV	ImpDef
MSA	X	(X)	-	-
Array CGH	-	X	-	-
Array SNP	UPiD	X	-	-
Array SNP trio	UPhD/UPiD	X	-	-
MS-MLPA	X	X	-	X
Sequencing Sanger	-	(X)	X	-
WES single	-	X	X	-
WES trio	(X)	X	X	-

FIGURE 1 The four different types of molecular alterations in ImpDis and methods to identify them in the prenatal workup. CGH, comparative genome hybridization; CNV, copy number variant; ImpDef, Imprinting Defect; MLPA, multiplex ligation-dependent probe amplification; MS, methylation specific; MSA, microsatellite analysis; SNP, single nucleotide polymorphism; SV, structural chromosomal variant; US, ultrasound; WES, whole exome sequencing. [Colour figure can be viewed at wileyonlinelibrary.com]

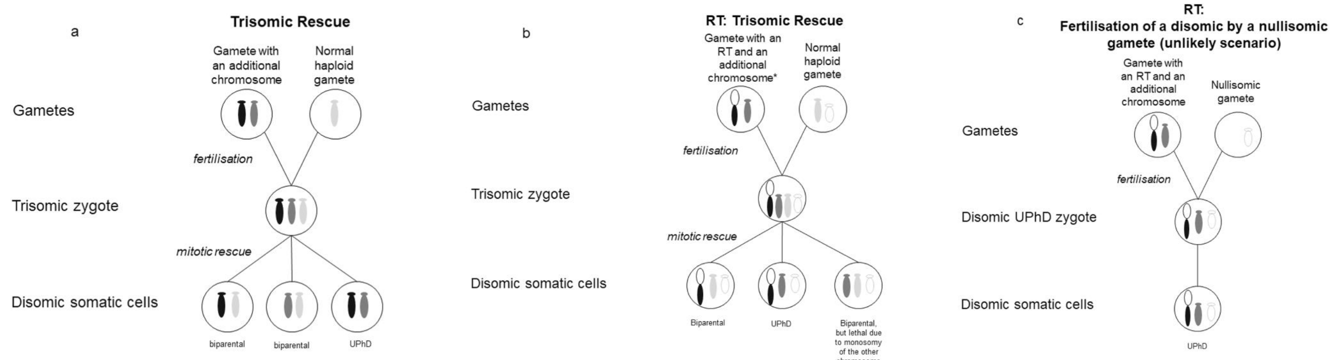

FIGURE 2 Simplified figure to illustrate UPD formation via selected mechanisms with a focus on the increased probability for UPD in families with RT (B,C). For clarity only one pair of the acrocentric chromosomes of the D- or G-group is depicted. In fact, the biological basis is more complex due to crossover events between chromatids (not shown). It should be noted that other UPD-formation modes, including monosomic rescue, are not shown here. (A) The most relevant mode of UPD formation in parents with normal chromosomal complements is rescue from a trisomic zygote. (B) Trisomic rescue can also occur in case one of the parents is carrier of a balanced RT. Additional rescue events, as well as mosaic formation with or without unbalanced cell lines are conceivable but not depicted. (C) Fertilization of a discomic gamete by a nullisomic one is an example of other possible but less likely formation modes. (black and gray shaded chromosomes represent the different homologous of a chromosomal pair (from different parents) (A–C), whereas the white and broader chromosome belongs to another pair (B,C)). RT, robertsonian translocations; UPD, uniparental disomy.

germ cells, reestablishment during gamete maturation, and maintenance during development (for review[20]). This fine-tuned regulation of imprinting signaling is prone to disturbances, and in the last years both environmental causes as well as genetic predispositions for ImpDef have been identified (for review[21]).

Though a pathogenic impact of environmental factors on the imprinting cycle of life is difficult to assess, an increasing number of data indicate that at least assisted reproduction (ART) might bear the risk for ImpDef in 11p15.5, in particular in BWS.[22–24] Though different pathomechanisms have been suggested for this association

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TABLE 2 Overview on the indications and molecular findings in pregnancies referred for testing for ImpDis to the three coauthoring centers according to their expertise in the last 5–13 years (Aachen/DE, Essen/DE, Milano/IT).

