Advanced Maternal Age and the Development of Prader-Willi Syndrome Resulting from Upd(15)mat through Non-disjunction at Meiosis 1

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Abstract: Advanced maternal age at childbirth is a risk factor for the generation of trisomies such as Down syndrome because of the high frequency of non-disjunction at maternal meiosis 1. Thus, it is predicted that increased childbearing age is also relevant to the generation of maternal uniparental disomies caused by trisomy rescue or gamete complementation with a disomic oocyte produced through non-disjunction at maternal meiosis 1. Here, we report our molecular genetic data for 117 patients with Prader-Willi syndrome. The results indicate that advanced maternal age at childbirth is a predisposing factor for the development of maternal uniparental disomy 15 because of increased meiosis 1 errors. This notion is applicable to other maternal uniparental disomies as well, and provides useful insight into the occurrence of imprinting disorders after artificial reproductive technologies (ART). In particular, it is recommended to perform maternal age-matched comparison for the evaluation of the potential influence of ART on the development of maternal upd.

Key words: Advanced maternal age, Trisomy rescue, Uniparental maternal disomy, Prader-Willi syndrome, Non-disjunction

Introduction

Genomic imprinting is a genetic phenomenon by which certain genes are expressed in a parent-of-origin-specific manner [1, 2]. This phenomenon is primarily observed in placental mammals, and human and other mammalian genomes contain multiple imprinted regions/domains [2]. Such imprinted regions/domains usually harbor differential methylation regions (DMRs) that are clearly methylated or unmethylated according to the parental origin, as well as paternally expressed genes (PEGs) (i.e., maternally imprinted genes) and maternally expressed genes (MEGs) (i.e., paternally imprinted genes) [2, 3]. Consistent with the parental origin specific methylation pattern, DMRs frequently function as imprinting control centers [3].

Imprinting disorders are caused by various mechanisms. They include not only intragenic mutations of imprinted genes and uniparental disomies, but also microdeletions and epimutations (hypermethylations or hypomethylations) involving DMRs and/or imprinted genes [4]. Among these mechanisms, it has been postulated that maternal uniparental disomies (upd) may increase with maternal age at childbirth, as has been observed in trisomies that are known to increase with maternal childbearing age, because the prevalent occurrence of non-disjunction at maternal meiosis 1 [5]. Here, we summarize our data which indicates that advanced maternal age at childbirth is a risk factor for the development of maternal upd in Prader-Willi syndrome (PWS) [6].
(Epi)genetic Causes of PWS

PWS is a representative imprinting disorder associated with various dysmorphic, neurologic, cognitive, endocrine, and behavioral/psychiatric features [7]. It is caused by a lack of expression of PEGs on the imprinted region at chromosome 15q11.2–q13, and previous studies have indicated that deletions of the paternally derived imprinted region and maternal upd for chromosome 15 (upd(15)mat) account for ~70% and ~25% of PWS patients, respectively [7]. The remaining PWS patients have rare abnormalities such as epimutations (hypermethylation) of the DMR encompassing exon 1 of SNRPN that functions as the PWS imprinting center (PWS-IC) and microdeletions involving the PWS-IC or HBII-85 snoRNAs distal to the PWS-IC [8–10].

Genetic Mechanisms Leading to Upd(15)mat

Upd(15)mat is defined as a situation in which both of the chromosome 15 homologs are derived from the mother. It is primarily caused by one of four mechanisms: trisomy rescue (TR), gamete complementation (GC), monosomy rescue (MR), or post-fertilization mitotic error (PE) (Fig. 1) [11]. TR refers to a condition in which chromosome 15 of paternal origin is lost from a zygote with trisomy 15 formed by fertilization between a disomic oocyte and a normal sperm. GC results from fertilization of a disomic oocyte with a nullisomic sperm. MR refers to a condition in which chromosome 15 of maternal origin is replicated in a zygote with monosomy 15 formed by fertilization between a normal oocyte and a nullisomic sperm. PE is an event after formation of a normal zygote.

