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ORIGINAL RESEARCH

Deletions and duplications of the 15q11–q13 region in spermatozoa from Prader–Willi syndrome fathers

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ABSTRACT: Prader–Willi syndrome (PWS) is a genomic disorder mostly caused by deletions of 15q11–q13 region (70%). It has been suggested that the particular genomic architecture of 15q11–q13 region, characterized to be flanked by low copy repeats, could predispose it to Non-Allelic Homologous Recombination (NAHR). However, no studies in gametes of fathers of PWS individuals have been published to date. The objective of the study was to assess the incidence of 15q11–q13 deletions and duplications in spermatozoa from PWS fathers and to appraise the value of the data obtained for the estimation of the risk of recurrence for the syndrome. Semen samples from 16 fathers of PWS individuals and 10 control donors, were processed by triple-color fluorescence *in situ* hybridization. A customized combination of probes was used to discriminate between normal, deleted and duplicated sperm genotypes. A minimum of 10 000 sperm were scored for every single sample. A significant increase in the frequency of 15q11–q13 deletions and duplications were observed in PWS fathers (0.90 ± 0.14%) compared with control donors (0.47 ± 0.07%). Ten out of 16 individuals contributed to this population increase ($P < 0.01$), suggesting a predisposition for 15q11–q13 reorganizations. Statistical differences were observed in the frequency of 15q11–q13 deletions and duplications in fathers of PWS individuals (0.59 versus 0.31%; $P = 0.001$), indicating that intra-chromatid NAHR exchanges also substantially contribute to the rearrangements. Results demonstrated the increased susceptibility of some fathers of PWS individuals to generate 15q11–q13 deletions, suggesting that the screening of anomalies in sperm should be advisable as a valuable complement for genetic counseling.

Key words: deletions and duplications / microdeletion syndromes / non-allelic homologous recombination / spermatozoa

Introduction

The concept of genomic disorder was proposed to describe human diseases caused by DNA reorganizations giving rise to a gain, loss or alteration of dosage-sensitive genes (Lupski, 1998). The number of genetic diseases identified as genomic disorders is increasing continuously (Sharp *et al.*, 2006) and those caused by chromosome deletions are part of these alterations (Ji *et al.*, 2000a). It has been observed that the DNA regions involved in genomic disorders caused by deletions are flanked by low copy repeats (LCR). Several authors have pointed out that the presence of LCR makes them prone to reorganizations (Inoue and Lupski, 2002; Gu *et al.*, 2008).

Prader–Willi syndrome (PWS) is a genomic disorder with an incidence of 1/15 000 newborns. The genetic cause is the lack of expression of paternally inherited 15q11–q13 genes which are under imprinting control. PWS can be caused by different genetic mechanisms: 15q11–q13 deletions (70%), maternal uniparental disomy (UPD; 25%), disruption of the imprinting center (4%) and other chromosome reorganizations that affect the integrity or function of genes within the 15q11–q13 region (1%) (Cassidy *et al.*, 2000).

The 15q11–q13 region is flanked by three LCR (LCR15-1, LCR15-2, LCR15-3) in which most of the deletion breakpoints (BP) in PWS cases take place (Amos-Landgraf *et al.*, 1999). Two types of deletions have been described according to the proximal BP (Fig. 1). Class I deletions have the proximal breakpoint within LCR15-1 (BP1), although Class II deletions have their breakpoint in LCR15-2 (BP2). Both Class I and Class II deletions have the distal breakpoint predominantly located within the LCR15-3 (BP3; 97%). Class II deletions are the most frequent ones (60%) and represent an ~6.5 Mb loss (Butler *et al.*, 2008). The 15q11–q13 LCRs have an approximate size of 400 kb and are mainly duplications of the gene/pseudogene *HERC2* forming blocks called END-repeats. Recently, the organization of these END-repeats and their orientation in each LCR15 have been established (Fig. 1; Makoff and Flomen, 2007). These blocks have more than 98% homology and act as hotspots for recombination (Emanuel and Shaikh, 2001). These features favor Non-Allelic Homologous Recombination (NAHR) between different copies of END-repeats, triggering reorganizations of the critical region (Ji *et al.*, 2000b; Inoue and Lupski, 2002; Stankiewicz and Lupski, 2002).

The orientation in which END-repeats are arranged determines the pairing and the consequent products of NAHR. When the END-repeats are oriented directly, it will give rise to complementary deletions and duplications if the NAHR took place between END-repeats of two chromatids (Fig. 2A.1), or only deletions if a pairing between END-repeats of the same chromatid take place (Fig. 2A.2). The indirect orientation of END-repeats favors the formation of inversions as a result of NAHR, caused by a pairing of END-repeats in the same chromatid (Fig. 2B).