Reason for testing	Targeted chromosome	Method	Total	Positive
Fetal imaging features				
Features of SRS	7, 11, 14	MS MLPA	44	1
Features of BWS	11	MS MLPA (*CDKN1C* sequencing)[a]	210	20: *KCNQ1OT1*:TSS-DMR LOM: 13 upd(11)pat: 4 *H19/IGF2*:IG-DMR GOM: 1 *CDKN1C*: 1[a]
Features of KOS14	14	MS MLPA	1	1
Familial history of ImpDis				
Previous child with AS or PWS	15	MS-MLPA	23	0
Other family member with AS or PWS	15	MS-MLPA	9	0
Previous child with UBE3A variant[b]	15	*UBE3A sequencing*	7	1
Chromosomal disturbances				
Paternal RT	14	MSA/(MS-MLPA)	52	1[c]
	15		6	0
	14, 15		6	0
Maternal RT	14		64	1[c]
	15		6	0
	14, 15		8	0
Fetal RT	14		33	0
	15		9	0
	14, 15		11	0
Other translocations in the fetus and/or parents	6	MSA/(MS-MLPA)	13	0
	7		33	0
	11		37	0
	14		20	0
	15		15	0
	20		12	0
Trisomy in invasive prenatal sampling	7	MSA	16	0
	11		5	0
	14		4	0
	15		8	0
	16		10	4
	20		2	0
Trisomy in NIPT	7	MSA	7	0
	16		4	2
	20		3	0

Abbreviations: AS, Angelman syndrome; BWS, Beckwith-Wiedemann syndrome; ImpDis, imprinting disorders; KOS14, Kagami-Ogata syndrome; MS MLPA methylation-specific multiplex ligation-dependent probe amplification; MSA, microsatellite analysis; NIPT, non-invasive prenatal testing; PWS, Prader-Willi syndrome; RT, Robertsonian translocation; SRS, Silver-Russell syndrome.

[a] *CDKN1C* was not screened routinely for all the samples, it is therefore not representative.

[b] In three families, the variant was inherited, the affected fetus was from such a family.

[c] Both parents were carriers of the same RT translocation, so the positive result is counted twice here for both chromosomes 14 and 15.

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(e.g. cell culture condition, infertility), the causative link is still unclear.

In contrast, the functional consequences of genomic variants in genes that orchestrate the establishment and maintenance of imprinting marks are becoming obvious. In the last years, numerous pathogenic variants in different members of the subcortical maternal complex (SCMC) and further factors involved in the oocyte maturation and early embryogenesis have been identified (for review[25,26]). In women these variants (maternal effect variants in *KHDC3L*, *NLRP2*, *NLRP5*, *NLRP7*, *PADI6*) cause reproductive failure (infertility, miscarriages, hydatidiform moles[27]) or aneuploidy and ImpDis in her offspring (for review[28,29]). Numerous patients with ImpDef born to mothers with SCMC variants have been published; the majority exhibit BWS or SRS phenotypes (for review[25]). Molecularly, nearly all these children show multilocus imprinting disturbances (MLIDs), that is, in these patients several other germline DMRs show aberrant methylation in addition to the region which is associated with the clinically diagnosed ImpDis.

3 | INDICATIONS FOR PRENATAL TESTING FOR IMPDIS

Reasons for referral for molecular prenatal search for ImpDis comprise chromosomal findings in the parents or in routine prenatal analysis, clinical observations, and a family history of ImpDis or predisposing genetic variants (Table 2).

3.1 | Chromosomal structural variants

The best-known predisposition factor for UPD formation is (familial)chromosomal translocations, that is, RT (Figure 2). For RTs, risk figures of up to 0.8% for UPDs of chromosomes 14 and 15 have been estimated[11] but are probably lower.[12] In our cohort of 195 families with RTs affecting chromosomes 14 and/or 15 only one upd (14)mat was detected, but this family was unique as both parents were first cousins, both carriers of RTs (Table 2).

