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Uniparental disomies 7 and 14

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Normally, one inherits one chromosome of each pair from one parent and the second chromosome from the other parent. Uniparental disomy (UPD) describes the inheritance of both homologues of a chromosome pair from the same parent. The biological basis of UPD syndromes is disturbed genomic imprinting. The consequences of UPD depend on the specific chromosome/segment involved and its parental origin. Phenotypes range from unapparent to unmasking of an autosomal-recessive disease to presentation as a syndromic imprinting disorder. Whilst paternal UPD(7) is clinically unapparent, maternal UPD(7) is one of several causes of Silver-Russell syndrome. Presentation of paternal UPD(14) ("Kagami syndrome") is a thoracic dysplasia syndrome with mental retardation and limited survival. Findings in maternal UPD(14) ("Temple") syndrome show an age-dependent overlap with the well-known maternal UPD(15) (Prader-Willi) syndrome and are dominated by initial failure to thrive followed by obesity, learning difficulties and precocious puberty. Diagnostic strategies to tackle the genetic heterogeneity of UPD(7) and UPD(14) syndromes will be explained. Management issues in UPD(7) and UPD(14) patients will be discussed, and finally areas requiring further research will be outlined.

Definitions

The regular chromosome make up of any human fertilised zygote and subsequent somatic cell comprises two haploid sets of chromosomes, one from each parent. On a karyotype level, this is called **diploidy**. When referring to an individual chromosome pair this status is called **biparental disomy**. If

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the two homologues of a chromosome pair originate from the same parent with no homologue from the other parent, this is called **uniparental disomy (UPD)**.[1] UPD may comprise an entire chromosome or part of a chromosome (segmental UPD).[2] UPD may be present as **isodisomy**, i.e. two copies of the same parental chromosome, or as **heterodisomy**, i.e. one copy each of the two homologues from the same parent (Fig. 1). **Nullisomy** describes the lack of a single chromosome, i.e. a haploid germ cell that contains 22 instead of 23 chromosomes is nullisomic for the lacking chromosome. **Mosaicism** describes the presence within an organism of at least two genetically different cell lines that are derived from a common precursor cell such as the fertilised zygote. Examples are mosaic trisomies with the trisomic cell lineage present in the extraembryonic tissues but not in the embryo proper.

Epigenetics is the regulation of gene expression through mechanisms other than DNA sequence changes. Well-known epigenetic mechanisms are de-/methylation of DNA residues such as of CpG dinucleotides and histone protein modifications. These epigenetics marks entail local changes of chromatin conformation and silencing of genes in the respective region.[3] Epigenetic marks such as

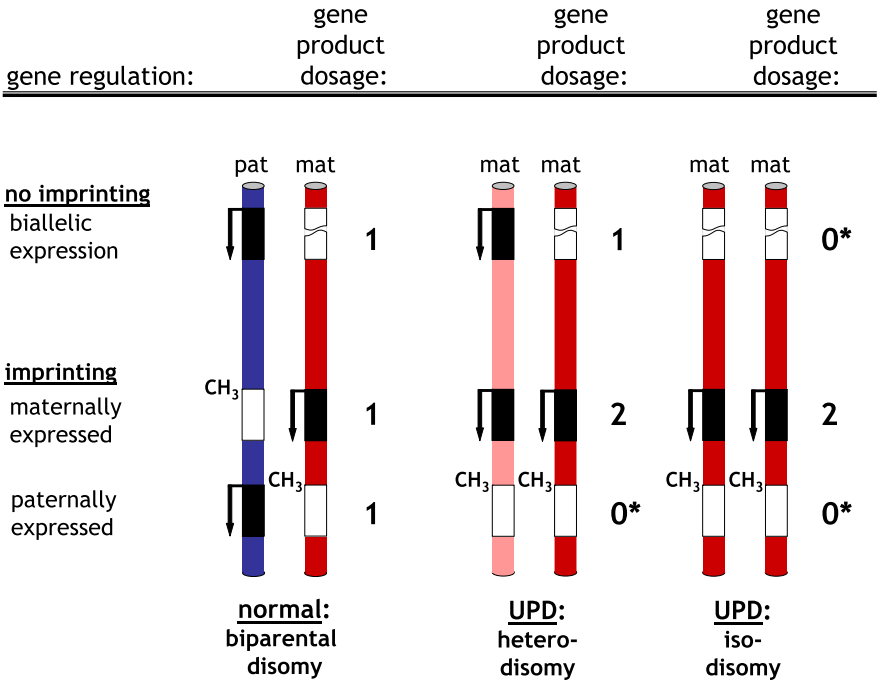

Fig. 1. Consequences of isodisomy and heterodisomy: imprinting disorder with or without unmasking of a mutant recessive allele. A pair of homologue chromosomes is symbolized by rods; blue indicates paternal, red maternal origin. Genes are drawn as rectangles, a mutated gene as an interrupted rectangle, an expressed gene as a blackened rectangle with an arrow alongside, and an imprinted gene as an empty rectangle with CH_3 symbolizing methylation. An asterisk indicates a contribution to the clinical phenotype. Let us assume, that in each case the mother is a heterozygous carrier for a gene mutation at an autosomal-recessive gene locus (top), whilst the father has two wildtype copies of this gene. The physiological state for an autosomal chromosome (biparental disomy) is shown on the left hand side and illustrates that differential gene regulation by imprinting may be of opposite parent-specific orientation in two adjacent genes. The heterodisomic proband in the middle has received both maternal homologues originating from the two maternal grandparents. This is the result of trisomic rescue after maternal meiosis I error. An imprinting disorder is caused by the lack of the paternally expressed copy of the gene that is physiologically imprinted (silenced by methylation) on the maternal chromosomes. The recessive mutation on one of the maternal chromosomes does not produce a clinical phenotype because the second maternal homologue carries a functioning wildtype copy of the same gene. In isodisomy on the right hand side two copies of the maternal chromosome with the recessive gene mutation have been passed on (e.g. trisomic rescue after meiosis II error). If the inherited maternal chromosome contains a recessive mutation, the proband will be homozygous for the recessive mutation thus having simultaneously an autosomal-recessive disease ("unmasking of a recessive allele") and an imprinting disorder. The two phenotypes may clinically be difficult to disentangle. The imprinting phenotype by itself is the same whether due to heterodisomy or isodisomy. Physiological recombination between homologue chromosomes in the germline prior to meiotic segregration errors means that there may be a combination of heterodisomy and isodisomy for different regions of the involved chromosome.

methylated CpG dinucleotides are stably inherited across cell divisions. The methylation pattern may be reset in the course of germ cell development or somatic de-/differentiation processes such as organ or tumour development. **Imprinting** is the epigenetic silencing of a gene in a parent-of-origin specific manner.[4] Thus, "imprinted" genes in the broader sense are genes which are subjected to transcriptional regulation by imprinting and which are expressed from one allele only: the cell or organism is by nature functionally **hemizygous** for these genes. *In sensu stricto*, "imprinted gene" means the allele that is transcriptionally inactivated or "silenced". Uniparental disomy for an imprinted allele is equivalent to functional nullisomy of this gene. A pathogenic change of the physiological methylation pattern leading to aberrant gene expression without any DNA sequence change involved is termed **epimutation.**

Birth weight below 2500 g is considered **low birth weight (LBW)**. This definition is independent of gestational age and comprises an array of different aetiologies such as premature birth, environmental causes or genetic conditions that affect intrauterine growth. **Small for gestational age** (**SGA**) neonates are those whose weight and/or height at birth is below the 10th percentile for gestational age. This comprises constitutional small size due to physiological reasons such as ethnicity. Another aetiology is pathological growth restriction (**intrauterine growth restriction** = **IUGR**) due to environmental or genetic factors that prevent a foetus from reaching its inherent growth potential.[5] SGA therefore is not a diagnosis but merely a descriptive term that requires investigations into its individual origin.

Background

Imprinting mechanisms regulate parent-of-origin specific gene expression

According to Mendel's laws of inheritance the contribution of autosomal genes to the developing embryo is equivalent independently of whether the particular chromosome region is of paternal or of maternal origin. However, in all placental (eutherian) mammals, humans included, there are some genes that are inactive (**imprinted**) or active (**expressed**) depending on the sex of the transmitting parent. In these cases, gene activity ("on" = expressed, "off" = silenced = imprinted) is regulated by epigenetic modification. Examples of epigenetic modifications are changes of regional chromatin conformation induced by addition or removal of methyl (CH_3) groups from Cytosin-Guanine dinucleotides (CpG). The latter are enriched in gene regulatory DNA regions such as promotor regions and other **differentially methylated regions** (**DMR**s) that may act as **imprinting centres** (**ICs**) *in cis* (on the same allele). ICs regulate the imprinting pattern of their immediate genomic environment.

