*Human Molecular Genetics, 2003, Vol. 12, No. 8* **849–858**
*DOI: 10.1093/hmg/ddg101*

# Genomic inversions of human chromosome 15q11–q13 in mothers of Angelman syndrome patients with class II (BP2/3) deletions

**Giorgio Gimelli[1], Miguel Angel Pujana[2,†], Maria Grazia Patricelli[3], Silvia Russo[4], Daniela Giardino[4], Lidia Larizza[4,5], Joseph Cheung[6], Lluís Armengol[2], Albert Schinzel[7], Xavier Estivill[2,6,8,*] and Orsetta Zuffardi[3,9]**

[1]Laboratorio di Citogenetica, Istituto G. Gaslini, Genova, Italy, [2]Programa Gens i Malaltia, Centre de Regulació Genòmica, Barcelona, Catalonia, Spain, [3]Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy, [4]Laboratorio di Citogenetica and Genetica Molecolare, Istituto Auxologico, Milano, Italy, [5]Dipartimeto di Biologia e Genetica, Università di Milano, Milano, Italy, [6]Program in Genetics and Genomic Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada, [7]Institute of Medical Genetics der Univiersitat Zurich, Zurich, Switzerland, [8]Departament de Ciencies Experimentals i de la Vida, Universitat Pompeu Fabra, Barcelona, Catalonia, Spain and [9]IRCCS Policlinico San Matteo, Pavia, Italy

Received December 9, 2002; Revised January 30, 2003; Accepted February 14, 2003

**Parental submicroscopic genomic inversions have recently been demonstrated to be present in several genomic disorders. These inversions are genomic polymorphisms that facilitate misalignment and abnormal recombination between flanking segmental duplications. Angelman syndrome (AS; MIM 105830) is associated with specific abnormalities of chromosome 15q11–q13, with about 70% of cases being mother-of-origin 4 Mb deletions. We present here evidence that some mothers of AS patients with deletions of the 15q11–q13 region have a heterozygous inversion involving the region that is deleted in the affected offspring. The inversion was detected in the mothers of four of six AS cases with the breakpoint 2–3 (BP2/3) 15q11–q13 deletion, but not in seven mothers of AS due to paternal uniparental disomy (UPD) 15. We have identified variable inversion breakpoints within BP segmental duplications in the inverted AS mothers, as well as in AS deleted patients. Interestingly, the BP2–BP3 region is inverted in the mouse draft genome sequence with respect to the human draft sequence. The BP2–BP3 chromosome 15q11–q13 inversion was detected in four of 44 subjects (9%) of the general population ($P < 0.004$). The BP2/3 inversion should be an intermediate estate that facilitates the occurrence of 15q11–q13 BP2/3 deletions in the offspring.**

## INTRODUCTION

Segmental duplications have focused intense research attention about the mechanisms of mutation of the human genome and the role of duplications in primate evolution (1). More than 30 human diseases, coined as genomic disorders (2), are the result of genomic rearrangements within segmental duplications (2,3). Several studies have demonstrated that closely located segmental duplications are one of the factors predisposing to the occurrence of genomic disorders. We hypothesize that, at least for some types of rearrangements, the second factor predisposing to rearrangements is the presence of heterozygous inversions at the region delimited by the segmental duplications. These inversions, which are submicroscopic due to the closeness of the segmental duplications, would interfere with the normal homologous synapses and would make misalignment and abnormal recombination more likely, just as it does for cytogenetically identifiable inversions. We have recently described that some *de novo* recurrent chromosome rearrangements, involving chromosome 8 or both chromosomes 4 and 8, are the recombinant products of submicroscopic heterozygous inversions present in the parent transmitting the disease-related

*To whom correspondence should be addressed at: Program in Genes and Disease, Center for Genomic Regulation (CRG), Passeig Marítim 37-49, 08003 Barcelona, Catalonia, Spain. Tel: +34 932240009; Fax: +34 932240089; Email: xavier.estivill@crg.es
†Present address: Cancer Biology Department, Dana-Farber Cancer Institute, Boston, MA 02115, USA.