Several studies have suggested that the particular genomic architecture of 15q11–q13 region predisposes it to NAHR phenomena, triggering different chromosome reorganizations: deletions (Amos-Landgraf *et al.*, 1999), duplications (Kotzot *et al.*, 2000), inversions (Gimelli *et al.*, 2003) and other reorganizations that emphasize the instability in this region (Ungaro *et al.*, 2001; Dennis *et al.*, 2006). Some authors suggest that structural variation within LCR, such as an increase in the number of repetitions in LCR segments, could be the cause of the predisposition to produce deletions in the germ line of some individuals (Amos-Landgraf *et al.*, 1999; Sharp *et al.*, 2007; Cusco *et al.*, 2008).

Nevertheless, it is considered that the deletions causing PWS, as well as other genomic disorders, have the same recurrence risk as

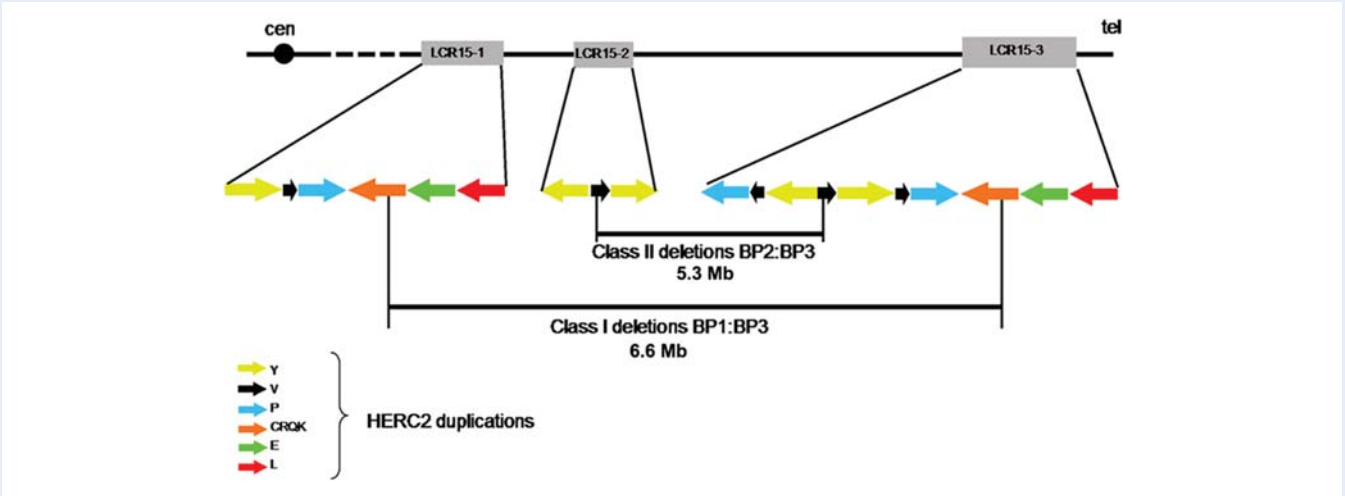

Figure 1 Schematic representation of 15q11–q13 region (Adapted from Makoff and Flomen, 2007). Color arrows represent HERC2 duplications and their orientation within the LCR15.