Maternal Age at Childbirth and the Development of Upd(15)mat

Of the four mechanisms leading to upd(15)mat, TR and GC are mediated by a disomic oocyte that is produced by non-disjunction at meiosis 1 (M1) or meiosis 2 (M2). Non-disjunction at M1 is known to increase with maternal age, probably because of a long-term (10–50 years) meiotic arrest at prophase 1 [12]. It is predicted, therefore, that the relative frequency of TR/GC type upd(15)mat through M1 non-disjunction is high in PWS patients born to aged mothers and is increasing in countries where childbearing age is rising.

Previous studies have revealed a significantly higher

Fig. 1. Schematic representation of normal and abnormal meiosis and the four mechanisms involved in the generation of upd(15)mat. Abnormal gametes, zygotes, and somatic cells are highlighted with light gray. Normal meiosis: The homologous chromosomes shown in red and orange recombine at the prophase and undergo meiosis 1 (M1) and meiosis 2 (M2), to produce gametes with a haploid set of chromosomes. Abnormal meiosis: When non-disjunction occurs at M1, this can produce nullisomic gametes and four types of disomic gametes. When non-disjunction occurs at M2, this can produce nullisomic gametes and two types of disomic gametes. Note, disomic gametes generated by M1 and M2 non-disjunctions can be distinguished by microsatellite data. When the pericentromeric region is present in the heterodisomic and an isodisomic conditions, this indicates the generation of disomic gametes through M1 and M2 non-disjunctions, respectively. In this instance, when two recombinations take place, this can influence the status (isodisomy or heterodisomy) of the middle to distal region, but not that of the pericentromeric region. Thus, the status of the pericentromeric region is informative about the timing of a non-disjunction. In contrast, it is impossible to discriminate between nullisomic gametes generated by M1 and M2 non-disjunctions, Upd15mat subtypes: Paternally derived chromosomes are depicted in blue and maternally derived chromosomes in red. In trisomy rescue (TR), a maternally derived disomic oocyte is fertilized with a normal sperm and, subsequently, the paternally inherited chromosome is lost from a trisomic zygote. In gamete complementation (GC), a maternally derived disomic oocyte is fertilized with a nullisomic sperm. Note that the disomic oocytes in TR and GC harbor a heterodisomic region(s), as shown in the schema of abnormal meiosis. In monosomy rescue (MR), a maternally derived normal oocyte is fertilized with a nullisomic sperm and, subsequently, the maternally inherited chromosome is replicated in a monosomic zygote. Thus, MR results in the formation of a full maternal isodisomy. In post-fertilization mitotic error (PE), a post-zygotic non-disjunction takes place with or without a recombination between non-sister chromatids, and is followed by the loss of an excessive chromosome containing a paternally derived region. Thus, PE respectively leads to either the generation of a segmental or full isodisomy.
maternal age in PWS patients with upd(15)mat than in those with deletions [13, 14], a significantly higher relative frequency of upd(15)mat in patients born to mothers aged ≥35 years than in those born to mothers aged <35 years [15], and a significantly increased relative frequency of upd(15)mat in PWS patients <5 years of age in United Kingdom where childbearing age is increasing [16]. In these studies, however, the underlying mechanisms for upd(15)mat were not examined, and it remains to be clarified whether or not advanced maternal age is relevant to the occurrence of TR/GC type upd(15)mat through M1 errors.

Japanese PWS Patients Examined

To examine the effect of advanced maternal age on the development of upd(15)mat mediated by M1 non-disjunction, we studied 117 Japanese PWS patients (72 male patients and 45 female patients) who satisfied the following selection criteria: normal karyotype in all the 50 lymphocytes examined; hypermethylated PWS-IC that was confirmed by methylation analysis for bisulfite treated leukocyte genomic DNA, using methylated and unmethylated-allele specific PCR primers [17]; and positive data on the maternal age at childbirth (parental age was not available in two aged patients who had left our follow-up).