How and when does imprinting occur? In eutherians, an imprinting switch takes place in germline cells during early gametogenesis due to the action of DNA demethylases ("**resetting**" = complete erasure of all methylation) and subsequently of DNA methylases ("**marking**" = establishment of sex-specific methylation).[6] Parent-of-origin dependent methylation is limited to specific gene regions through the interaction between imprinting centre (IC) and insulators. These DNA elements cooperate with proteins such as CTCF (OMIM 604167;[7]) and function as boundaries to the spread of chromatin conformation changes.[8] Such regional restriction of imprinting effects can produce "on" and "off" signals in immediately adjacent gene regions on the same chromosome (Fig. 1). The spermatocyte with paternally imprinted genes and the oocyte with maternally imprinted genes fuse to form the diploid fertilised zygote, which is then functionally hemizygous for the imprinted genes. Towards embryo implantation both parental genomes of the developing embryo first undergo demethylation, this time, however, exempting the imprinted regions ("**maintenance**" of imprinting). Then tissue-specific remethylation occurs in concert with other epigenetic mechanisms such as histone de-/acetylation or small regulatory RNA molecules depending on which genes are needed at the individual stages of embryo-/foetogenesis and postnatal development (epigenetic regulation of somatic gene activity).[9]

Imprinted genes are involved in extra-/embryonic growth control

Genes involved in regulating growth of the embryo versus growth of the nutrient-providing extraembryonic tissues (placenta) are enriched in imprinted chromosome regions.[10] Generally, it is postulated that paternally expressed genes promote whilst maternally expressed genes limit the

growth of the embryo (parental conflict theory[11]). The importance of imprinted genes for the development of a viable product of conception becomes particularly obvious in exceptional situations where uniparental disomy is not limited to single chromosomes or chromosome regions but includes all chromosomes. Parthenogenesis with two maternal chromosome sets, for example, results in digynic ovarian teratoma whilst diandric products of conception lead to complete hydatidiforme mole.[12] These outcomes exemplify the functional non-equivalence of the paternally and the maternally inherited genome.

In humans probably more than 100 genes are subject to transcriptional regulation by imprinting (http://igc.otago.ac.nz/Search.html, www.geneimprint.com/site/genes-by-species.Homo+sapiens). They are largely clustered in several chromosome regions that are conserved through evolution and that range from several kilo- to several megabases in size. This includes chromosome bands 1p36.33, 6q25.3, 7p12.2, 7q21.3, 7q32.2, 11p15.5, 11p13, 11q23, 14q32, 15q11-q13, 19q13.43, and 20q13. There are equivocal data on chromosomes 2, 16, and 18p with regard to imprinting, whilst imprinting has been largely ruled out for other chromosomes. Some of these data have been obtained in humans, some through observation in mice (www.mousebook.org/catalog.php?catalog=imprinting, www.geneimprint.com/site/genes-by-species.Mus+musculus).

The definition of an imprinted gene is complicated by the fact that some genes show tissue or development-specific expression, some with opposite imprinting patterns in different tissues, others with monoallelic expression in some but biallelic expression in other tissues. The *GRB10* gene on chromosome 7p11.2-p12 is an example for such a complex imprinting pattern.[13]

Mechanisms that lead to UPD and prenatal risk figures

Constitutional UPD can result from one of four different chromosome malsegregation events or from *de novo* or inherited structural chromosome aberrations (Fig. 2, modified from[14]).[15,16]

a) *Trisomic rescue* (aberrant zygote). The majority of trisomic products of conception are lost spontaneously in very early stages of implantation or embryogenesis. Occasionally trisomy is corrected spontaneously by postzygotic loss of one of the three homologues with ensuing survival of the embryo. Depending on how trisomy arose and on which homologue is lost, uniparental disomy may result either in the formation of isodisomy or heterodisomy. Mitotic rescue of trisomy in later embryogenesis leads to a mosaic product of conception. Sometimes trisomy persists in the placenta (confined placental mosaicism), sometimes trisomic mosaicism is present in the embryo proper. Prenatally detected mosaic trisomy indicates an increased risk for foetal UPD, although this may vary depending on the chromosome involved (11–25% for chromosome 15, no reliable figures for chromosome 7 or 14).[16] Trisomic rescue is probably the most prevailing mechanism leading to UPD both because of the frequency of non-disjunction events in the female germline and because of the better survival chances of a trisomic as opposed to a monosomic conceptus [see below: c) "Monosomic rescue"].

b) *Nullisomic gamete complementation* (aberrant zygote). Fertilisation between a disomic germ cell from one parent and a nullisomic germ cell from the other parent creates a disomic zygote with UPD. The chance of chromosome malsegregation occurring simultaneously in both the maternal and paternal germline and leading to complementary germ cells is very low, making this presumably the rarest mechanism.

c) *Monosomic rescue* (aberrant zygote). Fertilisation between a normal haploid germ cell and a nullisomic germ cell that lacks one chromosome produces a monosomic zygote. This may survive only if the unpaired chromosome then by chance undergoes mitotic endoduplication or isochromosome formation. Complete isodisomic UPD is the outcome. Nullisomy predominantly arises from non-disjunction in the maternal germline, hence resulting in paternal isodisomy/isochromosome. Yet, paternal UPD due to monosomic rescue is rare as opposed to maternal UPD secondary to trisomic rescue because the endoduplication event in monosomic rescue has to occur immediately after fertilisation in order to rescue the fertilised zygote from lethality. In conclusion, monosomy rescue is probably a rare mechanism.

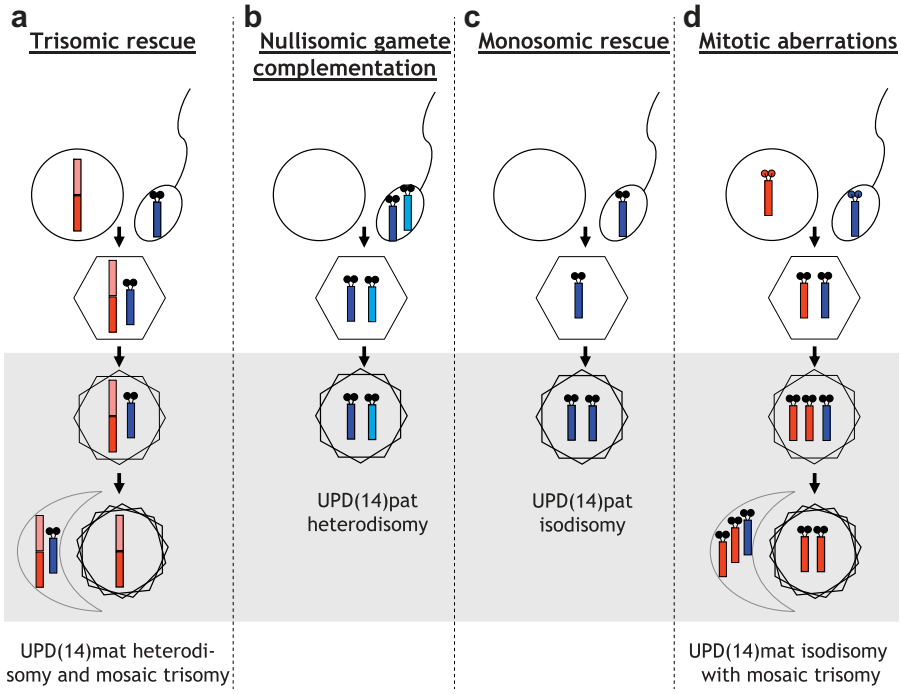

Fig. 2. Mechanisms leading to uniparental disomy (modified from[14]). The fertilised zygote prior to implantation is symbolized by a hexagon. Through mitotic division it develops into a blastula (dodecagon) that implants into the endometrium (gray lower half) and goes on to form the embryo proper (octadecagon) and extrambryonic tissues such as parts of the placenta (halfmoon-shaped object). Chromosome 14 with a paternal imprinting pattern is represented by a blue rectangle with satellited stalks. A homologous Robertsonian translocation inolving the two chromosomes 14 with maternal imprinting pattern is depicted as two connected rectangles shaded in different hues of red. (a) Trisomic rescue: it is assumed that the mother is a balanced carrier of an homologous Robertsonian translocation rob(14;14). Fertilisation of her disomic oocytes leads to a trisomic zygote, that may survive for serveral divisions before it is "rescued" (heterodisomic foetus) by the loss of the paternal chromosome 14, in this case with mosaic trisomy persisting in the placenta (confined placental mosaicism). (b) Nullisomic gamete complementation: in this particular case, paternal heterodisomy ensues, but only if malsegregation events occur simultationeously in both parental germlines. (c) Monosomy rescue: the monosomic fertilised zygote has to undergo immediate endoduplication of the paternal chromosome 14, otherwise it will not survive and not reach the stage of implantation. (d) Post-fertilisation error: two mitotic errors cancelling each other out have to occur and result in the early embryo. Mosaicism for a trisomic cell line is indicated in the placenta.

d) A *regular zygote* with biparental disomy suffers two postzygotic, i.e. mitotic, malsegregation events entailing formation of trisomy or monosomy and subsequent rescue by mitotic loss or mitotic endoduplication, respectively.
e) *Structural chromosome aberrations*. An extra structurally abnormal chromosome (ESAC) in the prenatal karyotype often derived from one of the acrocentric chromosomes is an example of increased risk of UPD in the foetus.[17] ESACs are detected in 0.2–0.7% of all pregnancies and are in ~50% derived from chromosome 15. The presence of a chromosome 15-derived ESAC in a foetal karyotype signals an estimated 5% risk for UPD(15) – whether the same risk magnitude applies to the rare chromosome 14-derived ESAC is unclear.[17] In larger ESACs with euchromatin, the clinical picture will be predominated by the partial trisomy rather than by UPD.

The report of a *de novo* nonhomologous Robertsonian translocation, such as 45,XX,der(14;21)(q10;q10), in foetal cells after amniocentesis means an elevated but in absolute terms still low empirical risk of 0.6–0.8% for a foetal UPD syndrome, in this example UPD(14).[18] Robertsonian translocations have a prevalence of about 1 in 1000 individuals in the general population.