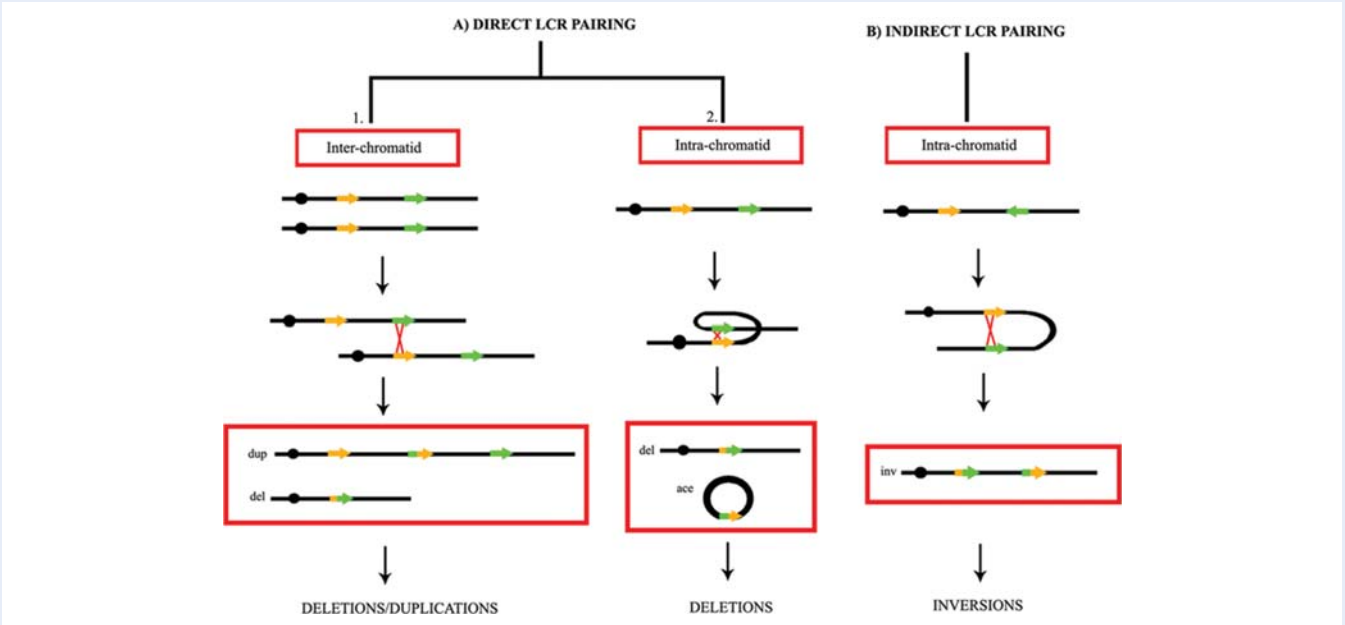

Figure 2 Schematic representation of different classes of NAHR according to the LCR orientation and the chromatids involved. Stable products of each reorganization are shown at the bottom of the figure.

that found in the general population, established from epidemiological studies, to be less than 0.5% (Gardner and Sutherland, 2004). However, population approaches for assessing the recurrence of these types of diseases show some limitations that hamper a reliable estimation of the risk (Rothlisberger and Kotzot, 2007): (i) Most rearrangements are very rare or even unique, (ii) Not all recurrence cases have to be reported and (iii) Families with one affected child might resign from having further children.

Studies in gametes, and especially in spermatozoa, have an advantage in obtaining large numbers of samples, and provide an alternative to studying the frequency of these reorganizations. The use of methodologies that allow fluorescence *in situ* hybridization (FISH) analyses in decondensed sperm nuclei enables an approach with high reliability and wide spectrum (Downie *et al.*, 1997; Egozcue *et al.*, 1997) and offers the possibility of direct assessment of gametes carrying deletions and duplications. Furthermore, an interphase analysis technique offers the possibility to analyze, cell by cell, a great number of sperm in the same individual (a great number of NAHR phenomena) and, as a result, assess phenomena that take place in a very low frequency with a high reliability.

The aim of this study was to assess the incidence of 15q11–q13 deletions and duplications in spermatozoa from fathers of PWS individuals. The analysis of the results has allowed the estimation of the recurrence risk for the syndrome and the assessment of the participation of intra or inter-chromatid NAHR in the production of anomalies.

Materials and Methods

Biological samples

Semen samples were obtained from 16 PWS fathers, aged 32–60 years old and 10 control donors of 24–50 years of age. Control donors were volunteers recruited from the general population. All subjects had normal karyotypes and were normozoospermic. To our knowledge, none of them were exposed to genotoxic agents and no history of chemotherapy, radiotherapy or chronic illness was recorded.

Patients gave their informed consent in writing to participate in the study and the protocol was approved by our Institutional Ethics Committee.

Fluorescence *in situ* hybridization

Samples were processed as described previously by our group; details of sperm fixation, nuclear decondensation and FISH protocol have been described elsewhere (Vidal *et al.*, 1993).

A triple-color FISH approach was used to determine the frequency of deletions and duplications of the 15q11–q13 region. We combined a *locus*-specific probe for the 15q11–q13 region (LSI D15S11, *Spectrum Orange*; Abott Molecular; Abott Park, IL, USA), a centromeric probe for chromosome 15 (CEP 15p11.2, D15Z1, *Spectrum Green*; Abott Molecular) and a centromeric probe for chromosome 6 (CEP 6, D6Z1, *Spectrum Aqua*; Abott Molecular). CEP15 and CEP6 probes were used as hybridization and ploidy control, respectively.

