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ARTICLE

Different distribution of the genetic subtypes of the Prader–Willi syndrome in the elderly

Margje Sinnema*[,1,2,3], Kees EP van Roozendaal[1], Marian A Maaskant[2,4,5], Hubert JM Smeets[1,3], John JM Engelen[1,3], Nieke Jonker-Houben[1], Constance TRM Schrander-Stumpel[1,3] and Leopold MG Curfs[1,2,3]

The Prader–Willi syndrome (PWS) is a genetic disorder caused by the absent expression of the paternal copy of maternally imprinted genes in chromosome region 15q11–13. The frequencies of different subtypes in PWS are usually given in literature as 70% deletion, 25–30% maternal uniparental disomy (mUPD) and 3–5% others (imprinting centre (IC) defects and translocations). Little is known about factors that influence the frequency of genetic subtypes in PWS. The study sample comprised 102 adults with clinically and genetically confirmed PWS, contacted through the Dutch Prader–Willi Parent Association and through physicians specialized in treating persons with intellectual disabilities. Genetic testing showed 55 persons (54%) with a paternal deletion, 44 persons (43%) with an mUPD and 3 persons (3%) with a defect of the IC. The observed distribution in our study differed from that in literature (70% deletion, 30% mUPD), which was statistically significant (*z*-score: $P<0.05$). This was mainly caused by a higher proportion of mUPD in the advanced age groups. Differences in maternal age and BMI of persons with PWS could not explain the differences in distribution across the age groups. Our study population had a much broader age range, compared with other studies, because of a predominance of elderly people (40+ years) with PWS. In other studies, these elderly persons might have been undiagnosed and/or underreported because of a lack of genetic diagnosis. The results underline both the need for correct genetic diagnosis in all persons with PWS and adjustment of the guidelines for preventive management in adulthood.
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INTRODUCTION

The Prader–Willi syndrome (PWS) is a genetic disorder caused by the absent expression of the paternal copy of maternally imprinted genes in chromosome region 15q11–13. PWS is characterized by neonatal hypotonia and feeding problems, childhood onset hyperphagia and obesity, short stature, hypogonadism, intellectual disabilities (ID) and behavioural problems.[1–3] In population studies,[4–6] birth incidence rates have been revised from one in 15 000 to one in 22 000–25 000. There are two main genetic subtypes causing PWS: paternal interstitial deletion of the 15q11–13 region[7] and maternal uniparental disomy (mUPD) of chromosome 15.[8] Less commonly, PWS arises from an imprinting centre (IC) defect[9] or from unbalanced chromosomal translocation.

The frequencies of different subtypes in PWS are usually given in literature as 70% deletion, 25–30% mUPD and 3–5% others (IC defects and translocations).[10]

At present, little is known about factors that influence the distribution of genetic subtypes in PWS. Most prominent is the association of mUPD with increased maternal age. Whittington *et al*[10] reported a higher frequency of the mUPD subtype of PWS in children under 5 years of age living in the United Kingdom compared with the frequencies reported in literature. They suggested an increasing maternal age at conception in this generation of mothers as a likely explanation of this change. Cassidy *et al*[1] also postulated a relationship between mUPD and advanced maternal age. This relation has been ascribed to the mechanism of nondysjunction during meiosis, forming a trisomic zygote and the subsequent loss of the paternal homologue, resulting in mUPD.[11]

In recent years, assisted reproductive technologies (ART) have been studied extensively as a potential factor involved in the molecular cause of genetic abnormalities. One report suggested an elevated risk of birth defects and genetic abnormalities associated with infertility and treatment with ART.[12] Other reports on increased numbers of children with Beckwith–Wiedemann syndrome and Angelman syndrome after ART also suggested a potential association between ART and an increased rate of imprinting abnormalities.[13] Finally, a relationship between the aetiology of PWS and paternal exposure to hydrocarbons has been reported in one study,[14] but this has never been supported by other studies.

We report the different distribution of genetic subtypes across age groups in the adult PWS population in The Netherlands and the differences with frequencies in literature. Possible explanations for this different distribution have been examined.

