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Why is There No Diploid Overdose Effect in Prader-Willi Syndrome Due to Uniparental Disomy?

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Abstract. Due to DNA technology, it is now apparent that the mechanisms of genetic disease are more complex than the model of a gene with biallelic expression in the diploid state. If a gene is imprinted, monoallelic expression is the norm when the chromosomes of a pair are inherited normally from each parent. Uniparental disomy (UPD) is the abnormal situation where both chromosomes of a pair come from the same parent. When the chromosome contains an imprinted gene, UPD may result in nullisomy or disomy for a functional copy of that gene. If there are two imprinted loci on the same chromosome, UPD for that chromosome results in nullisomy for one imprinted gene but functional disomy for the other a "diploid overdose" (DO). This situation has been well demonstrated in the Prader-Willi syndrome (PWS) which is the nullisomic phenotype for the PWS gene(s) on chromosome 15q11-13. Chromosome 15q11-13 also contains the gene for Angelman syndrome (AS) which has a phenotype distinct from PWS. Both loci are subject to imprinting – in PWS, the imprint is on the maternal chromosome 15, in AS it is on the paternal chromosome 15. All individuals with PWS due to maternal UPD, while functionally nullisomic for the PWS locus, are functionally disomic for the AS locus – a DO situation. Assuming that biallelic expression of an imprinted gene is harmful, one would expect DO for an imprinted gene to produce a phenotypic effect. Cases of PWS due to UPD do not appear to differ from those due to deletion (hypopigmentation in deletional cases can be explained by loss of D15S12 downstream from the critical region). There is no good evidence of DO for the AS locus in PWS due to UPD. Why then was it 'necessary' in evolutionary terms to imprint the AS locus and maintain the imprint faithfully for life. A similar situation of two imprinted genes on the same chromosome occurs with IGF2 and H19 on chromosome 11p15. Maternal imprinting for IGF2 and paternal imprinting for H19 is the norm. Paternal UPD in this situation does lead to a DO effect, namely Beckwith-Wiedemann syndrome. The possibility of a DO effect needs to be considered when assessing the phenotypic spectrum of UPD for other chromosomes currently under investigation.

Key words: Imprinting, Chromosome 15, Angelman syndrome, Beckwith-Wiedemann syndrome, H19

Biallelic versus monoallelic gene expression

The phenotypic expression of chromosomal and genetic disorders in humans has been of interest for many decades [1]. With the advent of DNA technology into clinical medicine during the 1980s, it has become apparent that mechanisms of genetic disease are more complex than the simple situation of two alleles, one on each chromosome of a pair, both of which are required to function for normal development [2]. Recessive disorders have shown that both alleles need not be functional for normal development, as heterozygous carriers of autosomal recessive mutations have normal growth, mental and physical development and fertility. For fully dominant genes, two normal copies are necessary for normal function. For certain other genes, physical deletion of one copy leads to an abnormal phenotype due to haploinsufficiency [3]. Hemizygosity is another facet of gene expression for genes such as those on the X chromosome which are inactivated (at random) in females and are active in males, so that one functional copy is the normal situation. Random X inactivation and two functional copies of a gene allow flexibility should mishap (eg. gene mutation, deletion) befall one copy.

Imprinting

A newly identified mechanism affecting gene expression is imprinting, whereby one allele of a pair, although physically present and apparently normal, is silenced. This occurs specifically and exclusively from one designated parent and is thus non-random [4-7]. Up to 25% of the mouse genome appears to be imprinted [8], but so far few human genes show this property [9]. The non-random silencing of one allele reduces flexibility for normal development so must serve an important function. The Prader-Willi syndrome (PWS) and Angelman syndrome (AS) loci, both on chromosome 15q11-13 are imprinted, the PWS locus always on the maternal chromosome and the AS locus on the paternal chromosome. On chromosome 11p15 IGF2 and H19 are also reciprocally imprinted, the maternal chromosome for IGF2 and the paternal for H19 [6, 10].

Imprinting occurs early in development, and is thought to be fundamental for normal human development [4, 5, 11, 12]. The process of imprinting requires establishment, which includes recognition of a difference between maternal and paternal chromosomes, maintenance and erasure as the gene passes through the germ line and somatic cell lines over the generations. Methylation plays an important role in the cellular imprinting process, usually by inactivating the gene [4, 6, 13-15]. The role of methylation in maintenance and erasure of the imprint through the action of methylases and demethylases appears to be well established [15-17]. Implementation of imprinting is not considered to be wholly the result of methylation as some studies have shown that methylation follows rather than precedes inactivation [17, 18]. Other mechanisms also involved in imprinting and parent-of-origin effects, include allele-specific replication [19], cis-acting gene regulation from an imprinter control element within a nucleosome domain of imprinting [7] or a chromatin effect [20]. These complex control mechanisms require cellular energy and organisation. It is clear that for specified genes, imprinting is not a trivial process. For the expression of imprinted genes on chromosome 15q11-13, not only is one allele sufficient, but it appears to be 'imperative' for some aspect of normal development and function influenced by these genes.