Analyses were carried out using an Olympus BX60 epifluorescence microscope equipped with a triple-band pass filter and specific filters for Aqua, FITC and Cy3.

A minimum of 10 000 sperm was scored for every single father and control. Samples were analyzed independently by two experienced observers, applying the following assessment criteria:

(i) Only spermatozoa with a well-defined boundary were evaluated. Overlapping spermatozoa were discarded from the count.
(ii) According to the number and distribution of the 15q11–q13 signals, the following genotypes were assigned (Fig. 3):
- Normal: spermatozoa displaying the 15q11–q13 signal and both centromeric signals for chromosomes 15 and 6 (Fig. 3a).
- Deletion of 15q11–q13 region: spermatozoa lacking the 15q11–q13 signal but displaying the centromeric signals for chromosomes 15 and 6 (Fig. 3b).
- Duplication of 15q11–q13 region: spermatozoa showing two 15q11–q13 signals, having the same size and intensity and being separated from each other by a distance longer than the diameter of each signal, plus a centromeric signal for chromosomes 15 and 6 (Fig. 3c).

(iii) In cases of disomy or diploidy, signals must be of the same size and intensity, and the distance between them must be at least the same as the diameter of the signal (Blanco *et al.*, 1996).

Sperm-FISH analyses were performed in a blind manner regarding the genetic origin of the syndrome in the children. These data were provided, once the study in gametes ended, by the Centre de Diagnòstic UDIAT from the Consorci Hospitalari Parc Taulí (Sabadell, Spain).

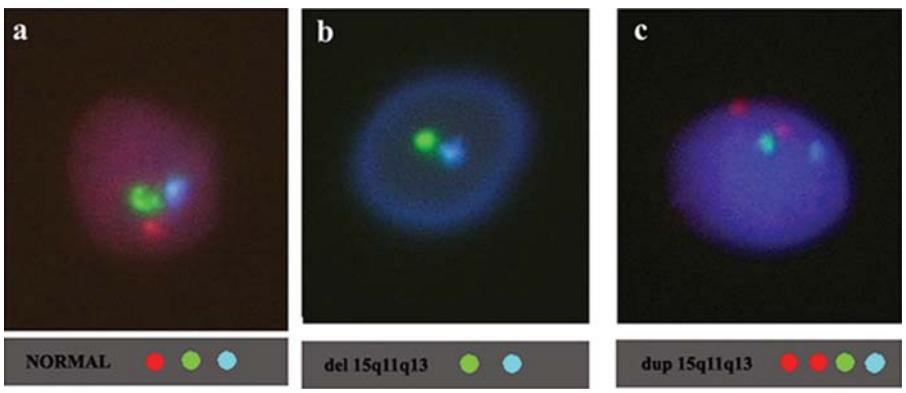

Figure 3 Spermatozoa classification regarding the signal combination observed.

Data analysis

Data were analyzed statistically using SPSS 14.0 (SPSS Inc.; Chicago, IL, USA) under the advice of the statistical service of the *Universitat Autònoma de Barcelona*.

To assess the participation of the NHAR interchromatid (deletions = duplications) and/or intrachromatid (deletions), in the generation of anomalies three comparisons were performed:

(i) A Pearson's correlation test of the frequency of 15q11–q13 deletions and duplications in control and PWS father series. The correlation was considered statistically significant when $P < 0.05$.
(ii) A Wilcoxon test to analyze whether the mean population frequency of 15q11–q13 deletions were different from that of duplications, both in control donors and PWS fathers. Differences were considered to be statistically significant when $P < 0.05$.
(iii) A χ^2 test to compare, at the individual level, the frequency of 15q11–q13 deletions and duplications. To avoid false positives due to the high number of spermatozoa analyzed per patient, differences were considered to be statistically significant when $P < 0.01$.

To assess the susceptibility of the 15q11–q13 region to generate deletions and duplications, the following statistical analyses were performed:

(i) The mean population frequency of deletions and the sum of deletions and duplications were compared between controls and PWS fathers by means of a Mann–Whitney test. Differences were considered to be statistically significant when $P < 0.05$.
(ii) A Pearson's correlation test of the frequency of deletions and duplications and paternal age was performed. Correlation was considered statistically significant when $P < 0.05$.