The aim of this study was threefold: (1) to explore the relationship between the distribution of genetic subtypes and age in an adult PWS population in The Netherlands; (2) to explore the relationship

[1]Department of Clinical Genetics, Maastricht UMC, Maastricht, The Netherlands; [2]Governor Kremers Centre, Maastricht University, Maastricht, The Netherlands; [3]GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands; [4]Department of Health Care and Nursing Science, Maastricht University, Maastricht, The Netherlands; [5]Stichting Pergamijn, Echt, The Netherlands
*Correspondence: M Sinnema, Department of Clinical Genetics – Governor Kremers Centre, Maastricht University Medical Centre, PO BOX 5800, Maastricht 6202 AZ, The Netherlands. Tel: +31 43 3875797; Fax: +31 43 3875800; E-mail: margje.sinnema@gen.unimaas.nl
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between the distribution of genetic subtypes and maternal age; (3) to explore the relationship between the distribution of genetic subtypes and BMI, as a possible indicator of selective survival of a certain genetic subtype.

METHODS

Participants

This study is part of a larger study in The Netherlands on 'Ageing in PWS'. Nominees with possible PWS were contacted through the Dutch Prader–Willi Parent Association and through physicians specialized in treating persons with ID (Figure 1). The individuals with PWS and their main caregivers (family and/or professional caregivers) were visited at home. Through semistructured interviews, data were collected on demographics, physical, behavioural and psychiatric conditions and adaptive skills. Some physical measurements, such as height and weight, were taken. The study was approved by the Medical Ethics Committee of the academic hospital Maastricht in The Netherlands, and written informed consent to participate in the study was given by the legal representatives (mostly parents) of the adults with PWS.

Genetic diagnosis

During the interviews, parents were asked whether genetic tests on PWS, including genetic subtype, had been undertaken previously. Written confirmation on genetic diagnoses was retrieved from genetic centres, with permission from the legal representatives. Genetic testing was undertaken in participants who did not have a confirmed genetic diagnosis (n=40). Cytogenetic and molecular analyses using the SALSA MLPA kit P245 (MRC Holland, Amsterdam) were performed to establish whether deletions were present. Diagnosis of PWS was confirmed by DNA methylation studies on the SNURF/SNRPN locus and mUPD was confirmed with microsatellite analysis at various loci on chromosome 15, when blood samples of parents were available.

RESULTS

The study population

Participants of the study were recruited through the Dutch Prader–Willi Parent Association and through physicians specialized in treating persons with ID (Figure 1). In total, 149 nominees with (possible) diagnoses of PWS were notified to us. After eliminating those nominees who had died and whose parents stated that PWS was excluded by genetic testing, we were left with 145 possible participants. Of them, 108 actually agreed to participate in the study (response rate 74.5%). The participants underwent genetic laboratory testing before (n=68) or during the study (n=40) to confirm the diagnosis and determine the genetic subtype. For 102 persons, the genetic diagnosis of PWS was confirmed and, consequently, this report is based on these 102 individuals. Of these 102 individuals, 32 were recruited through physicians specialized in treating persons with ID, 18 of these persons underwent genetic testing during the study.

Figure 2 shows the age distribution of the study group (standardized to 1 March 2009). A substantial proportion of the study population (44%, n=45) was $\geq$40 years of age. The oldest participant was 66 years old (Figure 2). Of the persons above the age of 40 years, 56% (25/45) underwent genetic testing because of an unconfirmed clinical diagnosis in the past.

The level of ID was mild (49%, n=50) or moderate (28%, n=29) in most participants. Eight (8%) participants had a severe ID. The other participants were functioning on a borderline ID level (10%, n=10) or did not have an ID (5%, n=5). The majority of the individuals were ambulant (86 of 97). Five persons needed minor adjustments for walking and five other persons needed major adjustments. One person was not ambulant anymore. Intellectual disability and mobility did not differ between genetic subtypes.

In total, 37 persons decided not to participate in the study. The mean age of participants did not differ from the mean age of nonparticipants, 36.2 *versus* 37.1 years (t-test: t=−0.413, P=0.68).