Uniparental disomy (UPD)

UPD is the abnormal situation where both chromosomes of a pair are derived from the same parent. First described in 1980 as a rare phenomenon in association with translocations of chromosome 22 [21], it has since received much discussion and investigation. UPD was shown to be associated with human disease when it was demonstrated in PWS [22], and then later in AS [23] and Beckwith-Wiedemann syndrome [10]. UPD is currently under investigation for trisomy 16, intrauterine growth retardation [24] and postnatal growth retardation [25, 26]. New cases are rapidly being reported for different chromosomes [26, 27] suggesting that UPD is more frequent than was thought and could be quite frequent in certain situations, such as Robertsonian translocations [28]. In the first description of UPD in PWS, the clinical phenotype was attributed to the lack of a paternal contribution to the 15q11-13 region [22].

The frequency of UPD in PWS is now known to be quite high – 25% of cases – but it is much lower in AS, accounting for only 2-3% of cases [7, 23]. UPD may be heterodisomic if the chromosomes are different and isodisomic if they are the same [11]. One mechanism is through rescue of an originally trisomic conceptus, reflecting maternal nondisjunction in PWS associated with advanced maternal age [29, 30].

Consequences of UPD

When UPD of a chromosome occurs, the effects may vary depending on whether there are imprinted genes on that chromosome and whether the UPD is heterodisomic or isodisomic (Table 1). The effect of maternal UPD in PWS is to delete the normal paternal copy of the gene, making the individual nullisomic for the one functional necessary copy of the imprinted PWS gene. In addition, when UPD of chromosome 15 occurs, another effect will be biallelic expression of the AS gene which normally shows only monoallelic expression (Fig. 1). This can be considered as a diploid overdose DO effect (DOE). DOE should be regarded as a special subtype of gene dosage effects specifically for the situation with a balanced karyotype and UPD. This is different to gene dosage effects of marker chromosomes, isochromosomes or chromosomal aneuploidies because in these cases there are additional whole chromosomes which may be present as mosaics or interfere with cell division [31, 32]. In sex chromosome aneuploidies, additional X chromosomes become inactivated through X inactivation [13]. Phenotype-karyotype correlations in autosomal aneuploidies are still uncertain and depend on multiple factors [33].

Table 1 - Consequences of chromosomal UPD on gene expression

None
Unmasking of a recessive
Nullisomic phenotype of an imprinted gene
Diploid overdose effect of an imprinted gene

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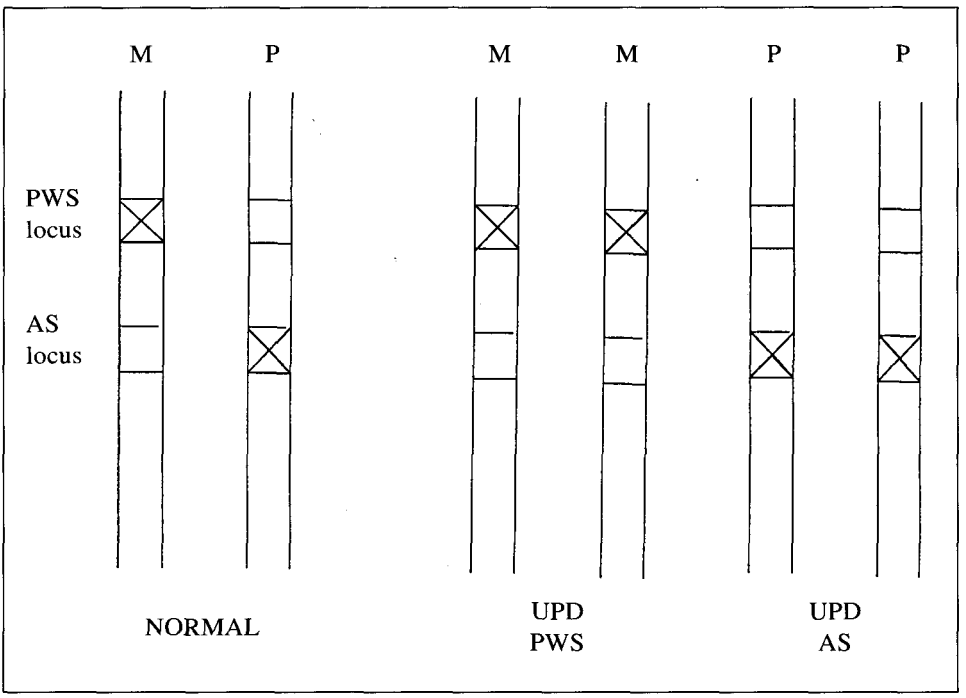

Fig. 1 - Diagrammatic representation of two chromosomes 15. The crossed square indicates imprinting with gene inactivation. The open square indicates the active locus. M = Maternal; P = paternal.