Results

A total of 101 505 sperm nuclei from the control donors were analyzed (Table I). The mean frequency of 15q11–q13 deletions was 0.22%, ranging from 0.08 to 0.38%, and with a standard deviation of 0.03%. In fathers of PWS individuals, a total of 163 542 sperm nuclei were analyzed (Table II). The mean frequency of 15q11–q13 deletions was 0.59% (±0.12 SD), ranging from 0.18 to 2.34%. The mean frequency of 15q11–q13 duplications in control donors was 0.24% (±0.04 SD) ranging from 0.12 to 0.53%. In PWS fathers, the mean frequency of 15q11–q13 duplications was 0.31% (±0.03 SD), ranging from 0.12 to 0.56%. A significant correlation was found between the frequency of 15q11–q13 deletions and the frequency of duplications in the control population ($P = 0.028$). Moreover, the frequency of 15q11–q13 deletions and duplications did not show statistical differences in the control population ($P = 0.609$). The correlation between the frequency of 15q11–q13 deletions and duplications was not significant in the fathers of PWS individuals, the frequency of deletions being significantly higher than the frequency of duplications ($P = 0.001$; Fig. 4a).

There was no correlation between the percentage of deletions and/or duplication and age, either in controls ($P = 0.381$) or in PWS fathers ($P = 0.329$).

At the individual level, significant differences between the frequency of deletions and duplications were observed in 7 out of 16 fathers analyzed ($P < 0.01$; Table II; Fig. 4b). In all of them, the frequency of deletions was significantly higher. Mean values for the frequency of 15q11–q13 deletions between controls (0.22 ± 0.03%) and individuals with PWS affected offspring (0.59 ± 0.12%) showed significant differences ($P = 0.0001$). However, the frequency of 15q11–q13 duplications was equivalent ($P = 0.097$).

When considering deletions and duplications as a whole (del + dup), a significant increase in the frequency of 15q11–q13 del + dup was also observed in fathers of PWS individuals (0.90 ± 0.14%), as compared with control donors (0.47 ± 0.07%; $P = 0.002$).

Comparing each father with the control values, moderate significant increases were found for the frequency of 15q11–q13 del + dup in sperm of 10 out of 16 cases analyzed ($P < 0.01$; Table II).

Discussion

Methodological considerations

FISH on decondensed sperm nuclei has been widely used as a tool for cytogenetic studies in spermatozoa (Martin, 2005), mainly involving meiotic segregation analyses in carriers of chromosome

Table I Sperm-FISH results in control donors.

Cases	Age	Normal	del 15q11–q13	dup 15q11–q13	del + dup[a]	Other[b]	Total
C-1	26	10 120 (99.07%)	31 (0.31%)	23 (0.23%)	54 (0.54%)	41 (0.40%)	10 215
C-2	24	10 105 (98.95%)	31 (0.30%)	30 (0.29%)	61 (0.59%)	47 (0.46%)	10 212
C-3	25	10 084 (99.22%)	8 (0.08%)	22 (0.22%)	30 (0.30%)	49 (0.48%)	10 163
C-4	23	10 155 (98.93%)	23 (0.22%)	38 (0.37%)	61 (0.59%)	49 (0.48%)	10 265
C-5	36	10 028 (99.31%)	25 (0.25%)	18 (0.18%)	43 (0.43%)	27 (0.27%)	10 098
C-6	28	10 016 (99.33%)	11 (0.11%)	12 (0.12%)	23 (0.23%)	45 (0.45%)	10 084
C-7	50	10 062 (99.25%)	24 (0.23%)	20 (0.19%)	44 (0.42%)	32 (0.32%)	10 138
C-8	50	10 038 (99.46%)	12 (0.12%)	16 (0.16%)	28 (0.28%)	27 (0.27%)	10 093
C-9	42	10 030 (99.36%)	17 (0.17%)	13 (0.13%)	30 (0.30%)	35 (0.35%)	10 095
C-10	26	10 022 (98.82%)	39 (0.38%)	54 (0.53%)	93 (0.91%)	93 (0.91%)	10 142
% ± SEM		99.17% ± 0.07	0.22% ± 0.03	0.24% ± 0.04	0.47% ± 0.07	0.38% ± 0.07	

[a]15q11–q13 reorganizations (deletions + duplications).
[b]Disomies, diploidies and nullisomies.

Table II Sperm-FISH results in PWS fathers.