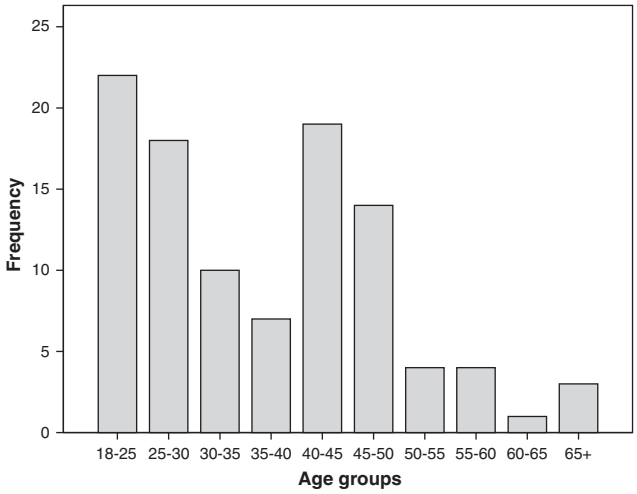

Figure 2 Frequencies by age group.

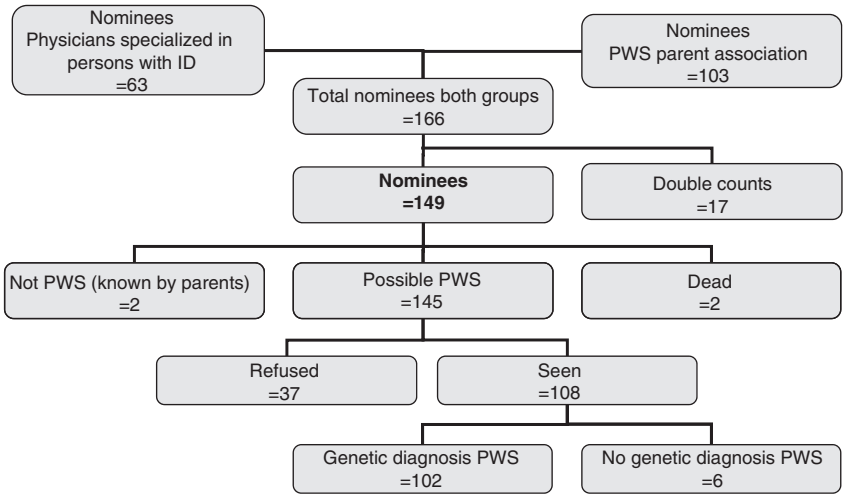

Figure 1 Background of the study population.

Relationship between distribution of genetic subtypes and age. Genetic testing showed 55 persons (54%) with a paternal deletion, 44 persons (43%) with a mUPD and 3 persons (3%) with a defect of the IC (Table 1). The observed distribution in our study differed from that in literature (70% deletion, 30% mUPD), which was statistically significant (*z*-score: $P<0.05$).

Table 1 also shows the distribution of persons per genetic subtype per age group. When comparing the deletion group and the mUPD group, the percentage of persons with the deletion subtype in the youngest age group (<25) was significantly higher than that in the older age groups. ($\chi^2=11.645$, df=3, $P=0.001$). In addition, the distribution ratio of deletion *versus* mUPD was 86:9 in the youngest *versus* 39:54 in the oldest age group.

The relationship between distribution of genetic subtypes and maternal age at birth. An analysis of the distribution of genetic subtypes in relation to maternal age at birth of persons with PWS showed that in all age groups, the mean age of mothers at birth of mUPD persons was significantly higher than the mean age of mothers at birth of deletion persons (Figure 3). The mean maternal age at birth of persons with a deletion was 28.9 years, compared with 35.2 years in persons with an mUPD ($t=-5.451$, $P=<0.01$).

To examine this effect of maternal age on genetic subtypes, we also analysed the effect in the different age groups of persons with PWS. The mean age of mothers at birth of deletion persons, as well as the mean age of mothers at birth of mUPD persons, did not differ significantly across age groups.

Table 1 Distribution of genetic subtypes by age groups

		Genetic subtype			
		Deletion	*mUPD*	*Other*	*Total*
Age groups (years)					
<25	N (%)	19 (86)	2 (9)	1 (5)	22 (100)
25–34	N (%)	15 (54)	13 (46)	0 (0)	28 (100)
35–44	N (%)	11 (42)	15 (58)	0 (0)	26 (100)
45+	N (%)	10 (39)	14 (54)	2 (7)	26 (100)
Total	N (%)	55 (54.0)	44 (43.1)	3 (2.9)	102 (100)

Abbreviation: mUPD, maternal uniparental dicomy.
Comparing deletion and mUPD: $\chi^2=11.645$, df=3, $P=0.001$.