 *© Oxford University Press 2003; all rights reserved*

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

**Table 1.** Results of FISH experiments in metaphase and interphase cells from mothers of Angelman syndrome patients

| Cells | Orientation of probes | Mothers of BP2/BP3 AS deletions (class II) | | | | | | Mothers of BP1/BP3 AS deletions (class I) | | Mothers of UPD AS patients |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9–15 |
| Metaphase | Normal | 0 | 0 | 0 | 0 | 13 | 9 | 9 | 10 | 93 |
| | Inverted | 8 | 10 | 10 | 7 | 0 | 0 | 0 | 0 | 0 |
| | Non-informative | 27 | 34 | 28 | 38 | 19 | 41 | 23 | 35 | 259 |
| Interphase | Normal | 3 | 2 | 0 | 2 | 38 | 38 | 41 | 33 | 215 |
| | Inverted | 34 | 45 | 38 | 40 | 3 | 0 | 1 | 2 | 35 |
| | Non-informative | 51 | 39 | 48 | 56 | 49 | 42 | 40 | 58 | 320 |

Columns 1–15 refer to the mothers of the different types of Angelman syndrome subjects. Normal, normal orientation of probe signals; inverted, inverted orientation of probe signals in one chromosome (metaphase) or in one set of signals (interphase); non-informative, non-informative orientation of probe signals; PB, break point; UPD, uniparental disomy.

chromosome (4,5). The inversions have the same breakpoints of the recurrent rearrangement and are flanked by paralogous segmental duplications. Moreover, they are present in a significant proportion of subjects of the general population, and can thus be considered genomic polymorphisms, which participate in the occurrence of the disease-associated rearrangements. Another cryptic inversion of 1.5 Mb on chromosome 7q11.23 has been found in four of 12 parents of patients with Williams-Beuren syndrome (WBS) (6). These findings suggest that genomic polymorphisms may be a common feature of some unstable regions associated with genomic disorders. To test this hypothesis we have studied the chromosome 15q11–q13 region in mothers of Angelman syndrome 15q11–q13 deleted patients.

Human chromosome 15q11–q13 appears rearranged in several disorders. Prader–Willi syndrome (PWS; MIM 176270) and Angelman syndrome (AS; MIM 105830) are neurobehavioral disorders that occur at a frequency of 1/10 000 to 1/20 000 live births (7). About 70% of PWS and AS patients have chromosome 15q11–q13 deletions of paternal and maternal origin, respectively (8). Most PWS/AS deletions are clustered on two proximal breakpoints (BP1 and BP2, class I and class II patients, respectively) and a common distal breakpoint (BP3) (9). Large (~400 kb) segmental duplications of high sequence identity (>90%) are located at these breakpoints (10,11). Evolutionary studies have revealed that these segmental duplications emerged ~20 million years ago (10), therefore from the emergence of the *Hominidae* family (12). Additional and partially common segmental duplications are present in multiple copies on 15q11–q14 (13). Despite the progress in the characterization of these segmental duplications, the molecular mechanism and possible susceptibility factors that could underlie the PWS/AS deletion remain unknown. For example, BP1/BP2 and BP3 15q11–q13 flanking segmental duplications seem to be located in inverted orientation, which would lead through intrachromosomal crossing-overs to paracentric inversions but not deletions. With this inverted orientation, other mechanisms such as intrachromosomal stem-loop intermediates have been proposed (10). Here, we demonstrate that a significant proportion of mothers of AS patients with the BP2/3 deletion carry an inversion of this region. The presence of the inversion should be an intermediate estate that facilitates the occurrence of BP2/3 deletions in the offspring.