If prenatal cytogenetic diagnosis reveals a homologous Robertsonian translocation such as 45,XX,der(14;14)(q10;q10), the risk of foetal UPD(14) is very high: 50% in a sonographically normal foetus, up to 100% in a liveborn child with phenotypic anomalies.[19]

In summary, UPD can be seen as the correction of aneuploidy at the potential cost of ensuing gene dysregulation at imprinted genome regions. The collective incidence for UPD of any human chromosome at birth is estimated to be in the range of 1/3.500.[15] However, there are no precise data on the incidence of chromosome-specific UPDs. The frequency of UPD may vary considerably from chromosome to chromosome depending on the incidence of trisomy and on the clinical severity of the associated phenotype. It is reasonable to expect that the incidence for maternal UPD increases with maternal age, reflecting the connection between maternal age, chromosomal non-disjunction events in meiosis and resulting trisomy.[20]

Clinical relevance of UPD and alternative mechanisms of gene dosage alteration

UPD for genes or chromosome regions that are not regulated by imprinting does not affect health. The exception from this rule is the unmasking of a recessive disease allele by uniparental isodisomy, often segmental UPD.[1,2] This is how the principle of UPD was first discovered in man. Patients homozygous for an autosomal-recessive disorder such as cystic fibrosis were reported who had only one carrier parent whilst the other parent was unexpectedly found not to carry the heterozygous mutation. Instead of non-paternity, in some of these patients molecular analysis revealed (segmental) isodisomy for the mutated chromosome from the carrier parent.[21,22] Current recommendations to confirm carrier status in parents of patients with an autosomal-recessive disorder[23] should lead to increased pick-up rates of isodisomy in the future.

However, UPD mainly comes to the attention of the clinician when imprinted gene regions are affected. The presence of two copies of an imprinted, i.e. silenced allele due to UPD means that the patient is functionally nullisomic for this gene. The opposite constellation leads to a duplicated gene product dosage: two transcriptionally active alleles instead of one active and one inactive allele (Fig. 1). Constitutional imprinting disorders are often pleiotropic developmental syndromes with the hallmark of disturbed pre- and postnatal growth. Undetected mosaicism for a chromosomally aberrant cell line either with trisomy or with an ESAC has to be kept in mind as possibly aggravating the clinical features of a patient with UPD.

There are several alternative mechanisms that can affect gene dosage regulation with the same functional outcome as UPD (Fig. 3).[6]

Heterozygous deletion of a chromosome fragment may appear as interstitial microdeletion, resolvable by fluorescence microscopy using the fluorescence *in situ* hybridisation (FISH) technique. Larger structural rearrangements are visible by light microscopy either as interstitial deletion/duplication of an existing chromosome or as extra structurally anomalous (marker) chromosome (ESAC) that contains chromosome regions regulated by imprinting.

Epigenetic mutations ("epimutations") affect methylation profiles thereby causing a paternal imprint pattern on a maternal chromosome and vice versa.

DNA mutations in imprinting centres (ICs) lead to secondary epimutations in the corresponding imprinted region.

The best known phenotypes that illustrate the clinical relevance of UPD and alternative mechanisms of epigenetic gene regulation disorders are Prader-Willi syndrome, i.e. functional loss of genes in the paternal 15q11-13 region as in UPD(15)mat, and Angelman syndrome, i.e. functional loss of genes in the maternal 15q11-13 region as in UPD(15)pat. These two syndromes have furthered our understanding of the molecular basis and clinical consequences of imprinting disorders and have triggered the development of diagnostic strategies for imprinting disorders in general.[24,25]

Molecular and cytogenetic diagnosis of UPD syndromes (Table 1)

Molecular diagnosis of UPD or functionally equivalent alterations (see above) is best performed by selected laboratories worldwide that are specialised in this field (www.genetests.org; www.orphanet.org; www.hgqn.de) and are able to carry out any of the techniques explained below (Table 1).[25]

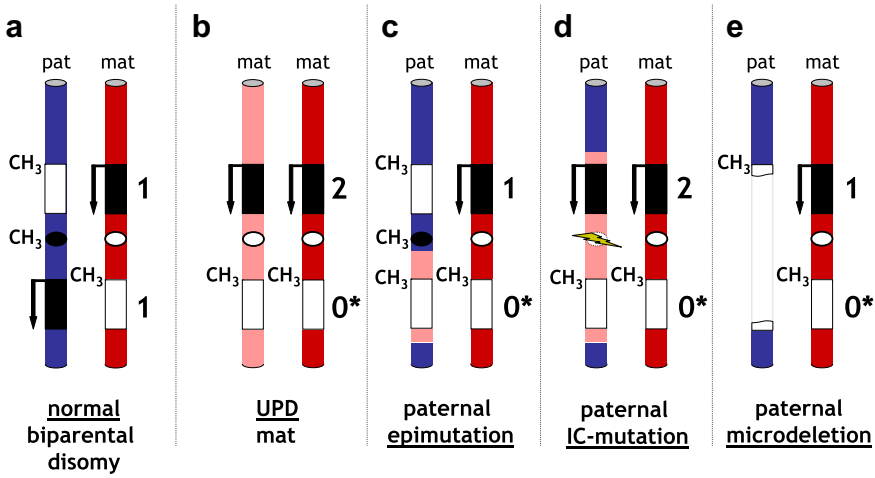

Fig. 3. Molecular defects underlying an imprinting disorder. Homologue chromosomes, imprinted genes and transcriptional gene activity are depicted in the same manner as in Fig. 1. An intergenic differentially methylated region (IG-DMR), symbolized by an oval between the two genes, functions as imprinting centre (IC), that controls the methylation patterns in the surrounding genomic region. A primary epimutation (c) means a change of methylation patterns (in this case from paternal to maternal pattern) without underlying DNA mutation of the IC on the paternal chromosome. A secondary epimutation (d) is a methylation change secondary to an IC mutation (serrated arrow across IC again in this case on the paternal chromosome).

a) Methylation-based methods such as methylation-specific PCR (MS-PCR) are now recommended as first-line screening techniques. Their advantage is that all classes of molecular alterations are detected that turn a biparental into a uniparental methylation pattern: UPD, microdeletions, epimutations, IC-/DMR mutations. No parental blood samples are required in the initial step of the analysis. Briefly, initial sodium bisulfite treatment of the DNA converts unmethylated cytosin into uracil whilst methylated cytosin residues e.g. in CpG islands of gene promotor regions or other

Table 1
Diagnostic tools for the molecular dissection of UPD syndromes.

	MS-PCR	MS-MLPA	Microsatellite marker analysis	Chromosomal FISH	Conventional cytogenetic analysis
Sample requirements	Patient only EDTA blood	Patient only EDTA blood	Patient and parents EDTA blood	Patient only Heparin blood	Patient only Heparin blood
Detects …	**UPD** **epimutation** **IC-/DMR deletion** **microdeletion**	UPD		IC-/DMR deletion Microdeletion	Large structural or numerical chomosome aberration
Distinguishes …		Epimutation IC-/DMR deletion Microdeletion	**Isodisomy** **Heterodisomy** (microdeletion)		**Isochromosome** **Robertsonian translocation** **ESAC**
Does not detect …	Gene mutation	(Gene mutation)	Epimutation IC-/DMR deletion Gene mutation	UPD Epimutation	UPD Microdeletion IC-/DMR deletion Epimutation Gene mutation
Prenatal application	Week 15 onwards (AC only)	Week 15 onwards (AC only)	Week 12 onwards (CVS or AC)	Week 12 onwards (CVS or AC)	Week 12 onwards (CVS or AC)

MS-PCR = methylation-specific PCR, MS-MLPA = methylation-specific multiplex ligation-primed probe amplification, DMR = differentially methylated region, IC = imprinting centre, CVS = chorionic villus sampling, AC = amniocentesis, ESAC = extra structurally abnormal chromosome, FISH = fluorescence *in-situ* hybridisation.

DMRs are protected against this DNA base transition. Specific PCR primers are designed over CpG dinucleotides in such a way that either methylated or non-methylated DNA is amplified only, with differing amplicon length. The two amplification reactions should be combined in one duplex PCR assay.[26] Depending on the specific locus being investigated, methylation-specific multiple ligation-dependent probe amplification (MS-MLPA) is sometimes performed giving simultaneous information on methylation status and DNA copy number. If either of these two tests yields a biparental methylation pattern, other diagnoses than an imprinting disorder of the tested chromosome have to be considered.

b) If a uniparental methylation pattern is detected by MS-PCR, DNA markers such as microsatellites are compared between the patient and his or her parents. Additional DNA samples from the parents of the patient are therefore required. A uniparental methylation pattern in connection with biparental microsatellite marker haplotypes strongly suggests an epimutation. If uniparental methylation pattern and uniparental microsatellite haplotypes are found, then UPD is likely. Haplotype analysis is capable of differentiating between isodisomy and heterodisomy and can be scaled up to genome-wide analysis.[27]

c) Differentiation between isodisomy and a heterozygous microdeletion is also possible by DNA-dosage specific techniques such as real-time PCR or chromosomal fluorescence *in-situ* hybridisation (FISH) with probes for the potentially microdeleted interval.

d) The diagnosis of UPD should be followed up by conventional cytogenetic analysis (Giemsa or Q-banded chromosome preparation from peripheral lymphocytes of a heparin blood sample). The purpose is to rule out a structural chromosome aberration as the underlying mechanism, such as a Robertsonian translocation or an extra structurally anomalous chromosome (ESAC). Cytogenetic analysis of parental blood samples is important when the imprinting disorder of a child is caused by a structural chromosome anomaly. In these cases, one of the parents might be a carrier of a chromosome aberration with a variably increased recurrence risk for the imprinting disorder depending on the nature of the parental chromosome aberration [see chapter “Genetic management issues of imprinting disorders”, paragraph Recurrence risks].