Relationship between distribution of genetic subtypes and BMI. An analysis of the distribution of genetic subtypes in relation to BMI (Figure 4) showed no difference between the mean BMI (kg/m^2) of persons with a deletion and that of persons with mUPD (32.3 *versus* 32.8, $t=-0.265$, $P=0.791$). A significant difference in mean BMI in the different age groups was observed for persons with a deletion, which were, respectively, 28.8, 31.5, 36.6 and 35.7 (ANOVA: $P=0.024$). Observed differences were mainly due to the difference between age group <25 years and age group 35–44 years ($P=0.045$)

Moreover, a significant difference in mean BMI in the different age groups was observed for persons with an mUPD, and were, respectively, 27.2, 37.8, 31.3 and 30.3 (ANOVA: $P=0.037$). Observed differences were mainly due to the differences between age group 25–34 years and age group >45 years ($P=0.078$).

Because the current BMI might not reflect weight status across the lifespan, we also examined the reported maximum BMI levels of these persons. The mean maximum BMI in deletion persons was 36.1, compared with 36.9 in mUPD persons ($t=-0.391$, $P=0.7$). In deletion persons, differences in maximum BMI between age groups were statistically significant (ANOVA: $P<0.01$), mainly because of differences between age group <25 years and age group 35–44 years, and between age group <25 years and age group >45 years (both $P<0.01$). In persons with mUPD, BMI maximum differences were not statistically significant between age groups (ANOVA: $P=0.145$).

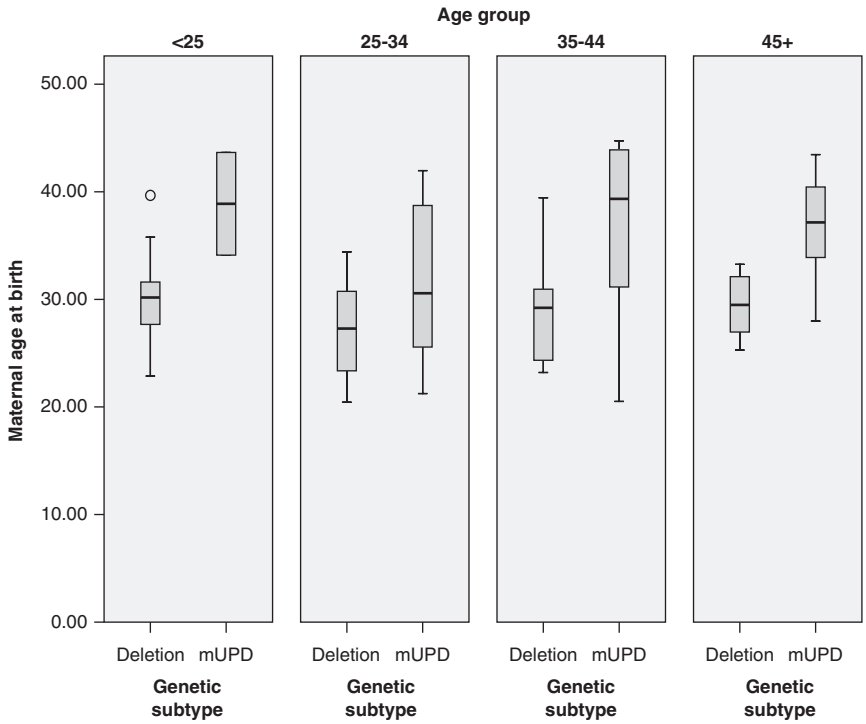

Figure 3 Maternal age at birth by genetic subtype and age group.