Classification of PWS Patients

We sequentially performed fluorescence in situ hybridization (FISH) analysis for a ~125 kb region encompassing SNRPN, microsatellite analysis for 13 loci on chromosome 15, and multiplex ligation-dependent probe amplification (MLPA) analysis for multiple segments including the PWS-IC. From the results, we identified deletion of the imprinted region in 84 patients (Deletion group), upd(15)mat in 25 patients, and epimutations in two patients (Fig. 2). In the remaining six patients without a deletion, further classification was not possible, because microsatellite analysis was not performed (Non-deletion group).

Furthermore, upd(15)mat was divided into three subgroups by the microsatellite data, as follows (Fig. 3) [18, 19]: heterodisomy for at least one of the three adjacent pericentromeric (<4 Mb from the centromere) microsatellite loci (D15S541, D15S542, and D15S1035) was regarded as indicative of TR/GC type upd(15)mat through M1 non-disjunction (TR/GC [M1] subgroup); the combination of isodisomy for the pericentromeric microsatellite loci and heterodisomy for at least one middle to distal microsatellite loci was interpreted as indicative of TR/GC type upd(15)mat through M2 non-disjunction (TR/GC [M2] subgroup); and isodisomy for all the informative microsatellite loci was regarded as indicative of MR/PE type upd(15)mat (MR/PE subgroup). It is usually impossible to distinguish between TR and GC and between MR and PE on the basis of microsatellite data, although identification of trisomic cells is specific to TR and that of segmental isodisomy or mosaicism with a normal cell lineage is unique to PE. Consequently, of the 27 patients with upd(15)mat, 15 patients were classified as TR/GC [M1] subgroup, seven patients as TR/GC [M2] subgroup, and three patients as MR/PE subgroup (Fig. 2).

Parental Ages at Childbirth

Distribution of parental ages in each group/subgroup is shown in Fig. 4A, and parental age data are summarized in Table 1. Maternal ages were invariably ≥35 in the TR/GC [M1] subgroup. Furthermore, comparison of maternal ages in the Deletion group, TR/GC [M1] subgroup, and TR/GC [M2] subgroup with >5 patients revealed a significant difference between the Deletion group and the TR/GC [M1] subgroup (P=1.0×10⁻⁷), but not between the Deletion group and TR/GC [M2] subgroup (P=0.19) (Mann-Whitney U test). In addition, while maternal ages at childbirth were grossly similar between the Deletion group, the TR/GC [M2] subgroup, and the Japanese general population,
they were obviously higher in the TR/GC [M1] subgroup than in the Japanese general population. Paternal ages showed a similar tendency, probably because of marriages between partners with 2–3 years age difference. However, while a significant correlation was observed between maternal and paternal ages in the Deletion group and the TR/GC [M2] subgroup, there was no significant correlation between maternal and paternal ages in the TR/GC [M1] subgroup because of relatively advanced maternal ages in this group (Spearman’s rank correlation test) (Fig. 4B).

We next compared the relative frequency of each group/subgroup between two different time periods (until the year 2002 and since the year 2003), because the maternal age producing the largest number of live-births changed from 25–29 years to 30–34 years, and that producing the third largest number of live-births changed from 20–24 years to 35–39 years, between these two time periods (Fig. 5A). The relative frequency of each group/subgroup markedly differed between the 75 patients born before 2003, and the 42 patients born since 2003 (Fig. 5B). TR/GC [M1] was indicated in three of the 75 patients born before 2003, and six non-deletion type patients were invariably born until the year 2002. Thus, the TR/GC [M1] group accounted for at least three and up to nine of the 75 patients born until the year 2002, and 12 of the 42 patients born since the year 2003; and the relative frequency of TR/GC [M1] was assessed to be significantly different, with the P values being 1.8 × 10⁻⁷ for 3/75 vs. 12/42 and 0.025 for 9/75 vs. 12/42 (Fisher’s exact probability test). In addition, there was no significant change in the parental ages of each group between the two time periods, although the maternal ages at birth of all the patients significantly differed between the two time periods (Table 1).