The hypothesis

The hypothesis proposed is firstly that for an imprinted gene, two functional alleles are harmful and secondly that this will produce an effect. The phenotypic effects of imprinted genes early in development are on survival, growth and placentation [5, 12, 16, 24, 34]. Later effects are not so well characterised, but have included growth retardation [25] and tumour development [10, 35, 36]. Effects on mental development, fertility and ageing are difficult to substantiate. Congenital anomalies and/or dysmorphic features are not considered to be a part of imprinting [12].

Cases of PWS due to UPD will have two active genes from the AS locus (Fig. 1) inherited from the mother. Can we assume that two copies of a gene that is normally imprinted, although of the parent of the other sex, does not have an adverse effect in some way on the phenotype at any time in the life of the individual? This aspect is rarely mentioned in discussions of UPD. It was mentioned in the original study of UPD in mice and considered to be without effect [37]. In a patient with an indefinite dysmorphic phenotype [38], maternal duplication for the AS locus was present and it was considered that this duplication could have produced a phenotypic effect in that patient.

Evidence for DOE

Comparison of deletional PWS and PWS due to UPD could potentially reveal a difference and thus provide evidence for DOE. Due to the frequency of UPD in PWS (about 25% of cases), the very rare occurrence of a nondeleted nondisomic form (maybe 1/1,000 cases) and the fact that the phenotype is well characterised [39, 40] provides an excellent model to look for evidence of a DOE. AS is not so suitable because UPD in AS is not frequent and the clinical diagnosis of AS is not straightforward [41]. The search for additional abnormalities in individual isolated cases of PWS due to UPD is being undertaken to unmask recessive disorders (Table 1) and aid in gene mapping of chromosome 15 [42]. The search for a DOE differs from this approach in that a feature or features sought should be present in all cases of PWS due to UPD.

Difference between deletional PWS and PWS due to UPD

Excluded from the following discussion is hypopigmentation, which is more common in deletional PWS than in PWS due to UPD [43]. Pigmentation has been shown to involve the P gene at the distal end of the chromosome 15 q11-13 region [44] which is deleted in over 90% of cases with the common large deletion [7, 45].

Few studies have looked closely at the phenotype of PWS and divided cases into deletional PWS and PWS due to UPD based on DNA testing. Two papers (Table 2) have addressed this point: Robinson et al. [46] for 7 cases of UPD and 19 of deletions, while Hamabe et al. [47] found 2 and 5, respectively. Together these cases amount to 9 UPD and 24 deletions. No clinical differences were brought to light. There is a distinct impression that such comparisons are well known and have been well done. Many studies have divided PWS patients into those with deletion and those with UPD – for example in one study there were 18 UPD and 5 deletions [48] and in another, 8 UPD and 18 deletions [49] but a clinical breakdown of features was not provided. Other reports have given clinical features but have lumped deletional and UPD cases together [45, 50]. After high-resolution cytogenetics (HRC) was introduced, many detailed clinical descriptions of PWS patients were based on the presence of cytogenetic deletions. On this basis, Butler et al. [51] first reported on the hypopigmentation of deletional patients and an apparently higher intelligence of patients with deletion than in those with normal chromosomes. No differences in anthropometric status were found in 38 PWS patients – 21 with apparent deletions and 17 non-deleted cases [52]. No difference in neuroen-

Table 2 - Clinical comparisons in PWS

Reference	UPD	Deletion
Robinson et al, 1991	7	19
Hamabe et al, 1991	2	5
	9	**24**

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docrine protein 7b was found between 13 cases of PWS with cytogenetic deletion and 13 non-deleted patients [53] or in calorie requirements between 25 patients with chromosomal deletion and 31 without deletion [54]. HRC is now known to be inaccurate for deletion detection with both false positives and false negatives reported when compared with DNA studies [7, 55].

The comparisons which have been made are rather superficial, lacking hard objective data such as the results of laboratory tests, neurological assessments, cerebral scanning techniques and personality traits. One excellent study on magnetic resonance imaging compared 6 AS patients with 4 PWS patients but no comparisons were made within each group [56]. Similarly, an excellent evaluation of autonomic system function, made on 14 subjects with PWS did not separate them into the two groups [57]. There are potentially a number of interesting areas where differences between PWS due to deletion and UPD could be sought. The development of psychoses [58], occurrence of dyslexia [59], sleep patterns [60], ageing [61] and response to treatment [62] are areas of interest, not studied in this comparative way. Reported heterogeneity among PWS patients could reflect differences between cases with deletion and UPD [63]. Clear, well-designed, detailed studies looking for differences between PWS with DNA deletion and UPD are required to exclude a DOE.