## RESULTS

### 15q11–q13 inversion in mothers of AS patients with BP2/3 deletions

By means of different probes located in 15q11–q13 according to their content of markers and genes from the region (14) (Fig. 1A), the 15q11–q13 deletion was defined as class I (BP1/3) in two AS patients and as class II (BP2/3) in six AS patients. Metaphase and interphase FISH experiments showed that four of six mothers (samples 1–4 in Table 1) of AS patients with the BP2/3 deletion had a heterozygous inversion of the 15q11–q13 region, which is deleted in their offspring (Fig. 2). However, none of the mothers of seven cases of AS showing paternal isodisomy (UPD) 15, nor the mothers of two patients with BP1/3 deletions had the 15q11–q13 inversion (samples 7–15 in Table 1). Interestingly, four of 44 unrelated subjects from the general population were heterozygous for the inversion, giving a frequency of 4.5% of inverted chromosomes in the general population. This leads to a significant difference of the frequency of the inversion in AS mothers of BP2/3 deletion patients, as compared with subjects from the general population ($P < 0.004$; OR = 20.00, 95% CI 2.75–145.5).

### Cluster of segmental duplications in the 15q11–q13 region

We have performed a BLASTN (15) analysis of the 15q11–q14 region against itself to identify the length and orientation of segmental duplications. We confirmed the presence of the three main segmental duplications (BP1, BP2 and BP3) and also detected two additional clusters (BP4 and BP5), as previously reported (10,11,13) (Fig. 1B). The latest assembly (build 30) of the draft human genome sequence corresponding to this region gives a size between BP1 and BP3 of ~4.8 Mb, and shows a tandem orientation of BP2 with respect to BP3. This orientation would be incorrect based on the detailed clone analysis of Christian *et al.* (10) and Amos-Landgraf *et al.* (11), although segmental duplication polymorphisms could also exist changing the orientations of these segments. The region that contains the BP3 segmental duplication is flanked by gaps in the build 30 assembly and it is considerably shorter compared with the physical map that has been constructed using YAC clones (14).