For prenatal diagnosis of UPD syndromes, methylation-based techniques are not to be used on DNA from chorionic villus samplings (CVS), but only on DNA from foetal cells collected through amniocentesis or foetal umbilical cord puncture. The first reason for this limitation is the uncertainty about the exact time point at which methylation patterns are stable in the early embryo. Secondly, some differentially methylated regions like the *MEG3*-DMR [see UPD(14) below] seem to be differentially methylated between foetal and placental cells.[28]

Clinical picture – UPD(7)

Paternal UPD (7), UPD(7)pat

UPD(7)pat in itself is most likely not associated with any clinical phenotype. Cases with UPD(7)pat have so far been ascertained through unmasking of a recessive mutation e.g. for chloride diarrhoea (*SLC26A3* gene on 7q31.1).[29] Although single cases with overgrowth have been reported[30], the absence of any persistent phenotype suggests that a double dose of growth-regulating genes that are paternally expressed and whose loss may cause the phenotype of UPD(7)mat [see next chapter] does not interfere with normal development.

Maternal UPD(7), UPD(7)mat phenotype, Silver-Russell syndrome (SRS; OMIM 180860)

Overview

UPD(7)mat is the most frequent clinically relevant UPD second to UPD(15) with more than 60 documented cases.[31] UPD(7)mat accounts for approximately 10% of patients with Silver-Russell syndrome (SRS, see also contribution in this volume by G Binder). SRS is (epi-)genetically heterogeneous, and imprinting defects or microduplications at chromosome band 11p15 account for another 35–50% of SRS patients[32] (Table 2, [33–38]). The classical SRS phenotype comprises prenatal-onset growth

Table 2
Genetic heterogeneity and recurrence risks of imprinting disorders

Clinical syndrome	**Silver-Russell syndrome** (n > 400)				**UPD(14)pat phenotype** "Kagami syndrome" ($n = 29$)		**UPD(14)mat phenotype** "Temple syndrome" ($n = 50$)		**Angelman syndrome** UPD(15)pat phenotype (prev. = 1: 15.000—20.000)		**Prader-Willi syndrome** UPD(15)mat phenotype (prev. = 1: 15.000—20.000)	
	UPD(7)mat phenotype		11p15.5 phenotype*									
Genetic defect	Proportion of all SRS cases	Recurrence risk	Proportion of all SRS cases	Recurrence risk	Proportion of cases	Recurrence risk	Proportion of cases	Recurrence risk	Proportion of cases	Recurrence risk	Proportion of cases	Recurrence risk
a) **UPD**	**~10%** UPD(7)	**<1%**	Single cases	<1%	**65—70%**	**<1%**	**70–80%**	**<1%**	3–7%	<1%	~25%	<1%
b) **Micro –deletion /duplication**	**<1%** dup(7p13)mat incl. *GBR10*	**<1%** if *de novo*	~1–2% dup(11 p)mat	<1% if *de novo*	**~14%** del(14q32.2)mat	**<1%** if *de novo*	**~10%** del(14q32.2)pat	**<1%** if *de novo*	70–75% del(15q11.2-q13) mat	<1% if *de novo*	~75% del(15q11.2-q13) pat	<1% if *de novo*
c) **Epimutation**	Single case of 7(q32)pat hypermethylation (*MEST*)	**<1%**	35–50% 11p15.5pat hypomethylation of ICR1	<1%	**~10%** 14(q32.2)mat hypermethylation	**<1%**	**~12 %** 14(q32.2)pat hypomethylation	**<1%**	2–3% 15(q11.2–q13)	<1%	~1% 15(q11.2–q13)	<1%
d) **IC-deletion**	?	–	?	–	**≤5% ?** del(IG-DMR)mat del(*MEG3*-DMR) mat	**≤50%** if present in mother	**≤2% ?** del(IG-DMR)pat	**≤50%** if present in father	0.5%	≤50% if present in mother	0.15%	≤50% if present in father
e) **Gene mutation**	Maternally inherited *GBR10* mutation	**50% ?**	?	–	No mutations found in *GTL2* or *MEG8*	–	No mutations found in *DLK1* or *RTL1*	–	10% *UBE3A*	50% if present in mother	2 cases of *SNORD116-1* deletion on paternal allele	≤50% if present in father
f) **Other /differential diagnoses**	**~1% :** various other chromosomal imbalances, e.g. del(15q26.3), but no *IGF1R* mutations non-syndromic SGA children				Jeune asphyxiating thoracic dystrophy		Autosomal-recessive syndromes with hypotonia, short stature and obesity (see text)		Mutations in *MECP2*, *CDKL5*, del(22q13.3)		**~5–10% of idiopathic cases: UPD(14)mat**	
g) **Unknown**	**35-45 %**				**?**		**?**		~ 10 %		?	
References	Yoshihashi et al., 2000[33] Kagami et al., 2007[34] Abu-Amero et al., 2008[35] Bruce et al., 2009[36] Spengler et al., 2010[37] Eggermann, 2010[38]		*see Binder, this volume or Eggermann, 2010[38]		Kagami et al., 2008[70] Ogata et al., 2008[77] Irving et al., 2010[72] Kagami et al., 2010[28]		Temple et al., 2007[80] Hosoki et al., 2009[81] Bena et al., 2010[82] Kagami et al., 2010[28]		Ramsden et al., 2010[25]		Ramsden et al., 2010[25] Hosoki et al., 2009[81] Sahoo et al., 2008[83] de Smith et al., 2009[84]	

? = unknown. **UPD** has a slightly increased recurrence risk (≤1%) if due to *de novo* or inherited Robertsonian translocations. If UPD is due to isochromosomes such as der(14;14)(q10;q10), the parents should be tested to exlude a parental carrier status that would indicate a ≤100% recurrence risk for UPD in liveborn children. **Microdeletions or microduplications** have a recurrence risk of ≤50% if present in the respective parent. The single case of paternal ***MEST*** hypermethylation (7q32.2) is debatable because *MEST* has not yet been proven to be involved in SRS and because partial hypermethylaton was also detected in the father of the patient.

retardation, which persists postnatally and is associated with delayed bone age. In SRS patients growth retardation is typically combined with BMI $\leq -$ 2 SD, characteristic craniofacial features including relative macrocephaly, delayed motor development, physical asymmetry and 5th finger clinodactyly. Even though the phenotypic profile across all patients with UPD(7) differs in some details from the profile in 11p15-associated patients [see "(epi-)genotype-phenotype correlation" below], the molecular (epi-)genotype cannot be reliably inferred from the phenotype in the individual SRS patient.[31] Clinical scoring systems for SRS have been put forward by several groups[39–42] to facilitate the clinical diagnosis (see Table 3).

Pathogenic mechanism

Potentially growth-regulating genes contribute to the phenotype. Several growth-related genes in three regions on chromosome 7 are expressed in parent-of-origin specific manner and hence are candidate genes for SRS. *GRB10, EGFR, IGFBP1, and IGFBP3* cluster in 7p11.2-p13. *SGCE* and *PEG10* reside in 7q22 and *PEG1* and *y2-COP* in 7q32.[43,14] These gene regions have been identified through rare patients with segmental isodisomy due to meiotic recombination. A maternally inherited p.P95S *GRB10* mutation (OMIM 601523) has been published for two independent SRS patients.[33] *GRB10* (growth factor receptor bound protein 10) codes for an intracellular protein that interacts with tyrosine–kinase receptors like IGF-1R. *GRB10* is an SRS candidate gene in 7p11.2-p12, but its role is not yet clear[44,45] On the basis of physical mapping and mouse model data, *MEST/PEG1* (mesoderm-specific transcript, paternally expressed gene 1; OMIM 601029) in the chromosomal region 7q32 has been discussed as another SRS candidate gene,[46,47] but final confirmation is missing.[48]

Growth

Growth retardation in SRS typically starts before birth, may be detectable by ultrasound as early as in gestational week 18, and suggests SRS especially in the presence of asymmetry.[49] Birth length of SRS patients shows a mean standard deviation of -2.94 SD (± 1.63; $n = 27$) with birth weight in a similar range of -2.62 SD (± 1.28; $n = 33$). Growth retardation continues postnatally (-3.39 SD $\pm$ 1.27 for

Table 3
Diagnostic scores for Silver-Russell syndrome (SRS)

Price et al., 1999[39]	Netchine et al., 2007[40]	Bartholdi et al., 2009[41]	Eggermann et al., 2009[42]
Original diagnostic score for classical SRS	Weighted diagnostic score for classical SRS	SRS severity score, maximum of 15 points	Relaxed diagnostic score to include SRS-like phenotypes
4 out of 5 criteria :		**≥ 8 points required for SRS :**	
Birth weight ≤ 2.3%ile	Birth weight ≤ 2.3%ile **and 3 out of 5 criteria :**	1pt = birth weight ≤ 10th centile 1pt = birth length ≤ 10th centile 1pt = rel. macrocephaly at birth	pre- and postnatal growth retardation (may be mild)
Postnatal growth ≤ 2.3%ile	Postnatal growth ≤ 2.3%ile	1pt = no catch up growth, height ≤ 3rd centile	
OFC within and parallel to normal percentiles	Relative macrocephaly at birth	1pt = OFD ≥ 3rd and ≤ 97th centile	**and**
Characteristic facial dysmorphism	Prominent forehead	1pt = triangular shaped face 1pt = prominent forehead 1pt = small chin or thin lips or late closure of fontanelle or …	prominent forehead and triangular face **or**
Physical asymmetry	Physical asymmetry	3pts = physical asymmetry (face/limb/body)	physical asymmetry
	Severe feeding difficulties or BMI ≤ 2.3%ile	*	
		1pt = attending regular school 1pt = 5th digit clinodactyly 1pt = genital abnormalities 1pt = others (e.g. pigmentary anomalies)	