Why no DOE?

If there is no difference between PWS due to deletion and UPD, as suggested by the bulk of current information, why is there no DOE?

(1) The basic assumption could be wrong, namely biallelic expression is not harmful either in fetal or postnatal life.

(2) Maybe the situation is the other way around and monoallelic expression is beneficial in some way (this still implies that biallelic expression is harmful). If biallelic expression were harmful in utero, three possible effects could accrue. (*a*) It could result in fetal demise. This is not the case, otherwise we would not see UPD for chromosome 15 in humans. It is interesting that overexpressed H19 does lead to death in utero [6]. (*b*) An effect on placentation. Placentation in PWS has not been examined, but monoallelic expression of the paternal locus (as in AS) possibly limits the size of the placenta to 'normal'. There is considerable evidence that the father's contribution is important to placental size and mother's contribution to fetal size [4, 5]. (*c*) Fetal growth. At birth, the PWS infant is usually of normal weight and length, although occassionally birth weight is reduced. Maybe two active maternal alleles result in a fetus too large for delivery and imprinting limits fetal growth to a 'normal' size [4, 5]. Imprinting of the PWS and AS loci possibly complement each other for this function – namely to produce a fetus and placenta of a size compatible with normal intrauterine development and ease of delivery – an example of 'nature vs. nurture' [12].

Is there no further requirement for imprinting? Postnatally, biallelic expression does not appear to be harmful, either in causing a degenerative disorder or cancer (PWS patients are not cancer prone as a group). The incidence of primary congenital malformations in PWS is low. To date, there is no benefit which can be attributed to PWS due to

UPD, compared to deletional PWS. It is interesting that preliminary data suggests a possible beneficial effect postnatally on brain function in AS due to UPD [41].

If imprinting has no purpose in postnatal life, why is the cellular machinery so faithfully geared to maintain the imprint with each cell division throughout life. Case studies have shown a tight fit of classical PWS with deletion or UPD [7] – in 15 patients with no deletion or disomy on DNA testing, all had atypical phenotypes [49]. Methylation studies have also shown that the imprint is tight, certainly with probe PW71 for the locus D15S63 [64, 65] and for exon α, SNRPN [66]. A description of heterogeneity of methylation on the maternal allele for intron 5 of the SNRPN gene [67] could represent loss of imprinting (LOI) in this context. LOI has been shown to occur in some cases of Wilms' tumour [6]. The differences in methylation in 2 of 3 affected AS sibs at ZNF127 in family AS013 [68] could also be interpreted as LOI. However, the evidence on the whole suggests a tight imprint for life.

(3) The nullisomic phenotype is predominant and overrides any DOE. This is possible as in both PWS and AS, the phenotype is severe. Early survival in PWS is in large part dependent on intensive neonatal care. The PWS transgenic mouse does not survive more than 2-3 days postpartum [69]. With two reciprocally imprinted loci on chromosome 15, UPD will always produce a nullisomic phenotype and if this overrides a feature due to DOE, we shall never know.

Other imprinted loci and UPD

What is the expectation of a phenotype due to DOE of the AS locus. We do not know, but it would certainly not exhibit any of the features of the nullisomic phenotype. Atypical cases of PWS cannot be ascribed to a DOE. Perhaps we should be looking for UPD of chromosome 15 in abortions, abnormally sized placentas, in large babies or other sporadic conditions involving brain function or tumour development without congenital anomalies or marked dysmorphic features.

If there is no DOE for the AS locus on chromosome 15q11-13 does this then mean that there is no DOE for other loci which are imprinted? For chromosome 11p, there is a DOE, namely Beckwit-Wiedemann syndrome, which is distinct from the deletional phenotype seen in some abnormal chromosomes involving 11p15 [70]. Cases of UPD with a normal phenotype, for example maternal UPD for chromosome 22 [71] and maternal UPD for chromosome 13 [72], do not exclude imprinted gene(s) on the other (paternal) chromosome. Neither do they exclude an imprinted gene on the maternal chromosome – they only exclude an imprinted gene with a nullisomic phenotype.

Conclusion

Genomic imprinting has opened new doors to unravelling factors involved in gene expression and mechanisms of disease but we are only just starting out on this path. UPD is only 6 years old in terms of awareness among geneticists as being something to positively look for. One consequence, namely the unmasking of a recessive disorder, has just recently been appreciated and is now being actively researched. It seems timely not to

assume that not all phenotypes with UPD are due to nullisomy of a putative imprinted gene but could be due to a DOE of a putative imprinted gene. The current lack of an example from the good model of PWS should not detract from the possibility of DOE or deter conscious thought about it. Considerably more research is required to follow the natural history of human genetic syndromes associated with imprinting and the significance and consequences may vary from one gene to another.

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