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

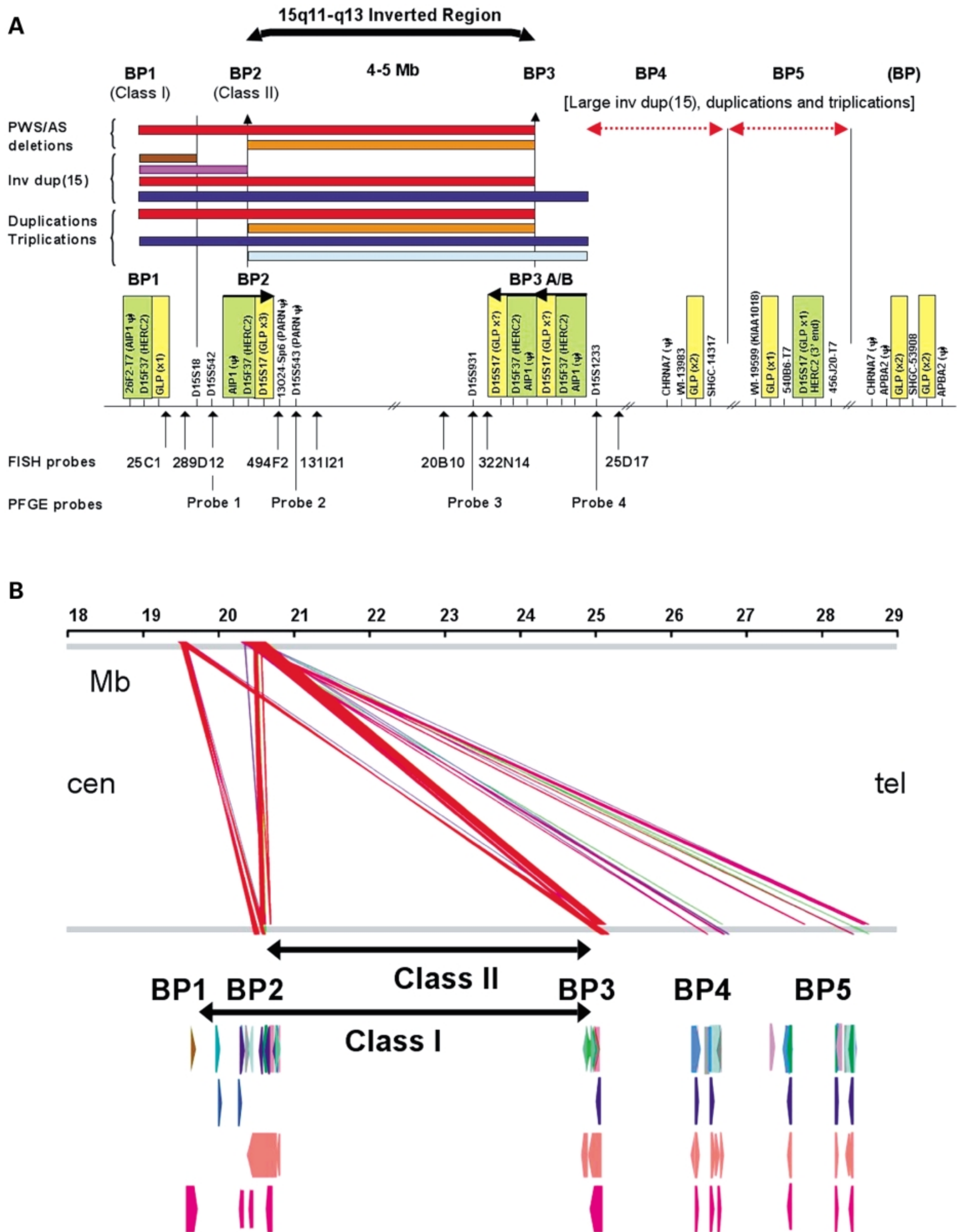


**Figure 1.** (**A**) Genomic map of the 15q11–q13 region, rearranged in PWS and AS patients and other human disorders. BP1–BP3 breakpoints involved in class I (BP1) and class II (BP2) PWS/AS deletion types are shown. Other breakpoints (BP4, BP5 and BP) are also indicated. The location of probes used for FISH (RP11 BAC clones) and PFGE (probes 1–4, see Material and methods) analysis are shown. Distances between segmental duplications, BPs and markers are not scaled. The LCR15 sequences are marked by yellow rectangles (*GLP*, *golgin like protein*, *x* possible number of copies) and the *HERC2*-related segmental duplications by green rectangles. (**B**) GenomePixelizer display of the intrachromosomal BLAST results from chromosome region 15q11–q13 (red = 100% sequence identity, purple 95–99%, green 90–94%). The correspondences between locations at the panel are only approximate. Coloured arrows indicate regions of sequence identity and orientation between segmental duplications in build 30. The same colour of arrows indicates segmental duplications that are related to each other. Other segmental duplications in the region, that are unrelated to the PWS/AS deletions are not shown.