* feeding problems, hypoglycaemia, excessive sweating were considered characteristic for SRS, but omitted because of unreliable reporting.

height and −3.11 SD ± 1.66 for weight) combined with delayed bone age.[50] Average values for final adult height and weight in untreated patients are 151 cm for males and 140 cm for females (−4.2 SD scores[51]). Especially in early childhood, there is relative macrocephaly. Relative macrocephaly in SRS is a disproportionately large neurocranium not only in relation to the body length (OFC on average 2 standard deviations above height[50],) but also in relation to the facial skull that is often triangular in shape with a pointed chin. Physical asymmetry (face, body, or limbs) is a diagnostically valuable feature in about 30–70% of the SRS patients.[52]

Psychomotor and cognitive development

Infancy and early childhood are variably marked by severe feeding difficulties and delayed motor development. If left untreated, recurrent neonatal hypoglycaemic episodes may affect cognitive outcome, which is otherwise within the normal range in the majority of patients. There are no systematic studies on cognitive potential of SRS patients according to (epi-)genotype. A left shift of the IQ distribution in SRS patients by about 8–15 IQ points compared to the control group has repeatedly been confirmed.[53,54] Two patients with complete UPD(7)mat in the study of Noecker and Wollmann achieved IQ scores (81 and 84) that were significantly below the mean of SRS patients (95.7) in this study (total of 36 children with SRS). The authors did not detect any significance of the severity of the physical SRS features or of growth hormone treatment on the IQ score, but this may be due to the small sample size.

Others

Features that are more frequently but not regularly observed in the group of SRS patients than in healthy probands are fifth finger clinodactyly and brachydactyly, delayed bone age and delayed closure of the anterior fontanelle, high-arched palate or cleft palate, urogenital malformations such as hypospadias or cryptorchidism, precocious puberty, a high-pitched squeaky voice, congenital ptosis, and café-au-lait spots.[42,52]

(Epi-)genotype–phenotype correlation

A prospective study of 66 molecularly proven SRS patients[52] revealed that 60% of patients with 11p15 hypomethylation but only 20% of UPD(7)mat patients presented with four out of the five diagnostic features defined by Price et al..[39] The group of patients with 11p15 hypomethylation differed significantly from the group of UPD(7)mat patients in the frequencies of physical asymmetry (68% versus 30%), triangular face (59% versus 90%), low-set or posteriorly rotated ears (36% versus 75%), global delay (20% versus 65%), fifth finger clinodactyly (75% versus 45%), and additional congenital anomalies (36% versus 10%). Severe feeding problems, excessive sweating and hypoglycaemic episodes were all relatively more frequent in UPD(7)mat patients although not to a level of statistical significance. These data largely confirmed earlier results from a comprehensive retrospective evaluation of previous studies.[31] With a view to UPD(7)mat patients, Kotzot pointed out an interesting additional finding: Patients with heterodisomy of chromosome 7 display developmental delay more frequently than patients with isodisomy 7 (50% versus 35%). He proposed that undetected mosaicism for trisomy 7 may be responsible for this observation. As opposed to heterodisomy, which results from trisomic rescue, isodisomy is based on monosomy rescue or gamete complementation, both mechanisms that carry a lower risk for mosaicism.

Phenotypes of patients with hypomethylation of 11p15.5 were also subclassified further according to the extent of the methylation defect.[41]

On average, UPD(7)mat children as opposed to SRS children with 11p15 anomalies show a postnatal deceleration of height gain possibly in part accounted for by pronounced feeding difficulties and gastro-oesophageal reflux.[55] The postnatal accentuation of growth failure results in relative macrocephaly later on rather than at birth. But whilst children with 11p15 hypomethylation seem to have an abated response to growth hormone treatment possibly due to IGF-I insensitivity, UPD(7)mat children respond to growth hormone treatment in a way not different from non-syndromic SGA children. Hence, the potential final heights are comparable for all SRS children independent of the genetic heterogeneity.

Diagnostic strategy

The diagnostic scoring systems for SRS (Table 3) are useful to distinguish between patients with classical and with milder SRS-like phenotype for future studies on genotype–phenotype correlations.

Sequential genetic testing for 11p15.5 imprinting defects and UPD(7)mat, however, is currently recommended for both groups of patients.[38,52] In other words, molecular testing for SRS should be offered also to patients who present with mild intrauterine and/or postnatal growth retardation ≥ -2 SD in combination with characteristic craniofacial features or body asymmetry.[42] Lowering the threshold for molecular testing in clinical practice means trading off testing sensitivity against specificity. Using the strict clinical diagnostic criteria for SRS results in a detection rate of approximately 50–60% (see Table 2) whilst the inclusion of patients with a less specific phenotype yields a detection rate of as low as 18%.[42]

Differential diagnoses

The combination of intrauterine growth retardation with signs of asymmetry, postnatal feeding problems, failure to thrive, and fifth finger clinodactyly is also compatible with diploid/triploid mosaicism.[56] Relative macrocephaly, triangular facial contour (often present in UPD(7); absent in diploidy/triploidy) and pigmentary anomalies (more likely in diploidy/triploidy than in UPD(7)) are discriminatory features. The phenotypic overlap between UPD(7) and diploid/triploid mosaicism may be due to UPD affecting several chromosomes in the diploid cells of a patient with diploidy/triploidy. If diploidy/triploidy is suspected, a cytogenetic analysis on cultured skin fibroblasts should be offered. An alternative strategy is microsatellite marker analysis on DNA from buccal smears or saliva samples.

Three further differential diagnoses are MULIBREY nanism (OMIM 253250; autosomal-recessive *TRIM37* mutations, mostly Finnish patients), autosomal-dominant SHORT syndrome (OMIM 269880; responsible gene unknown), and 3M syndrome (OMIM 273750; autosomal-recessive *CUL7* mutations). All three share intrauterine and postnatally persisting growth retardation, relative macrocephaly, triangular face, hypotonia, motor delay, feeding difficulties, risk for hypoglycaemic episodes in infancy, speech delay but mostly normal intelligence, delayed bone age and decreased subcutaneous fat with SRS.[57] Distinguishing features may be asymmetry in SRS patients, constrictive pericarditis and fibrocystic long bone changes in MULIBREY nanism, Rieger anomaly in SHORT syndrome, and full lips as well as bony chest abnormalities in 3M syndrome.

Various constitutional submicroscopic structural chromosome imbalances such as chromosome 10q26 deletion syndrome (OMIM 609625) or chromosome 12q14 microdeletion show phenotypic overlap with SRS.[37] Molecular karyotyping by array-CGH should therefore be routinely offered in SRS patients negative for a chromosome 7 and 11 imprinting disorder, especially when there is no relative macrocephaly. Microdeletion/duplication syndromes tend rather to be associated with microcephaly and more often than in SRS with mental retardation as discriminatory features from chromosome 7 or 11 aetiologies.

Medical care

No specific formal guidelines are available for the management of children with SRS. The medical team should involve a community paediatrician, paediatric endocrinologist, clinical geneticist, nutrition specialist, physiotherapist and should enlist the help of paediatric orthopaedicians in cases with significant asymmetry. There are currently no sufficient data to determine whether tumour risk might be slightly increased in SRS patients with asymmetry. The latter is sometimes decribed as hemihypertrophy, sometimes as hemihypotrophy.

A postnatal renal ultrasound to exclude urogenital malformations and regular check ups for cryptorchidism are recommended.

Evaluation of the patient for and education of the parents about clinical symptoms and signs of neonatal hypoglycaemia is strongly recommended in order to prevent long-term neurological damage. The main contributory factors to hypoglycaemia during the neonatal period and infancy of SRS patients are low birth weight with muscular hypoplasia and hence reduced glycgogen stores, also reduced growth hormone levels and reduced caloric intake due to poor feeding and gastro-oesophageal dysmotility.[58,59] In the absence of an unanimously accepted definition of clinically relevant neonatal hypoglycaemia, thresholds are suggested for plasma glucose concentration just prior to meals of 40 mg/dL (2.2 mmol/L) during the first 24 h of life and 50 mg/dL (2.8 mmol/L) after 24 h of age.[59] Manifestations of hypoglycaemia such as nocturnal sweating, irritability, hypotonia, weak suck, tachypnoea, jitteriness, hypotonia, hypothermia, lethargy, and vomiting are unspecific and partly overlap with primary SRS symptoms and signs.[60] Treatment starts with frequent, i.e. two hourly, oral feeds day and night. In severe cases of hypoglycaemia, i.e. plasma glucose concentration of less than 20–25 mg/dL (1.1–1.4 mmol/L) or

if hypoglycaemia persists, parenteral glucose infusions are added. In SRS patients with severe ongoing failure to thrive, nasogastric or percutaneous gastrostomy tube feeding may be necessary. In case of any surgical procedure special arrangements to reduce perioperative fasting times should be made (first on the waiting list etc.). Parents need to be alerted to an increased likelihood of hyoglycaemia in stress situations such as infections with high fever. The idea that growth hormone, the main anabolic hormone, could possibly be started earlier than usual [see second next paragraph] in SRS patients with pronounced hypoglycaemia[60] has not yet been systematically examined.

SRS-specific growth charts can be found at www.magicfoundation.org/www/docs/7.1371/russell-silver-syndrome-growth-charts.html. They should be used to monitor growth and the effect of growth hormone treatment, but also to detect additional growth-related problems that may be unrelated to SRS.