Cases	Age	Normal	del 15q11–q13	dup 15q11–q13	del + dup[a]	Other[b]	Total	Etiology
PW-1	41	10 027 (98.17%)	**54 (0.53%)**	**32 (0.32%)**	86 (0.85%)*	51 (0.50%)	10 214	UPD
PW-2	35	9873 (98.42%)	**79 (0.79%)**	**45 (0.45%)**	124 (1.24%)*	35 (0.35%)	10 032	Deletion
PW-3	44	10 554 (98.96%)	32 (0.30%)	27 (0.25%)	59 (0.55%)	52 (0.49%)	10 665	Deletion
PW-4	35	10 037 (99.47%)	18 (0.18%)	15 (0.15%)	33 (0.33%)	22 (0.22%)	10 090	Deletion
PW-5	30	9948 (98.57%)	**80 (0.80%)**	**23 (0.23%)**	103 (1.03%)*	32 (0.32%)	10 092	Deletion
PW-6	33	10 120 (98.77%)	42 (0.41%)	36 (0.35%)	78 (0.76%)*	48 (0.47%)	10 246	Deletion
PW-7	47	10 061 (99.08%)	43 (0.42%)	28 (0.28%)	71 (0.70%)*	22 (0.22%)	10 154	Unknown
PW-8	50	10 164 (99.07%)	32 (0.31%)	27 (0.26%)	59 (0.57%)	36 (0.35%)	10 259	Deletion
PW-9	60	10 049 (99.16%)	28 (0.28%)	32 (0.32%)	60 (0.60%)	25 (0.25%)	10 134	Deletion
PW-10	60	9867 (96.56%)	**239 (2.34%)**	**43 (0.42%)**	282 (2.76%)*	70 (0.68%)	10 219	UPD
PW-11	42	10 549 (99.04%)	37 (0.35%)	30 (0.28%)	67 (0.63%)	35 (0.33%)	10 651	UPD
PW-13	53	9986 (98.32%)	**81 (0.80%)**	**26 (0.26%)**	107 (1.06%)*	64 (0.63%)	10 157	Deletion
PW-14	55	10 031 (99.22%)	**47 (0.46%)**	**12 (0.12%)**	59 (0.58%)	41 (0.40%)	10 130	UPD
PW-15	47	9918 (98.28%)	**51 (0.51%)**	**26 (0.26%)**	77 (0.77%)*	97 (0.96%)	10 092	Deletion
PW-16	44	10 177 (98.85%)	46 (0.45%)	39 (0.39%)	85 (0.84%)*	34 (0.33%)	10 295	Deletion
PW-17		10 020 (98.12%)	59 (0.58%)	57 (0.56%)	116 (1.14%)*	76 (0.74%)	10 212	Unknown
% ± SEM		98.63% ± 0.17	0.59% ± 0.12	0.31% ± 0.03	0.90% ± 0.14	0.45% ± 0.05		

Data on the offspring etiology are shown in the last column.
[a]15q11–q13 reorganizations (deletions + duplications).
[b]Disomies, diploidies and nullisomies.
*Significant increases versus control values ($P < 0.01$).
In bold are shown the significant differences between deletions and duplications within the same row ($P < 0.01$).