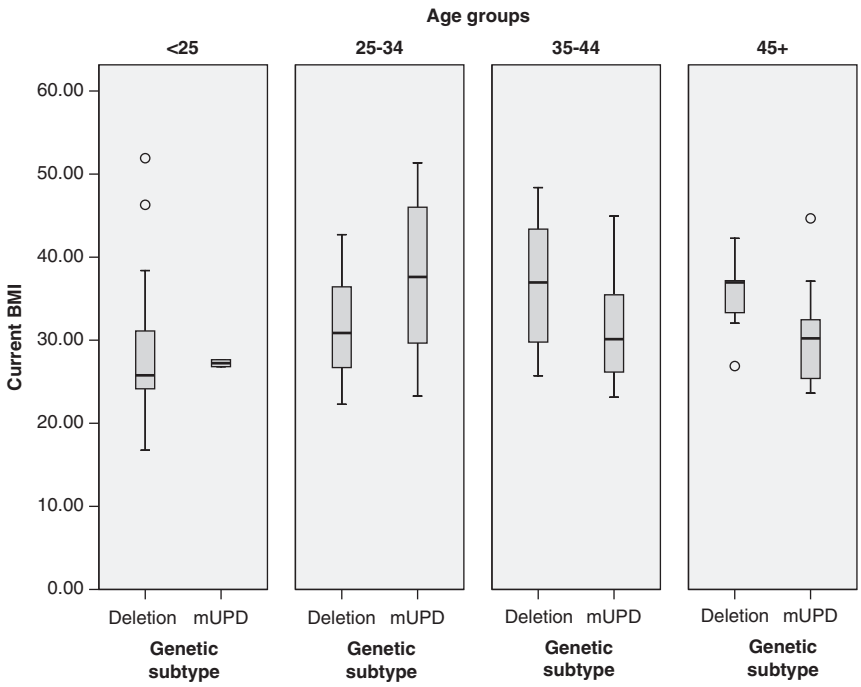

Figure 4 Current BMI by genetic subtype and age group.

Table 2 Distribution of PWS genetic subtypes in recent population studies

Location	*Sample total*	*Sample ascertained*	*Deletion (%)*	*mUPD (%)*	*Other (%)*	*Age range*	*Above 40 years of age*
United Kingdom	96	58	74	22	3	0–47	7
Flanders	78	73	69	19	5	0–56	23
Australia	30	24	70	10	0	0–15	0

DISCUSSION

In our study population of 102 adults with PWS in The Netherlands, a different distribution of genetic subtypes across the different age groups was found when compared with the results of previous population-based studies (Table 2).[4–6] In this study, a relatively high proportion of mUPD in the older age groups was found. Possible explanations for the differences will be discussed.

Bias of study population

The differences in the distribution of genetic subtypes across age groups could be attributed to a selection bias. Bias of the study group may include one of the following: sampling error in our study or selective diagnosing in our study and/or in previous literature.

Sampling error. Participants of our study were recruited through the Dutch Prader–Willi Parent Association and through physicians specialized in treating persons with ID. The response rate of the study was 75%. We have no reason to assume a sampling error in our study, either by nonresponse or by the selection of cases. However, we did not analyse the genetic PWS subtypes in the nonresponse group nor did we perform a population study in which all possible cases of PWS were included.

Selective diagnosing. Selective diagnosing (that is, undiagnosed deletion cases in our study or undiagnosed mUPD cases in other studies) is unlikely, as most studies used broad inclusion criteria. Major clinical criteria (such as obesity, hyperphagia, ID, hypogonadism) are common to both deletion and mUPD subtypes and are not likely to be missed. However, in the elderly, information on hypotonia and feeding problems at a young age is not always known by professional caregivers or siblings. In our study, we found a wide age range; in particular, there were relatively many elderly (40+ years) with PWS compared with other studies (Table 1). More than half of these elderly (56%) did not have a confirmed genetic diagnosis in the past. In other studies, these elderly might have been undiagnosed and/or underreported. This may especially be the case in studies that collaborated only with a PWS parent association as the source for their participants. This kind of study should be conducted in other populations over different countries in order to increase the number of patients and to reduce a probable way of recruitment.

Additional factors reported in literature

Additional factors that might influence the distribution of genetic subtypes comprise maternal age, environmental variables causing genetic errors and the use of reproductive technologies.[10,12,14,15] Although the age of mUPD mothers at birth of the PWS baby is significantly higher than the age of deletion mothers in this study, this does not explain the differences in distribution across the age groups. Reproductive technologies were not used in our study population and so cannot have influenced these results. Furthermore, we have no

indications of environmental factors affecting the relatively high proportion of mUPD in our study.

Selective mortality

A possible explanation for the observed differences in the distribution of genetic subtypes across age groups may be differences in survival of the genetic subtypes. As causes of death in adults with PWS are usually stated as 'obesity related',[5,16,17] we compared the BMI rates of individuals by subtypes across the different age groups. We found no statistically significant differences indicating a difference in survival between the genetic subtypes. In contrast with our results, a possible relationship between higher death rates in the young adult age range and the mUPD subtype has been stated by Smith *et al.*[18] However, in other studies, no significant differences in mortality rates in relation to the genetic subtype were found. Most studies are limited by sample size or report on highly selective cases,[16,17] and thus these data in the literature may be insufficient to compare differences in survival. Further studies on survival in relation to genetic subtypes of PWS are highly recommended.