Growth hormone (GH) treatment in children with SGA of unknown cause is approved as early as from two years (FDA, USA) or four years (EMEA, Europe) of age, if there has not been any spontaneous catch-up growth until then.[61] However, in patients in whom SGA is due to classical SRS, no catch-up growth is to be expected. A question for future studies might therefore be to explore the effects of GH treatment already from the first months of life in proven SRS patients especially with a view to helping to reduce the likelihood of hypoglycaemic episodes. There are so far no sufficient data as to whether the GH dose for older SGA children (0.035 mg/kg/d in Europe, 0.070 mg/kg/d in the USA[62]) would be suitable also for infants. Experience gained from GH treatment of SGA children indicates that therapy should be continued until adult height is achieved in order to preserve height gain.[63] Long-term GH treatment does not seem to increase the tumour risk in SGA patients[64,65] or to accentuate limb asymmetry in SRS patients.[66] Uncertainty about supraphysiological IGF-1 levels and the incidence of cancer means that GH dose should be adjusted to keep IGF-1 levels within or near physiological concentrations[67] (reviewed in[62]). A note of caution: As of July 11, 2007 the American Association of Clinical Endocrinologist argued that there are insufficient data on both efficacy and safety of long-term use of growth hormone in short children who do not have growth hormone deficiency. The AACE concludes that any treatment ought to be carefully and individually monitored (www.aace.com/pub/positionstatements/ShortChildren.php).

Psychological support re short stature may be useful especially around puberty, and patients should be informed about SRS interest groups. In general, because of their small stature children with SRS tend to be underestimated in age by others including medical staff and teachers which may in the long-term affect their cognitive development.

Practice points – UPD(7) syndromes

- UPD(7)pat patients: careful monitoring for overgrowth and developmental delay.
- Patients with unexplained SGA presentation and/or persisting growth retardation: evaluation for Silver-Russell syndrome (SRS) using one of the clinical diagnostic scoring systems (see Table 3).
- Molecular genetics in the hand of expert laboratories will identify an chromosome 7 or 11 (epi-)mutation in approx. 50–60% of cases that fulfil the clinical diagnostic criteria for SRS.
- A lower threshold than the required diagnostic score for SRS should be considered for molecular testing in order to detect mild/atypical presentations.
- Assessment of recurrence risk for SRS: parental chromosome analysis should be offered if structural chromosome 7 or 11 anomalies are the cause.
- Idiopathic SRS: microarray-CGH for submicroscopic genome imbalances to be offered.
- Feeding problems with hypoglycaemia and low body mass index, short stature and motor retardation are the main issues for preventative management in infancy and early childhood. Use SRS-specific growth charts.
- Growth hormone therapy in SRS patients: supervision by a paediatric endocrinologist. For guidelines see consensus statement for SGA children.[61]
- Data on natural history and response to GH therapy in the individual SRS patient should ideally be collected in a central database. For contacts see (www.gesundheitsforschung-bmbf.de/de/2050.php#Imprinting or Tauber et al.[68]

Clinical picture – UPD(14)

Paternal UPD (14), UPD(14)pat phenotype ("Kagami syndrome", OMIM 608149)

Overview (Table 4)

UPD(14)pat is a rare but distinctive foetal malformation syndrome that has been reported in at least 29 independent cases.[69,70,28] The combination of features that may lead the prenatal sonographer or neonatologist to the diagnosis include polyhydramnios, a bell-shaped chest with angulated ribs ("coat-hanger configuration") resulting in pulmonary hypoplasia, and abdominal wall defects (omphalocele, diastasis recti). Placental weight and body weight at birth are often elevated for gestational age.[71] Additional signs after birth are craniofacial dysmorphism (microtia, prominent forehead with low hairline, blepharophimosis, depressed nasal bridge, prominent philtrum, puckered mouth) and often (mesomelic) limb shortening, but long fingers with contractures, and lax skin.[69,72] The pathognomonic coat-hanger rib sign, which may be visualized as early as in gestational week 23[69], helps to distinguish between UPD(14)pat and other short rib syndromes such as Jeune thoracic dysplasia.[73] The average clinical course of UPD(14)pat is dominated by the severe, often lethal respiratory insufficiency, marked feeding difficulties with postnatal growth failure and moderate to severe mental retardation. The prognosis in most cases is very limited. However, children with UPD (14)pat who survive the critical stage of infancy may show improvements of their thoracic configuration and reach a stable state.[74]

Genetic basis and (epi-)genotype-phenotype correlation

The critical region for the UPD(14)pat phenotype was mapped by comparing the effects of segmental UPD(14)pat and chromosome 14 microdeletions with the picture of full UPD(14)pat (Fig. 4). Segmental UPD, maternal epimutations or microdeletions of 14q32.2 containing the differentially methylated genes *DLK1* and *RTL1* (both paternally expressed) as well as *MEG3* (= *GTL2*), *RTL1as* and *MEG8* (all maternally expressed) are necessary and sufficient for the characteristic UPD(14)pat phenotype.[70,72,75,76] Up to date there are no indications that the phenotype differs depending on whether UPD, a microdeletion or epimutation is the cause. We therefore suggest the name of "Kagami syndrome" for the UPD(14)pat phenotype (Table 2).[28,70,72,77]

Table 4
Age-dependant UPD(14)pat phenotype ("Kagami syndrome")

	prenatal	postnatal	prognosis
Cardinal features			
> 90 %	polyhydramnios, placentomegaly, premature birth		
	thoracic dysplasia ("coat hanger sign") → respiratory failure		
	abdominal wall defect		
		feeding difficulty, growth failure	
		mental retardation, limited survival	
Additional features			
~ 50-90 %		craniofacial dysmorphism (short neck, small ears, frontal hypertrichosis and bossing, depressed nasal bridge, micrognathia, puckered mouth)	
		distal arthrogryposis, mesomelia, scoliosis	
~ 35%		congenital heart disease	

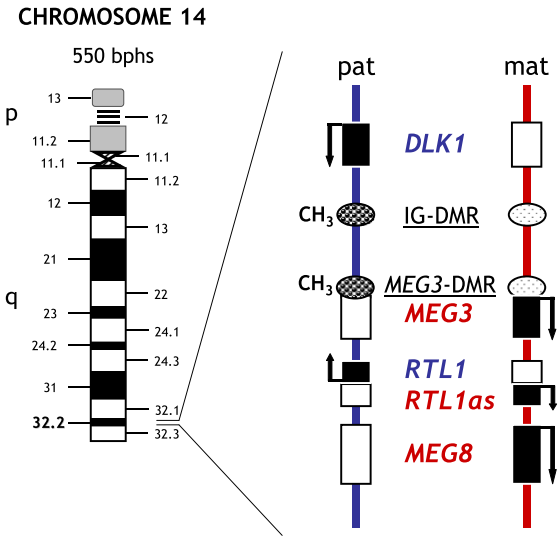

Fig. 4. Schematic view of the imprinted region 14q32.2; modified from Kagami et al., 2010.[28] Depicted are the following paternally expressed genes: *DLK1* = delta like, OMIM 176290, gene product is a human epidermal-growth-factor-like protein found in secretory granules of pancreatic beta cells; *RTL1* = retrotransposon-like gene, OMI 611896, gene product is highly expressed in the foetus and placenta and essential for maintenance of the foetal capillaries at least in mice.[75] The maternally expressed genes comprise: *MEG3* = maternally expressed gene 3, OMIM 605636, the functional gene product is possibly an RNA molecule with regulatory function for pituitary growth. *RTL1as* = RTL1 antisense transcript containing microRNAs that regulate *RTL1* gene activity by RNA interference (RNAi) mechanism.[76] *RTL1as* thus acts as a repressor for *RTL1*. *MEG8* = maternally expressed gene 8, non-protein coding imprinted transcript of yet unknown function. Also schematically depicted are the intergenic differentially methylated region (IG-DMR) and *MEG3*-associated differentially methylated region (MEG3-DMR). The IG-DMR is hierarchically superior to the MEG3-DMR in controlling the imprinting pattern of this gene cluster.[28] Both IG-DMR and MEG3-DMR are physiologically hypermethylated on the paternal allele and hypomethylated on the maternal allele in the foetus.

Medical care

In view of the limited prognosis, the extent of medical interventions has to be individually discussed with the parents. Early and if necessary long-term artificial ventilation may gain time for the chest deformity and pulmonary function to stabilize[74], and gastrostomy tube feeding might ameliorate the catabolic situation. Little is known about long-term cognitive development. In patients surviving the first three to five years cognitive and speech development might be better than previously assumed (unpublished personal observation in a molecularly proven case, RH).

Maternal UPD(14), UPD(14)mat phenotype (Temple syndrome)

Overview (Table 5)

The incidence of UPD(14)mat is probably underestimated because the phenotype is variable, relatively mild and age-dependant. At least 43 cases have been documented[78], the majority of them diagnosed through structural chromosomal aberrations such as balanced Robertsonian translocations or extra structurally abnormal chromosomes (ESACs).[79] Findings that are shared by the majority of UPD (14)mat patients are pre- and postnatal growth delay, hypotonia and joint hypermobility, psychomotor delay, feeding problems, truncal obesity setting in, around or after the third year of life, recurrent middle ear infections, facial dysmorphisms, premature puberty and advanced bone age leading to stunted growth. Intelligence is within the normal range in the majority of UPD(14)mat patients, however the proportion of children experiencing learning difficulties or mild mental retardation is increased compared to the general population.