De-novo chromosomal aberrations might also prompt testing for ImpDis as they might predispose to UPD formation (Table 2).[30] These include small supernumerary marker chromosomes,[14] and aneuploidies with the chance for trisomic rescue (Figure 2A). Prenatally, aneuploidies are detectable in the course of chorionic villous sampling (for review[31]), and in case the subsequent amniocentesis gives a normal euploid result, a UPD formation is well conceivable. In the last years, there is also an increasing demand for UPD testing after a positive Non-Invasive Prenatal Testing result. In own cohorts of families ascertained with the diagnosis of trisomies for chromosomes harboring imprinted regions, UPD was detected only for chromosome 16 (Table 2). This finding corresponds to the observation that trisomy 16 is the most frequent trisomy in human conceptuses and always of maternal origin.[32]

3.2 | Findings in prenatal imaging

Clinical observations indicating an ImpDis are mainly based on prenatal imaging (Dufke et al., in the same issue). Omphalocele is the most frequent indication for prenatal diagnosis as it is associated with BWS, but also with transient neonatal diabetes mellitus and KOS14. Molecularly, hypomethylation of the IC2 and pathogenic *CDKN1C* variants are typically associated with this feature in BWS. Another pathomorphological sign in BWS and KOS14 is placental mesenchymal dysplasia (PMD), a placental vascular anomaly characterized by normal biparental trophoblast and abnormal mesenchyme due to an overexpression of paternal genes within the stromal component. PMD and omphalocele are rarely diagnosed in the first trimester, but more commonly after the 13–14th week. During the 2nd trimester, visceromegaly, macroglossia, and placentomegaly can be observed, in the third trimester intrauterine growth restriction (IUGR) and oligo or polyhydramnios can be diagnosed. However, molecular prenatal testing is more commonly asked for those imaging signs with onset during the first and 2nd trimesters of pregnancy. The correlation between the aforementioned findings in fetal imaging and the occurrence of molecular alterations in the sense of specific ImpDis is confirmed by the authors' experiences with a detection rate of up to 10% in prenatal samples referred for BWS testing (Table 2).

3.3 | Family history

In case of a family history or a predisposing genetic constitution for an ImpDis, the molecular nature of the alteration (SNV, CNV, SV) and its mode of inheritance have to be considered as they have a significant impact on the probability of a child with an ImpDis (Dufke et al., in the same issue). For familial variants affecting imprinted loci, the impact of the imprinting status on the clinical phenotype or the possible lack of it in that generation has to be considered. An exception is MLID and maternal effect variants. Though more data are needed to allow general suggestions about reproductive counseling in this family, in specific situations MLID in the family or maternal effect variants can be considered as indication for prenatal testing.

In case of familial chromosomal aberrations affecting an imprinted region, the prediction of the clinical phenotype has to consider the sex of the parent contributing the altered allele, but also the size and functional content of the variant. As different case reports on chromosomal alterations in 11p15.5 show, the same variant can either cause BWS or SRS depending on the sex of the transmitting parent, but in case of different sizes even healthy carriers have been documented (e.g. Ref. 33). This is also the case for TS14 and KOS14 where differently sized CNVs have been reported.[34] In AS and PWS on the other hand, the IC-deletions can lead to PWS or AS or no phenotype depending on the transmitting parent and the functional content.[5]

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3.4 | Mode of conception

Though the causal link has not yet been identified, ART appears to be more frequent in cohorts of specific ImpDis patients in comparison to the general population, for example, in BWS.[22,24] Accordingly, the mode of conception should be considered in the course of clarification of pregnancies suspicious for ImpDis.

4 | METHODS APPLIED IN TESTING FOR IMPDIS

Different assays are available for postnatal molecular testing of ImpDis,[35] addressing the four types of molecular alterations by either targeting aberrant methylation marks or genomic alterations (Figure 1).

The most widely used assay to identify CNVs, UPDs and ImpDef in patients referred for ImpDis testing is methylation-specific multiplex ligation-dependent probe amplification (MS MLPA).[36] MS MLPA assays are commercially available, and the different kits address all currently known clinically relevant DMRs. The advantage of MS MLPA is that they detect CNVs, UPDs and ImpDef in the same run. However, MS MLPA often does not allow to discriminate between UPD and ImpDef. For that purpose, further methods have to be applied.[5,35]

Quality assessment schemes organized by the European Molecular Quality Network have shown that the majority of laboratories offering diagnostic testing for ImpDis use MS MLPA, though other assays are also in use and provide complementary results (e.g. Ref. 37).

CNVs might also be addressed by quantitative assays, in particular in case of larger duplications or deletions array genotyping might help to determine the extent and the breakpoint of the rearrangement if needed.