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

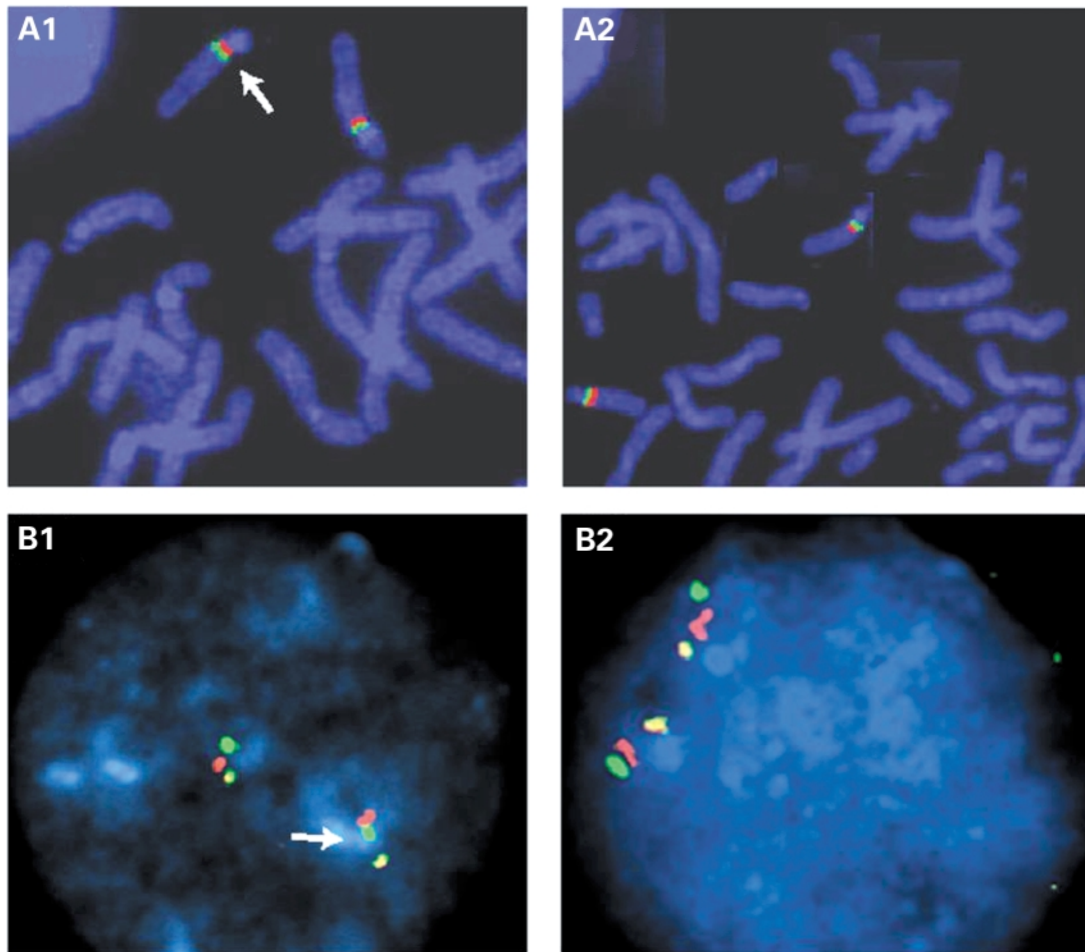


**Figure 2.** Identification of the 15q11–q13 inversion in mothers of AS patients with the BP2/3 deletion. FISH hybridization results on metaphase (**A**) and interphase (**B**) chromosomes from mothers of AS patients with BP2/3 deletion with (A1, B1) and without (A2, B2) the 15q11–q13 inversion. In (A) probes RP11-494F2 (green) and RP11-322N14 (red), at the two ends of the BP2–BP3 region, were used. In (B) probes RP11-494F2 (green), RP11-131I21 (red), within the proximal BP2–BP3 region, and RP11-25C1 (yellow), between BP1 and BP2, were used. White arrows indicate the inverted chromosome.

## Identification of 15q11–q13 inversion and deletion breakpoints

To further characterize the 15q11–q13 region involved in the inversion and deletions, we performed pulsed field gel electrophoresis (PFGE) analysis of samples from AS mothers with and without the BP2–BP3 inversion, AS BP2/3 and BP1/3 deleted patients, and subjects from the general population.

Firstly, by means of probe 1, located near marker *D15S542* (on the proximal end of BP2), we detected the probable proximal deletion breakpoint of two class II patients (from three analysed) using three different restriction enzymes in one case, which gave additional fragments of ~0.6 Mb *Not*I, ~1.7 Mb *Nru*I and ~0.7 Mb *Sgr*AI, and with an additional ~1.5 Mb *Mlu*I fragment in another class II patient (Fig. 3). Therefore, if this probe detected the class II breakpoints, then it should also reveal the BP2–BP3 genomic inversion in the mothers of AS class II patients. Thus, probe 1 gave additional bands in three different BP2–BP3 inverted mothers: ~1.8 Mb *Mlu*I in sample LB327, three different *Nru*I extra-bands (~1.0, 1.5 and 1.7 Mb) in sample LB325, and a ~1.6 Mb *Nru*I extra-band in sample LB320. We excluded the possibility of *Nru*I partial digestion by hybridizing the same filter with the other probes designed in this study.