Table 5
Age-dependant UPD(14)mat phenotype (Temple syndrome)

	prenatal	infancy	childhood	prognosis
Cardinal features				
> 90 %	pre- and postnatal growth retardation		short stature	
50-90 %		feeding problems, dystrophy	obesity and hypercholesterolemia	
		hypotonia, hyperextensible joints, motor retardation		
70-90 %		small hands		
≥ 90 %			precocious puberty	fertility
Additional features				
~ 33%	premature birth			
inconsistently reported		craniofacial dysmorphism (frontal bossing, high arched palate, short philtrum)		
~ 33 %		hydrocephalus and macrocephaly		
~ 40 %			scoliosis	
≤ 33 %				mild to moderate mental retardation
< 20%		microcephaly		

Growth and somatic features

Approximately 90% of children with UPD(14)mat suffer from intrauterine growth retardation.[78] There is no persistent pattern with respect to head circumference. Hydrocephaly which may be associated with macrocephaly, occurs in about one-third of patients. Hands and feet are often short. After initial failure to thrive approx. 60% of the patients develop truncal obesity but without the compulsory eating habits typically seen in patients with Prader-Willi syndrome. In more than 90% of UPD(14)mat patients, pubertas praecox sets in often very briskly and contributes to short stature unless treated. Hence, the trias of short stature, of age-dependant change from failure to thrive to obesity and of brisk precocious puberty is a strong clinical diagnostic handle, but only in later childhood. This is reflected in the relatively late average age at which diagnosis is made (at nine years of age according to[78]). Dysmorphic facial features (mild blepharophimosis, fleshy tip of the nose, high forehead)[14] are frequent, but inconsistent(ly reported) and thus not reliable.

Psychomotor and cognitive development

In 74% of UPD(14)mat patients statomotoric development and/or speech are delayed. In probably no more than one-third of cases there is mild to moderate mental retardation. Untreated hydrocephalus, isodisomy with unmasking of a recessive allele for a disorder that interferes with cognitive development, or mosaic trisomy 14 all potentially affect developmental outcome.

Genetic basis and (epi-)genotype–phenotype correlation

More than 35 patients with (segmental) UPD(14)mat, five patients with a paternal microdeletion of the imprinted region 14q32.2, and six cases with a paternal epimutation (hypomethylation) encompassing the *MEG3*-DMR all presented as UPD(14)mat syndrome (Table 2).[28,80–82] Parental TGG DNA repeat polymorphisms have been suggested as a predisposing factor for 14q32.2 microdeletions.[82] The crucial role of the 14q32.2 region (Fig. 4) was further confirmed by the finding of a UPD(14)pat phenotype in the son of a mother who herself had signs compatible with a UPD(14)mat phenotype. Mother and son shared the same heterozygous 8.5 kb deletion involving only the intergenic differentially methylated region (IG-DMR) in 14q32.2.[28] The 14q32.3 IG-DMR controls the methylation status of the *MEG3*-DMR and the imprinting status of genes in this region.[28]

The exact molecular aetiology (UPD versus microdeletion versus epimutation versus DMR deletion) cannot be deduced from the phenotype in the individual patient. In view of the first description both of UPD(14) and of 14q32 epimutations by Karen Temple the UPD(14)mat phenotype is also called Temple syndrome.[81] UPD, microdeletions and epimutations show different frequencies in Temple syndrome compared to Prader-Willi syndrome or Angelman syndrome (PWS/AS) (Table 2)[25,81,83,84] but as in PWS/AS methyl-specific PCR enables molecular testing for Temple syndrome irrespective of the (epi-) genetic heterogeneity.

Diagnostic strategy

Like for the other imprinting syndromes, a tiered approach is taken for molecular testing to confirm a suspected UPD(14)mat syndrome (Table 1). The first step is methylation-sensitive PCR for the *MEG3* locus. An abnormal methylation pattern is followed up by microsatellite analysis on DNA from the patient and parents to distinguish between an epimutation/MEG3 deletion on the one hand and a UPD (14)mat on the other hand. In case of UPD(14)mat, cytogenetic analysis is performed to exclude a structural chromosome anomaly such as a Robertsonian translocation involving chromosome 14 of maternal origin. Quantitative PCR analysis of the *MEG3* gene differentiates between a primary epimutation and a 14q32 microdeletion that may be inherited from the father.

Differential diagnoses

Two recent studies looked at the combined number of 111 patients who were clinically classified as Prader-Willi syndrome (small for gestational age, hypotonia, feeding problems and development of obesity in later childhood) but were negative for 15q12 imprinting anomalies.[78,81] Methylation-specific PCR revealed that 9/111 patients had Temple syndrome. In conclusion, the functional lack of a paternally imprinted region 14q32 leads to Temple syndrome that may be indistinguishable from PWS in infancy and childhood until precocious puberty (Temple syndrome) versus delayed or incomplete puberty (Prader-Willi syndrome) sets in.

The infant with hypotonia, severe feeding problems, developmental delay and growth retardation as initial presentation is seen in a number of differential diagnoses apart from Prader-Willi syndrome. The most important amongst them is autosomal-recessive congenital hypothyroidism (CH) because it is relatively frequent (1:4000 live births[85];) and treatable. Usually, CH will be picked up by routine neonatal hypothyroidism screening. Transient hypothyroidism may occur in patients with Temple syndrome[78].

Bardet-Biedl ~ (OMIM 209900), Cohen ~ (OMIM 216550), and Alström syndrome (OMIM 203800) are autosomal-recessive syndromes with varying degrees of hypotonia, develop-/mental retardation and short stature (and obesity). Although clear diagnostic combinations of features exist for each of these syndromes, the findings are either not present in all patients with the same disorder or are not necessarily present in infancy or early childhood. When present, distinguishing features are polydactyly, renal abnormalities and hypogonadism (Bardet-Biedl), impaired vision and sensorineural deafness (Alström), progressive retinal dystrophy (Bardet-Biedl, Cohen, Alström).

Medical care

Preventive management for patients with Temple syndrome needs to be adapted to the age-dependent presentation (Table 5). Complications early on in the natural history of Temple syndrome are hypoglycaemia due to premature birth and/or poor feeding as well as hydrocephaly with raised intracranial pressure. In mid-childhood learning difficulties, short stature and truncal obesity predisposing to scoliosis, hypercholesterolaemia, diabetes and obstructive sleep apnoea become the prevailing problems. Guidelines for the management of paediatric obesity are available at www.endo-society.org/guidelines/final/upload/FINAL-Standalone-Pediatric-Obesity-Guideline.pdf or www.uni-duesseldorf.de/AWMF/ll/050-002.pdf but do not pay special attention to patients with imprinting disorders. No UPD(14)mat–specific growth charts are available. It remains to be investigated whether for monitoring purposes PWS-specific charts for height, weight and body mass index could be used instead. For a collection of PWS growth charts specific for various ethnicities see Butler, Phillip, Whitman, Management of Prader-Willi syndrome, Springer, 3rd edition, 2006.

Precocious puberty defined as puberty starting before eight years in girls and before nine years in boys may be a crucial diagnostic clue in hitherto unrecognized patients, but is also affected by ethnic and familial factors. Precocious puberty is more easily recognized in girls (breast growth) than in boys (testicular enlargement prior to penile growth and appearance of pubic hair). Endocrine laboratory studies including gonadotropins and CNS imaging should be performed for possible pituitary anomalies. Patients with imprinting disorders are not separately considered in guidelines for the management of precocious puberty. (www.ich.ucl.ac.uk/clinical_information/clinical_guidelines/scg_guideline_00003) Given the frequent combination of short stature and precocious puberty in patients with Temple syndrome, a combined treatment with growth hormone and gonadotropin-releasing hormone (GnRH) analoga[86] should be considered to postpone the pubertal growth spurt.

Practice points – UPD(14) syndromes

- UPD(14)pat patients: lack of knowledge about long-term prognosis demands an open and individual approach respecting parental attitudes towards medical interventions.
- Patients with unexplained PWS-like presentation: low threshold for genetic testing for UPD (14)mat (Temple) syndrome in expert laboratory.
- Assessment of recurrence risk for Temple syndrome: parental chromosome analysis whenever a structural cytogenetic anomaly involving chromosome 14 is the cause.
- The typical UPD(14)mat phenotype is age-dependent. Hypoglycaemia, growth retardation, obesity with obstructive sleep apnoea and hypercholesterolaemia, scoliosis, and central precocious puberty (CPP) are the main issues for preventative management in infancy and childhood.
- Suspected central precocious puberty (CPP): do endocrine studies including bone age assessment and cranial MRT to make the diagnosis at an early stage. Especially important in boys, in whom clinical signs of CPP are less obvious.
- Growth hormone and/or GnRH analoga therapy in patients with Temple syndrome should be supervised by a paediatric endocrinologist. For guidelines see consensus statements.[61,87]

General diagnostic considerations with regard to UPD

A patient presents with an autosomal-recessive disorder. The gene locus is in a known region of genomic imprinting. In this situation, unexplained features such as physical asymmetry, congenital failure to thrive, childhood obesity and developmental delay in a patient with a well-known recessive phenotype should trigger heterozygosity testing of the parents, if this has not been done already.[23] If one parent unexpectedly is not a heterozygous mutation carrier, UPD for the mutated allele of the carrier parent should be considered.