For UPD testing, microsatellite analysis is the method of choice as it is reliable, rapid and economic. By comparing fetal microsatellite alleles with those from the parents, a UPD for the region of interest can be confirmed or excluded. However, microsatellite analysis does not cover whole chromosomes; therefore, segmental UPDs might escape detection by microsatellite typing. SNP array analysis is an alternative approach to identify uniparental isodisomy, and by typing of the parents in parallel (trio analysis) also uniparental heterodisomy can be diagnosed but is generally challenging (for review[38]).

SNVs are routinely identified by sequencing methods. As the number of currently known imprinted genes harboring pathogenic variants (Table 1) is small, the most appropriate technique to target these variants is Sanger sequencing. For known familial variants, it is recommended that Sanger sequencing should be restricted to the SNV of interest as this approach is fast and avoids incidental findings.

As clinical features of ImpDis become more specific after birth, targeted methylation-specific (MS) tests are commonly applied for postnatal molecular testing. The situation is different prenatally as most ImpDis do not present specific clinical features at the time of invasive diagnostics; therefore, exome- and genomewide next generation sequencing (NGS)-based techniques (preferably whole exome sequencing [WES] or whole genome sequencing) are used as first tier assays to search for disease causing variants (e.g. Ref. 39). Thus, in contrast to the postnatal diagnostic workup, prenatally MS assays are rather applied as second line tests in case of negative NGS results, with the exception of pregnancies with positive family histories or features strongly indicating a specific ImpDis.

In the prenatal testing workup, the use of the aforementioned assays depends on the molecular subtypes to be addressed (CNVs, SNVs, UPDs, ImpDef) (Figure 1), the DMR of interest, and the reason for the prenatal analysis.

Comparable to the diagnostic workup of patients suspicious for monogenetic diseases, the decision on the prenatal testing procedures has to consider their advantages and limitations. In the prenatal situation, the test should be appropriate, that is, it has to be adapted to the available sample type to keep the turn-around-time as short as possible and to avoid incidental findings. Additionally, the decision on the test used is influenced by the equipment and validation politics of the laboratory running the test, and national rules.

5 | CHALLENGES AND LIMITATIONS IN PRENATAL MOLECULAR TESTING FOR IMPDIS

One of the main challenges in prenatal testing is identifying a fetus suspected of having an ImpDis, if there is no known chromosomal aberration or pathogenic SNV in the family. Most fetal imaging anomalies at the time of invasive diagnostics are not that specific and therefore, a targeted ImpDis test will hardly be requested prenatally.

5.1 | Type and time of prenatal sampling

A major factor with an impact on the reliability of prenatal testing for ImpDis is the sample type, either extraembryonal chorionic villi sampling (CVS) or fetal amniotic fluid.[35] As CVS is an extraembryonic tissue, it might not reflect the (epi)genetic constitution of the fetus. Hand in hand goes the question of the time of sampling, as methylation is not completed in all DMRs and cytosine-phosphate-guanine (CpGs) motif before the 13th week of pregnancy,[40] and CVS analysis might give false positive results for the chromosome 11 and 14 associated ImpDis.

Special attention should also be directed to the locus analyzed. For example, DMRs such as the *MEG3*:TSS-DMR, which is postnatally analyzed for diagnosis of TS14 and KOS14, is a secondary DMR and its methylation is not completely set in CVS.[41] For amniotic fluid, there is insufficient data yet. For prenatal testing of AS and PWS, secondary DMRs like *NDN*:TSS-DMR and *MAGEL2*:TSS-DMR on chromosome 15 are also not suitable, whereas methylation ratios of *MAGEL2*:TSS-DMR in amniotic fluid has to be judged carefully and only in context with the other probes for the *SNRPN* DMR (*SNURF*:TSS-DMR) where the methylation imprint is stably set in chorionic villi and amniotic fluid.[5]

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5.2 | Mosaicism

Another diagnostic challenge of ImpDis is mosaicism for ImpDef, and upd(11)pat in BWS. This mosaic distribution can lead to false-negative diagnostic results caused by insufficient sensitivity of testing methods, the time of mosaic formation, and severe discrepancies between different tissues.[42] Deep sequencing assays to circumvent the lack of sensitivity are in development,[43] and further studies will show their appropriateness. However, a negative testing result does not exclude the existence of a molecular alteration in the sense of an ImpDef, and this has to be stated clearly in the report, particularly in a prenatal situation.