Breakpoint fragments were also detected with three additional probes flanking the inverted region, located at the distal end of BP2 and at the proximal and distal ends of BP3. The identification of different extra fragments using different restriction enzymes in the same samples should indicate the existence of genomic rearrangements rather than simply restriction site polymorphisms. Probe 2, located in the vicinity of marker *D15S543*, detects different additional fragments with several restriction enzymes in two inverted mothers: additional ~2 Mb *Nru*I and >2 Mb *Not*I fragments were identified in sample LB325, and an ~1.8 Mb *Not*I extra fragment in sample LB327. Probe 3, located in the proximal side of BP3 detected an additional ~1.5 Mb *Sgr*AI fragment in the inverted LB327 sample. Interestingly, probes 3 and 4 detected other polymorphic fragments in samples from the general population or in the mothers tested (inverted and non-inverted), further confirming the complexity of the 15q11–q13 region. Thus, probe 2 detected a ~0.3 Mb *Nru*I fragment in the LB327

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

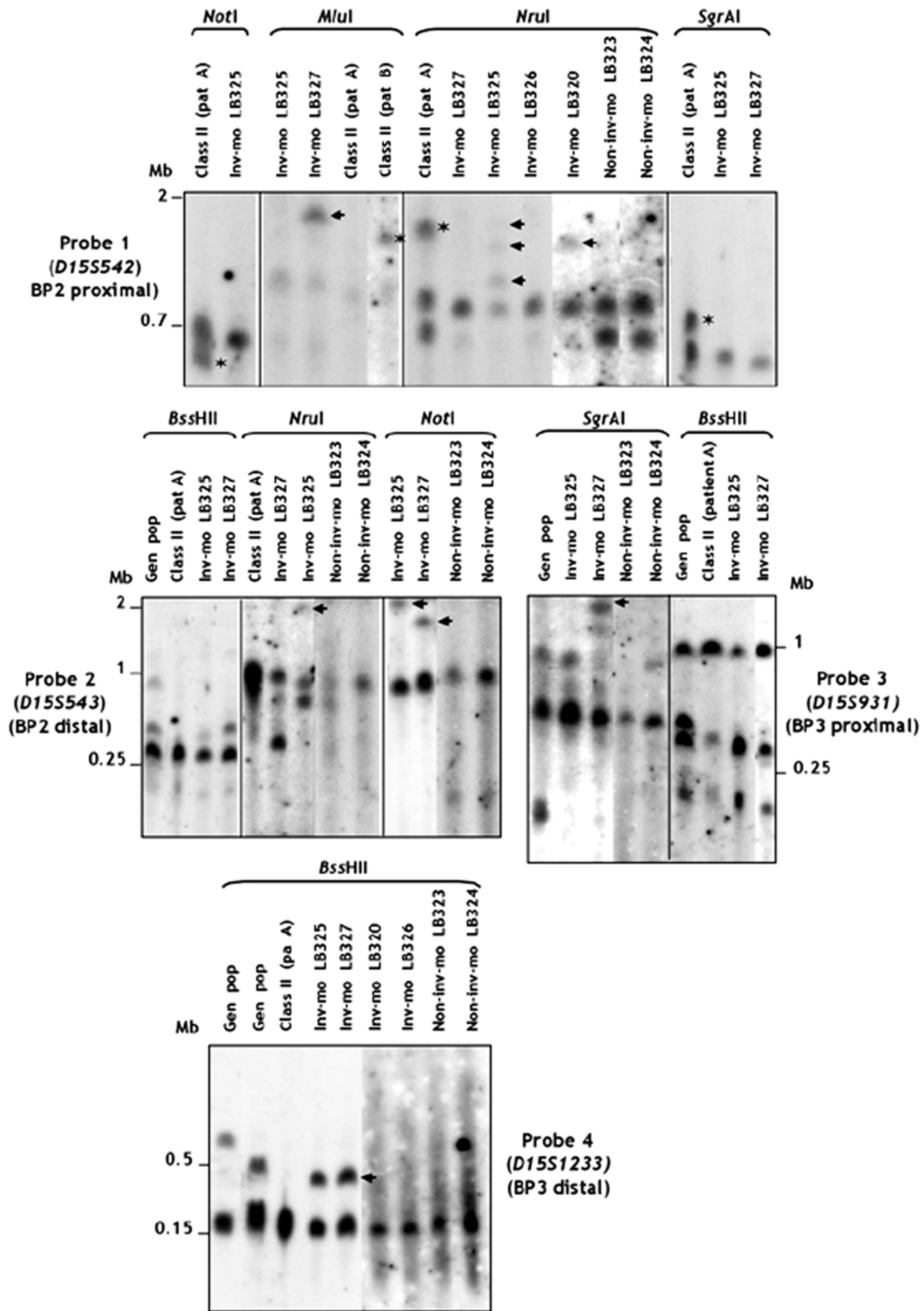


**Figure 3.** Pulse-field gel electrophoresis (PFGE) results for probes located at the ends of the 15q11–13 inverted region. Probes 1 and 2 are located proximally and distally to the BP2 segmental duplication, respectively. Probes 3 and 4 are located proximally and distally to the BP3 segmental duplication, respectively (non-inverted chromosome configuration). Inv-mo, inverted mother; LB, sample number; Non-inv-mo, non-inverted mother; class II, AS BP2/3 deleted samples; Gen pop, general population sample; pat, patient. Inversion breakpoints (specific extra bands of inverted mothers) are marked by arrows, and deletion breakpoints (specific extra bands of class II patients) by asterisks. Other polymorphic bands that appear in inverted and non-inverted mothers, class II or samples from the general population are not marked. Wild-type restriction patterns correspond to lanes without arrows, asterisks or additional polymorphic bands mentioned in the text.

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