Vice versa, findings in a patient with proven UPD, that are not accounted for by UPD alone, warrant a review whether the UPD segment includes an autosomal-recessive gene that could explain the additional symptoms. If this is not the case, chromosome analysis in a peripheral heparin blood sample or in cultured fibroblasts from a skin punch biopsy might reveal mosaicism for trisomy or for a mosaic marker chromosome. Lastly, it should be kept in mind that a patient with a UPD syndrome and additional features might have a second independent genetic condition as in the case of a patient with UPD(14)mat and autosomal-recessive rod monochromacy. This patient was frequently and wrongly cited as an example for “unmasking of a recessive allele” by UPD until a homozygous mutation in the *CNGB3* gene on chromosome 8q was identified.[88]

Genetic management issues of imprinting disorders

Patients with a diagnosis of UPD(7) or UPD(14) syndrome and their families should be offered genetic counselling, ideally in the setting of a multidisciplinary childhood development clinic that brings together a community paediatrician, a paediatric endocrinologist, a dietary specialist, and a clinical geneticist. The specific role of the clinical geneticist covers three areas:

a) making the clinical diagnosis and communicating the results of the molecular genetic testing to the patient (see previous chapters);
b) advice re recurrence risk of UPD in future pregnancies and if appropriate re prenatal diagnostic options;
c) liaising the family of an affected child with other families in similar situations to enable an exchange of experience.

Recurrence risks

The large majority of imprinting disorders is sporadic and does not have a measurably increased recurrence risk. However, a thorough work up of the underlying molecular mechanism in the individual case is always necessary in order to identify the few ones with a clearly increased recurrence risk (Table 2). Two scenarios in the UPD patient may be associated with an increased recurrence risk of UPD for future siblings or children: if UPD results from a chromosomal translocation or ESAC, or if the UPD syndrome is due to a chromosomal microdeletion of the imprinted gene region. In these cases, karyotyping or microdeletion analysis should be offered to the parents. Empirically, an inherited Robertsonian translocation entails an elevated but low recurrence risk of <1% just like a *de novo* Robertsonian translocation (see chapter 2 "Background", paragraph "Mechanisms that lead to UPD and prenatal risk figures").[18] A high recurrence risk of up to 50% is associated with an inherited microdeletion. Occurrence of UPD(14)mat and UPD(14)pat in the same family has been reported several times as a result of an inherited microdeletion encompassing imprinted genes or as a result of a deleted *cis*-acting imprinting centre.[70,28]

Prenatal diagnosis

The individual recurrence risk and the range of clinical severity of the UPD syndrome in question are factors that the parents of an affected child will consider when deciding whether to opt for prenatal diagnosis and thereby implicitly whether to continue or terminate an affected pregnancy.

In purely medical terms, the decision process about prenatal diagnosis seems to be more clear-cut for an increased recurrence risk for a severe condition, e.g. heterozygous maternal 14q32.2 microdeletion with a 50% risk for UPD(14)pat syndrome in the offspring, than for a combination of a low recurrence risk with a relatively good prognosis in case of an affected child, such as UPD(14)mat as a sporadic event. However, the intrafamilial phenotypic variability of the Temple syndrome means that the long-term challenge for parents of a second affected child cannot be reliably predicted. In the end, the decision is always a personal one that the informed parents take.

Assisted reproductive technology (ART)

An increased absolute risk of <1% for a foetal imprinting disorder in ART conception has been suggested by the authors of an in-depth review on this topic.[89] However, confounding factors such as ovulation induction prior to ART, increased parental age and/or sub-/infertility, which are often the reason for ART in the first place, put in question whether the link to ART as such is causal or at least partly a selection bias.[90,91] Idiopathic male infertility, e.g., is strongly associated with aberrant methylation of *MEST* (7q32) and *IGF2/H19 ICR1* (11p15.5).[92]

Assuming a causal role of ART, an induction of primary epimutations is more conceivable than an induction of DNA mutations such as microdeletions.[93] This hypothesis is confirmed by the study of patients with Beckwith-Wiedemann syndrome (BWS; OMIM 130650), a multigenic imprinting disorder that is caused in ~60% of cases by loss of maternal methylation at the so called DMR2 locus on 11p15.5: In ART-conceived BWS patients the proportion of this molecular subgroup is increased to 83–100% of cases.[89]

As each imprinting disorder is rare, a relative risk increase through ART should be more easily detected for those imprinting disorders with a relatively high proportion of epimutations already in natural conceptions such as Beckwith-Wiedemann, 11p15.5-associated Silver-Russell syndrome, and possibly UPD(14) syndromes. In fact, an association with ART has been demonstrated for Beckwith-Wiedemann and Angelman syndrome, but not convincingly for Prader-Willi syndrome. Only two ART-conceived SRS patients (one with equivocal molecular data and one with mild phenotype) and no ART-conceived UPD(14) patients have been published.[93] In summary, there are as yet no data for UPD(7) or UPD(14) syndromes to prove or to exclude a causal link with ART. Animal models are of limited value because of species-specific differences in natural reproductive biology.[94]

Non-medical support

Patients' organisations are an important source of support for parents of a child with a congenital developmental disorder. Addresses of the national organisations for patients with UPD(7) or UPD(14) are listed in www.orpha.net. There is a tendency for the families of the more severely affected spectrum of either condition to join the respective patients' organization thus distorting the true picture somehow. For information on clinical trials or on research projects the parents should be pointed towards http://clinicaltrials.gov and www.orpha.net.

Conclusion and outlook

Silver-Russell syndrome and UPD(14) syndromes are complex imprinting disorders with characteristic age-dependent phenotypes, genetic heterogeneity and low recurrence risk in the majority of cases. The diagnostic strategy includes sequential epigenetic and DNA-based locus-specific tests and has been modelled on diagnostic algorithms for Prader-Willi/Angelman syndrome. DNA-based testing or cytogenetic analysis of parents is sometimes required for accurate determination of recurrence risks. Owing to the rarity of SRS and UPD(14) syndromes, no specific therapeutic guidelines have been established. Prevention of hypoglycaemia and dystrophy in early life, growth hormone treatment for short stature, orthopaedic interventions for physical asymmetry, and developmental support are the therapeutic essentials in SRS. Whilst a palliative approach is an option in UPD(14)pat syndrome, UPD (14)mat syndrome therapy focuses on the initial failure to thrive and on preventing subsequent obesity, short stature and precocious puberty.

Fields of further research

Clinical diagnosis and research into (epi-)genotype–phenotype correlations of imprinting disorders such as SRS or UPD(14)mat syndrome would benefit from the development of age-specific clinical diagnostic scoring systems, as have been established for Prader-Willi syndrome.[95] Currently, four different scoring systems are being used for suspected SRS patients (Table 3).

Two studies suggest that the milder SRS-like phenotypes are preferentially found in UPD(7)mat patients[41,52]: Among the patients with hypomethylation 11p15 only 1 out of 42 (2.4%) did not reach the diagnostic threshold of 8 points in the scoring system by Bartholdi. Only 4 out of 44 (9%) showed merely 1 or 2 criteria in the study of Wakeling, who employed the scoring system of Price et al. In contrast, for UPD(7)mat patients the corresponding figures were 3 out of 10 (30%) and 5 out of 20 (25%), respectively. It could be argued therefore that for practical reasons patients with a mild SRS-like phenotype and absence of asymmetry should be tested in reverse order: first for UPD(7)mat and only then for 11p15 hypomethylation.

Technological progress now allows analysis of methylation patterns on an epigenome-wide scale.[96] However, just like in genome-wide testing for DNA imbalances by array-CGH, there are two prerequisites for a meaningful interpretation of the molecular data: (a) Definition of physiological epigenome patterns. This is a considerably more complex challenge than on DNA level, because methylation patterns are partly dynamic and may depend on the tissue and the age of the proband.[97] (b) Carefully phenotyped patients who allow correlation between molecular and clinical data. Especially for the rare imprinting disorders such as SRS and UPD(14) specific international registries might be valuable tools to collect phenotypic and epidemiological data (incidence, frequency after assisted reproductive technologies) despite the inherent reporting bias.

The goals of phenotypic and molecular studies are to explore the full extent of the clinical variability in Silver-Russell and UPD(14) syndromes, and to better understand their molecular basis and natural history. SRS might be caused by dysregulation of a gene network in which allele-specific gene expression is not only controlled by a *cis*-acting imprinting centre but also by *trans*-acting genetic factors.[98] Molecular dissection of SRS might make it possible to attribute individual clinical features to specific (epi-)genetic alterations. This knowledge in turn would help to optimize preventative clinical management, e.g. by predicting from the (epi-)genotype of an individual SRS patient whether she/he has a low or high risk of developing hypoglycaemia.

Research agenda

- Define clinical criteria when to initiate molecular genetic testing for Silver-Russell syndrome in SGA children.
- Chromosome 7 or chromosome 11 associated Silver-Russell syndrome: what are the molecular pathomechanisms?
- What degree of hypoglycaemia is clinically significant and needs to be treated? This becomes particularly relevant with the new option of continuous glucose monitoring in neonates.[99]
- SRS patients with asymmetry: hemihypertrophy or hemihypotrophy? Can the hypothesis be confirmed that tumour risk is not significantly elevated?
- (Epi-)genotype–phenotype studies in SRS patients: corroborate suspected IGF-1 insensitivity/reduced GH responsiveness in patients with 11p15 hypomethylation.
- UPD(14)pat (Kagami) syndrome: study effect of interventions such as temporary artificial ventilation and tube feeding document on long-term cognitive outcome and quality of life.
- Molecular pathophysiology of age-related endocrine phenotypes in UPD(14)mat (Temple) syndrome.
- The therapeutic long-term benefit of hormone therapy in patients with Temple syndrome needs to be investigated.
- Idiopathic cases of Silver-Russell syndrome or Temple syndrome: microarray-CGH studies and genome-wide methylation analysis to understand the contribution of genomic microdeletions/-duplications and multilocus epimutations.
- A comprehensive cytogenetic and phenotypic database has been set up with $>$ 1,100 UPD cases for all chromosomes combined: http://www.med.uni-jena.de/fish/sSMC/00START-UPD.htm. This database should in future prevent unintentional multiple reporting of the same case and facilitate epidemiological analyses.

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