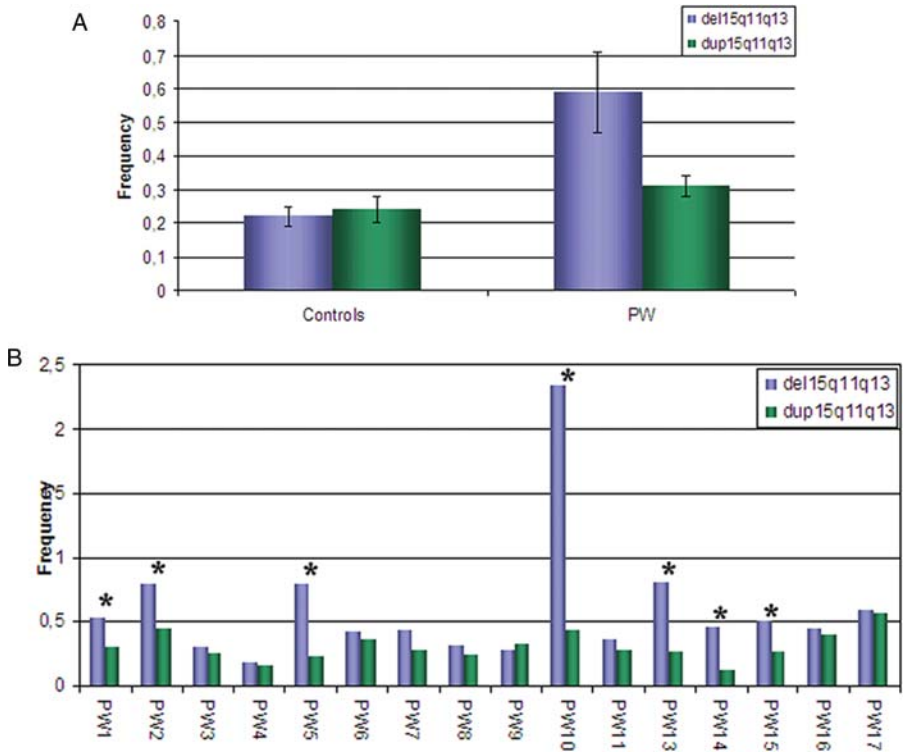

Figure 4 (**A**) Mean frequencies of 15q11–q13 deletions and duplications in Control donors and PWS fathers. Error bars represent the standard error of mean (SEM). (**B**) Frequencies of 15q11–q13 deletions and duplications observed in every single PWS father. Asterisks indicate cases with significant differences between the frequency of deletions and duplications.

reorganizations (Anton *et al*., 2007) as well as aneuploidy rate evaluation in infertile men (Sarrate *et al*., 2009). The results obtained in our work have proven that it is also a valuable and powerful methodological approach to evaluate the frequency of deletions and duplications in sperm cells, provided that an accurate FISH design is used: probes spanning the region to be examined, control probes for the chromosome involved and a well defined and strict scoring criteria.

In our study, the assessment of deletions and duplications was performed by using a combination of two probes: a centromeric probe for chromosome 15 indicating the presence of this chromosome, and a LSI probe for the 15q11–q13 region. The presence of the centromeric signal and the absence of the LSI probe were identified as deletions. The same experimental design was used for the evaluation of the frequency of duplications of the 15q11–q13 region. Taking scoring criteria into account (Blanco *et al*., 1996), two signals should be considered as independent if they shown separated by a distance greater than the diameter of each signal. In this sense, it is important to state that the resolution of the FISH technique on interphase nuclei is 100 kb (Andreeff and Pinkel, 1999). Considering that a duplication of the 15q11–q13 region will separate the signals to a distance of ~4 Mb (Fig. 1), a separation far greater than the resolution limit, we were able to clearly distinguish two signals from the LSI probe and thus, to assess duplications of the region.

Starting from the assessment of the control population, the basal frequency of deletions and duplications of the 15q11–q13 region were established. The results in the control population showed very clearly that there was little variability (Table I). This suggests a high homogeneity and an optimum hybridization efficiency of the LSI probe used in sperm cells. As a result, data obtained in this work indicate that this technique is also useful in determining the recurrence risk of syndromes caused by deletions of paternal origin and in inferring the type of NAHR involved in the appearance of deletions and duplications in spermatozoa.

Mechanism of origin of deletions and duplications of the 15q11–q13 region

The significant correlation between the frequency of deletions and duplications of the 15q11–q13 region in the control population indicates that inter-chromatid NAHR is the main mechanism originating deletions in these individuals (with complementary deletions and duplications). Thus, as expected for this region, a basal level of NAHR in meiosis can be assumed and established at 0.47%.

In PWS fathers, a significant increase was observed in the frequency of deletions with respect to the frequency of duplications, indicating that intra-chromatid NAHR would also participate in the increase of the frequency of deletions in these individuals. If it is taken into account that the frequency of duplications in PWS fathers is not different from that seen in controls, although the frequency of deletions is significantly greater in the former, our results suggest that

intra-chromatid NAHR would be the mechanism mostly involved in the significant increases of reorganizations of the 15q11–q13 region.

It is well-known that during spermiogenesis, programmed double strand breaks (DSBs) are induced to facilitate the chromatin remodelling that takes place in elongating spermatids (Leduc *et al.*, 2008). It was observed that the proteins involved in the Homologous Recombination (HR) repair machinery are present during this stage of spermatogenesis, pointing out the participation of this DNA repair system in spermatids (Srivastava and Raman, 2007). Our results suggest that 15q11–q13 deletions, which are generated in a higher frequency in PWS fathers, could be triggered at this stage by intra-chromatid NAHR, because only one chromatid is available to repair the DSBs. As this phenomenon was not observed in control donors, our results indicate that these individuals could be susceptible to generating these anomalies, probably related to the presence of specific haplotypes that predispose this region to NAHR.