sample (also seen in non-inverted mothers, not shown), and probes 3 and 4 detected polymorphic fragments of ~0.4 and ~0.6 Mb with *Bss*HII in subjects from the general population, respectively (Fig. 3).

### Chromosome region BP2–BP3 15q11–q13 is also inverted in the mouse genome sequence

We have aligned the public sequence of human 15q11–q13 with the syntenic region of mouse chromosome 7. This alignment revealed that the BP2–BP3 region is also inverted in the mouse sequence with respect to the human sequence (Fig. 4). This inversion was also detected using the mouse sequence data from Celera (data not shown). Although these regions still need further sequencing and assembling, the inversion limits fit well with the *HERC2* pseudogene locations, corresponding to BP segmental duplications. As expected, the gene and sequence tagged sites (STS) order in the mouse radiation hybrid map support the data of the assembled sequence and therefore the inverted orientation of this region with respect to the human sequence. Thus, the gene block *P-Gabrg3-Gabra5-Ube3a-Snprn-Ndn-Magel2* appears inverted when compared against the human map, but not with respect to flanking genes, such as *Chrna7* (distal) and *MGC35570* (proximal), and genes located distal or proximal to these ones, respectively. Although the available sequence on the proximal region is still incomplete, the alignment between mouse and human shows that the inversion does not include the BP1–BP2 region. Interestingly, the order of the BP3–BP4 and BP4–BP5 segments is altered in the mouse syntenic region. The BP3–BP4 mouse sequence is located distal to the BP4–BP5 region, which is contiguous to the BP2-BP3 (Fig. 4). The boundaries of these rearrangements correlate with the position of BPs and clusters of low copy repeat 15 sequences (LCR15s) partially related to the BP segmental duplications (13).