Psychiatry

The most striking difference between the deletion and mUPD subtypes in adulthood is the occurrence of psychotic episodes, which are more associated with the mUPD subtype.[19–21] In the general population, psychotic illnesses result in a substantially increased risk of death at a relatively young age. Overall, people with schizophrenia have a two- to threefold increased risk of premature dying.[22] Suicide contributes to this increased premature mortality. Moreover, people with schizophrenia are thought to be less inclined to seek health care, to consume less medical care, to engage in high-risk behaviours and to be less compliant with their treatments. Combined, these lifestyle factors elevate the risk of a wide range of somatic conditions and consequently increase the risk of early death.[22] However, in our adult PWS population, the proportion of mUPD in the elderly is higher than in younger age groups. Although psychotic illness attributes to an elevated risk of premature death in the general population, this may not hold true for the PWS population. Suicide is probably rare in people with PWS. Practically all adults with PWS live under close supervision of their parents or professional caregivers. Lifestyle factors such as smoking, alcohol consumption, access to food and compliance to therapy are usually carefully monitored by parents and professional caregivers and might therefore not influence the life expectancy, as observed in psychiatric persons in the general population.

Differences in gene expression

Phenotypical differences that exist between genetic subtypes might arise from a variety of proposed genetic mechanisms. The PWS phenotype results from the absence of expression of a set of genes in the 15q11–13 region, which are paternally expressed and maternally imprinted. Although no single gene alteration has been found that accounts for all key PWS characteristics,[3,23] several unique translocation and deletion persons have narrowed a 'key' region to explain much of the PWS phenotype to the HBII-85 snoRNA gene.[24] Persons with PWS either have no inherited copy of the paternally expressed genes (deletion) or two maternal gene copies because of a maternal disomy (mUPD). In addition, imprinting of the respective genes may be aberrant as a result of an imprinting defect in the 15q region.

The phenotypic differences between persons with either the deletion or mUPD subtype may be related to the underlying differences in gene expression of the genetic subtypes. In the deletion subtype, haploinsufficiency of nonimprinted genes in the PWS region may contribute to phenotypic differences between the deletion and mUPD subtype.[25] Conversely, maternally expressed genes, such as UBE3A, show an elevated expression in the mUPD subtype and have been suggested to contribute to specific phenotypic characteristics.[26,27]

Furthermore, any imprinted genes distal to 15q11–13 would be affected in persons with UPD but not in persons with a deletion.[25] In addition, differences in expression have been reported because of parental biased expression of nonimprinted genes outside the deletion region.[27] Consequently, persons with mUPD have a lower level of expression from chromosome 15, compared with individuals without PWS and those with a deletion subtype. A more severe phenotype may be associated with a reduced life expectancy. A milder clinical phenotype in persons with Angelman syndrome due to paternal UPD compared with persons with a deletion has been reported.[28]

CONCLUSION

We did not find a proper explanation for differences in the distribution of genetic subtypes across age groups. Further research on morbidity and causes of death in relation to the genetic subtypes is required.

The increasing number of elderly people with PWS and the different distribution of genetic subtypes across age groups raise some important issues. Our findings underline the need for correct genetic diagnosis in persons with PWS. Over the past 10 years, the age of diagnosis has fallen significantly and the majority of cases are now diagnosed during the first months of life. The results of this study show a substantial number (n=45) of persons above the age of 40 years and even up to the age of 66 years. We noticed that 25 of these elderly persons had not had a confirmed genetic diagnosis before this study. The clinical diagnosis of PWS is based on core features such as hypotonia, failure to thrive and undescended testes in boys. However, this clinical information is not always available later in life. Genetic testing for PWS in adults should also be considered in case of a less-marked phenotype, characterized by behavioural and psychological problems, in addition to obesity and delayed or incomplete puberty.[29]

The results also underline the need for adjustment of the guidelines for preventive management in adulthood. For example, special attention should be given to psychiatric problems, because of the high proportion of mUPD persons among elderly people with PWS. A correct genetic diagnosis, followed by efficient use of preventive management programmes, should allow the ageing PWS population to enjoy a longer and healthier life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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