Conclusions and Perspectives

Our data imply that advanced maternal age at childbirth constitutes a risk factor for the development of TR/GC [M1] subgroup type upd(15)mat. Indeed, maternal ages were significantly higher in the TR/GC [M1] subgroup than in the Deletion group that is free from maternal age effect. Furthermore, the relative frequency of the TR/GC [M1] subgroup has been significantly higher since the year 2003 have been delayed childbearing age became obvious, and advanced maternal ages at birth since the year 2003 have been primarily associated with the high frequency of the TR/GC [M1] subgroup. Although it was impossible to distinguish between TR and GC and between MR and PE [20], this would not pose a major problem as the patients with M1 non-disjunction are included only in the TR/GC [M1] subgroup.

The results provide a useful insight into the risk assessment of artificial reproductive technology (ART) in the occurrence of imprinting disorders [21]. Even if the frequency of maternal upd is apparently higher in children born after ART than in the general population, ART is primarily performed for aged couples [22]. Indeed, according to the 2007 National Survey
Fig. 4. Parental ages at childbirth
A: The distribution of parental ages in each group. The light pink and blue vertical bars represent the mean maternal and paternal ages at childbirth from 1970–2008.
B: Correlation between maternal and paternal ages at childbirth. Significant correlation is observed in the TR/GC [M2] and Deletion groups, but not in TR/GC [M1] group because of the relatively advanced maternal ages.

Fig. 5. Relative frequency of underlying genetic causes between two time periods
A: Secular trend in the number of live-births according to maternal ages in Japan. Constructed by the authors on the basis of the Annual Nationwide Survey Data from the Ministry of Health, Labour and Welfare.
B: Relative frequency of each group in 75 patients born until the year 2002 and in 42 patients born since the year 2003.
performed by Japan Society of Obstetrics and Gynecology, the frequency of children born after ART is ~1.8% of neonates in the general population and ~4.5% of neonates born to mothers aged ≥35 years. Thus, maternal age-matched comparison is necessary for the evaluation of the potential influence of ART on the development of maternal upd.

In summary, the results imply that the advanced maternal age at childbirth is a predisposing factor for the development of upd(15)mat because of increased M1 errors. This notion is applicable to maternal upd in general as well as to trisomies [23]. Further studies will permit a better assessment of the (epi)genetic risk of advanced maternal age at childbirth.

References


Table 1. Parental ages (year) at childbirth

<table>
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<tr>
<th></th>
<th>Deletion</th>
<th>TR/GC</th>
<th>TR/GC</th>
<th>MR/PE</th>
<th>Epi-mutation</th>
<th>Non-Deletion</th>
<th>All patients</th>
<th>General population</th>
</tr>
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<td>Maternal age</td>
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<td>36</td>
<td>32</td>
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<td>7</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>117</td>
</tr>
<tr>
<td>Until 2002</td>
<td>Median</td>
<td>29</td>
<td>37</td>
<td>32</td>
<td>29</td>
<td>–</td>
<td>36</td>
<td>30</td>
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<tr>
<td>Since 2003</td>
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<td>32.5</td>
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<td>35.5</td>
<td>33.5</td>
<td>38.5</td>
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<td>2</td>
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| Paternal age   | Median   | 32.5  | 40    | 35.5  | 31           | 41.5         | 36           | 33              | 30.6–33.0       |
| Number         |          | 82    | 15    | 6     | 3            | 2            | 6            | 114–b          |
| Until 2002     | Median   | 32.5  | 43    | 35.5  | 28           | –            | 36           | 33              |
| Number         |          | 58    | 3     | 4     | 1            | 0            | 6            | 72–b           |
| Since 2003     | Median   | 32.5  | 39.5  | 35.5  | 34           | 41.5         | –            | 34.5            |
| Number         |          | 24    | 12    | 2     | 2            | 2            | 0            | 42              |

The data of the general population indicate the range of the mean parental ages at childbirth from 1970 to 2008.

a Paternal age was not available for in two old patients who had left our follow-up and whose hospital records had been discarded.
b Paternal age was not identified for one patient who was born after artificial insemination with donated semen.

P = 0.00017.