5.3 | Effect of cell culture

Furthermore, one has to consider the effect of cell culture. Some cell lines might proliferate better than others, or culturing might have a direct effect on the methylation. Especially for chromosome 14, it could be shown that the DMRs acquire hypermethylation (e.g. Ref. 44), but an influence of cell culturing on imprinting marks can also be observed for other DMRs. Thus, native cells (e.g. amniotic fluid) should be preferred and are suitable for routine testing, but in some situations might have the disadvantage of yielding small amounts of DNA/test material. The optimal would be to analyze both native and cultured tissues because rarely altered cells are present in a very low amount and become visible only after culturing.[45]

5.4 | Maternal contamination and twin pregnancies

In accordance with prenatal testing in monogenetic disorders, the diagnostic workup should comprise testing for maternal contamination to avoid false-negative results. In twin pregnancy, molecular testing requires the unambiguous sampling and sample identification for each fetus, but the interpretation of the molecular results might be additionally hindered by the observation that monozygotic twins can be clinically discordant but show the same aberrant molecular pattern in blood.[46] This observation is unique for ImpDis and can be explained by vascular connections in the placenta resulting in haemotopoetic stem stells. Accordingly, the healthy twin should not exhibit molecular alterations in other tissues than blood, but for the prenatal period this has not yet been documented.

6 | OUTLOOK

The decision on prenatal testing has to weigh the benefits for the families and the methodological limitations. With the diagnostic implementation of high-throughput and deep-sequencing molecular approaches in methylation and prenatal testing[43,47,48], further improvement of testing can be expected. However, the suitability of each test has to be monitored, and each test needs appropriate validation.

In addition to the targeted prenatal testing for ImpDis, the addressing of another group of gene-specific imprinting changes, so-called episignatures, might be relevant in the future (for review[49,50]). Germline mutations in an increasing number of genes (currently >50), often with chromatin-related function, have been shown to result in specific methylation profiles in DNA isolated from leukocytes. These signatures can be used to interpret variants of unknown significance but can also be used to establish diagnoses in cases where the genomic variant cannot be found.

Given the appearance of signatures in leukocyte DNA, it would seem to be possible that such signatures can also be detected in DNA from fetal origin, either from chorion villus biopsy or amniocentesis. An important caveat, however, is that DNA methylation is grossly different between tissues. Therefore, it is unlikely that the CpGs which are sensitive in leukocyte DNA will overlap completely with CpGs sensitive in prenatal sources of DNA. In addition, there may be important differences between directly isolated DNA, and cultured DNA, which is often used in prenatal situations because the amounts are so limited.

We do however consider prenatal genetics an important field for the application of these signatures, since it may provide a fast and reliable method of classification of variant of unknown significance and may lead to fewer false-negative prenatal testing results. Therefore, we hope that the attention of the episignature field will partially shift to the prenatal situation, even though obtaining sizable numbers of positive, as well as negative, controls will be challenging.

7 | CONCLUSIONS

Prenatal testing for ImpDis is challenging due to the molecular heterogeneity and mosaicism for several molecular subgroups, therefore sampling and diagnostic workup has to be adapted to the reason for referral and the knowledge about the methodological limitations. Additionally, the referring clinicians have to be aware of the fact that the prediction of the clinical outcome of a pregnancy is difficult. Furthermore, the molecular disturbance might escape detection due to cellular mosaicism, particularly in the case of ImpDef and upd(11)pat. Thus, in these constitutions false-negative results always have to be considered, and therefore fetal imaging should be the diagnostic tool on which decisions on the management of the pregnancy should be based. However, several ImpDis do not exhibit clinical features detectable by prenatal imaging (e.g. AS) or occur late in pregnancy (e.g. IUGR).

In summary, the decision for molecular prenatal testing for ImpDis should be based on close exchange between the clinicians, the lab conducting the test, and the families before the initiation of the test. These discussions should weigh the chances and challenges of the prenatal test, with focus on the need of the family.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available on request.

ETHIC STATEMENT

The study was approved by the ethical committee of the Medical Faculty of the RWTH Aachen (EK303-18).

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