Susceptibility in generating deletions and duplications of the 15q11–q13 region

In population terms, PWS fathers showed a significant increase of 15q11–q13 del + dup. Ten of the 16 individuals analyzed contributed to this increase. It has been suggested that structural variations, such as inversions of critical regions with similar features (Osborne *et al.*, 2001; Gimelli *et al.*, 2003) or variations in the number of repetitions of the LCRs (Amos-Landgraf *et al.*, 1999; Sharp *et al.*, 2007; Cusco *et al.*, 2008) could be predisposing factors for the appearance of deletions in descendants. These numerical and structural changes could make homologue pairing during the recombination process difficult, thus favoring heterologous pairing with nearby chromosomal segments that share a very high degree of homology, such as is the case of the LCRs. In this way, individuals who are carriers of changes of this type could be susceptible to different degrees to the phenomena of NAHR, and the consequent increase of reorganizations in the region involved.

As regards the comparison of the results obtained in spermatozoa with the PWS etiology, no relation between an increase of deletions and duplications in sperm and the etiology was observed. Furthermore, some of the individuals who show increases of 15q11–q13 del + dup in spermatozoa are fathers with descendants affected by PWS caused by maternal UPD (Table II). Among these is the case with the highest frequency of deletions of all of the series analyzed (PW-10: 2.76%). As has been previously described, LCRs are regions which are susceptible to DSB which increase the processes of HR in the repair these breakages (Baumer *et al.*, 1998; Christian *et al.*, 1999; Gu *et al.*, 2008). A predominance has been described for HR in repairing DSB in the first stages of embryonic development (Essers *et al.*, 2000). LCR pairing of homologous chromosomes to repair by means of HR will generate partial UPDs, which in the case of genes controlled by imprinting will give rise to different pathologies (Feuk *et al.*, 2006). In our situation, a repair process for post-zygote HR could generate the partial UPD of the 15q11–q13 region. Cases have been published of PWS caused by partial UPD (Gregory *et al.*, 1991; Nazarenko *et al.*, 2004; Salavoura *et al.*, 2008) and cases of partial UPD implicated in other pathologies (Kotzot, 2008). In our study, the genetic origin of the syndrome was mainly performed using internal markers (data not shown). In one out of the four UPD cases, both internal and external markers were analyzed in order to discriminate between total and partial UPD (in the remaining cases no DNA was available for the analysis). Interestingly, in one case in this study a partial UPD was identified in a father (PW-14) who also displayed an increased incidence of del15q11q13 in spermatozoa (Table II).

Thus, our results indicate that the increased frequency of deletions of the 15q11–q13 region in spermatozoa is in fact an indicator of the instability of this region that predisposes it to different types of reorganizations: deletions, duplications, inversions and probably partial UPDs.

Clinical significance

Despite the increases observed being moderate (range: 0.70–2.76%), these individuals should be considered at risk of transmitting PWS to their descendants. Our results suggest a relationship between the presence of PWS descendants and an increase of deletions and duplications of the 15q11–q13 region in the spermatozoa. In these individuals an increased frequency of NAHR may occur, affecting regions with a similar genomic architecture to the 15q11–q13 region, giving rise to a high number of gametes carriers of reorganizations. Presumably, the vast majority of them would lead to non-viable embryos or fetuses but those related to genomic disorders compatible with life, such as deletions of the 15q11–q13 region, must be also present. In this situation, the recurrence risk could reach clinical relevance. In this sense, it will be interesting to study whether these individuals show an increase of deletions in spermatozoa for other regions of the genome with similar characteristics, such as 7q11.23 region (involved in Williams–Beuren syndrome) or 22q11.2 (involved in DiGeorge/Velocardiofacial syndrome). Similar situations have been observed in other studies where moderate increases of chromosomal anomalies have been found in spermatozoa of fathers with offspring affected by different syndromes: chromosome 21 disomies in spermatozoa from Down syndrome fathers (Blanco *et al.*, 1998) or sex chromosomes disomies in spermatozoa from fathers with descendants affected by Turner syndrome (Martinez-Pasarell *et al.*, 1999) and Klinefelter syndrome (Arnedo *et al.*, 2006).

In conclusion, although additional cases should be analyzed, our results demonstrate the increased susceptibility of some PWS fathers to generate 15q11–q13 deletions, probably related to the particular genomic architecture of the region.

These preliminary results deserve to be taken into consideration and the screening of 15q11–q13 anomalies in spermatozoa should be suggested in fathers of PWS individuals seeking genetic advice, both to generate larger data sets and to gather information that can further help the genetic counseling of these families.

Authors' roles

O.M. was responsible for adquisition of data, data analysis and interpretation, final approval.

J.B. was responsible for conception and design, data analysis and interpretation, final approval.

F.V. was responsible for conception and design, revision of data and interpretation, final approval.

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