## DISCUSSION

We describe here a 15q11–q13 genomic inversion in mothers of AS BP2/3-deleted patients and in 9% of subjects of the general population. Since BP2 and BP3 segmental duplications are usually in a tail-to-tail orientation (10,11), the inverted chromosomes are likely to arise by non-homologous recombination events between the high sequence identity of these segmental duplications. To explain the predisposition to deletions in the subjects carrying BP2/3 inversion, a partial tandem orientation within the BP2 and BP3 inverted blocks should be proposed. Thus, the inversion between BP2 and BP3 would change the relative orientation of an internal portion of the segmental duplications that, through unequal cross over events between them, would lead to deletions or duplications in the offspring.

Since the 15q11–q13 inversion is present in 4.5% of chromosomes of subjects from the general population, and since there are very few cases of recurrence of the 15q11–q13 AS deletion (risk of less than 1%) (16), the penetrance of the genomic inversion regarding the risk to suffer a BP2/BP3 deletion in the offspring of the carrier mothers is likely to be low (0.1–0.2%). Individual variability could exist in the recombination process or machinery, which could further modify the susceptibility to the rearrangements. It must be noted that the complex organization of duplicons may lead to different rearrangements and that, as already discussed by Giglio *et al.* (5), some of them may not be compatible with relatively normal embryo development. Chromosome 15q11–q13 duplicons might mediate not only deletions and inv dup(15) chromosomes but also acentric chromosomes of the 15q13-qter portion. Thus, the heterozygous inversion mothers might indeed have several embryos unbalanced for chromosome 15 rearrangements, but either the embryo is precociously aborted or the abnormal chromosome 15 is lost due to the absence of a functional centromere. It is also possible that embryos with the 15q11–q13 deletion are less viable than normal embryos, which could perhaps explain the low frequency of recurrence of the deletion in mothers carrying the 15q11–q13 BP2/3 inversion. Another factor that could influence the susceptibility to deletion or duplication in gametes of females that carry the BP2–BP3 inversion could be the specific location where the recombination leading to the inversion occurred. Although the sample studied is relatively low, it seems that the inversion events occurred in different cases, in different positions within BP2 and BP3, as detected by the variability of breakpoints. Thus, the different position of the breakpoints of the inversion in BP2 segmental duplication should lead to different length of regions of sequence identity oriented in tandem between BP2 and BP3 segmental duplications. This could affect the presence of recombination-prone sequences in direct orientation within the blocks of inverted segmental duplications.

We have found that the BP2–BP3 inverted mothers have variable breakpoints. This indicates that there is not a major recombination hot spot, which probably is due to the large size (over 400 kb) of the BP2 and BP3 segmental duplications (10,11). This is supported by independent observations of other investigators analyzing PWS and AS patients (17). The study of Mewborn *et al.* (17) detected seven different breakpoints (from 24 PWS/AS deleted patients) and they proposed the existence of inverted chromosomes as a susceptibility factor for the deletions. We have also shown here that there is variability for the class II deletion breakpoints within the BP2 segmental duplication. The demonstration of potential inversion or deletion hot spots within the BP2 and BP3 segmental duplications will need the analysis of a large number of samples and a detailed characterization of the segmental duplications. The presence of other PFGE extra-bands in samples of the general population denotes the complex organization of the region. Other large polymorphic regions have been found proximal to BP1 (18) and distal to BP3 (19).

We have detected two cases of BP2/3 AS patients and two cases of BP1/3 AS patients in which the mothers do not carry 15q11–q13 inversions detectable by FISH with the probes used in this study. Since the region containing these segmental duplications is extremely complex, with multiple other partially common segmental duplications (LCR15s) (13), it is possible that these cases have specific variants within blocks of segmental duplications. This hypothesis should be tested once we know the detailed organization of these segmental duplications in a large number of subjects with deletions and controls samples.

Interestingly, the mouse/human sequence alignment shows an inverted orientation of the sequence between the BP2 and BP3

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

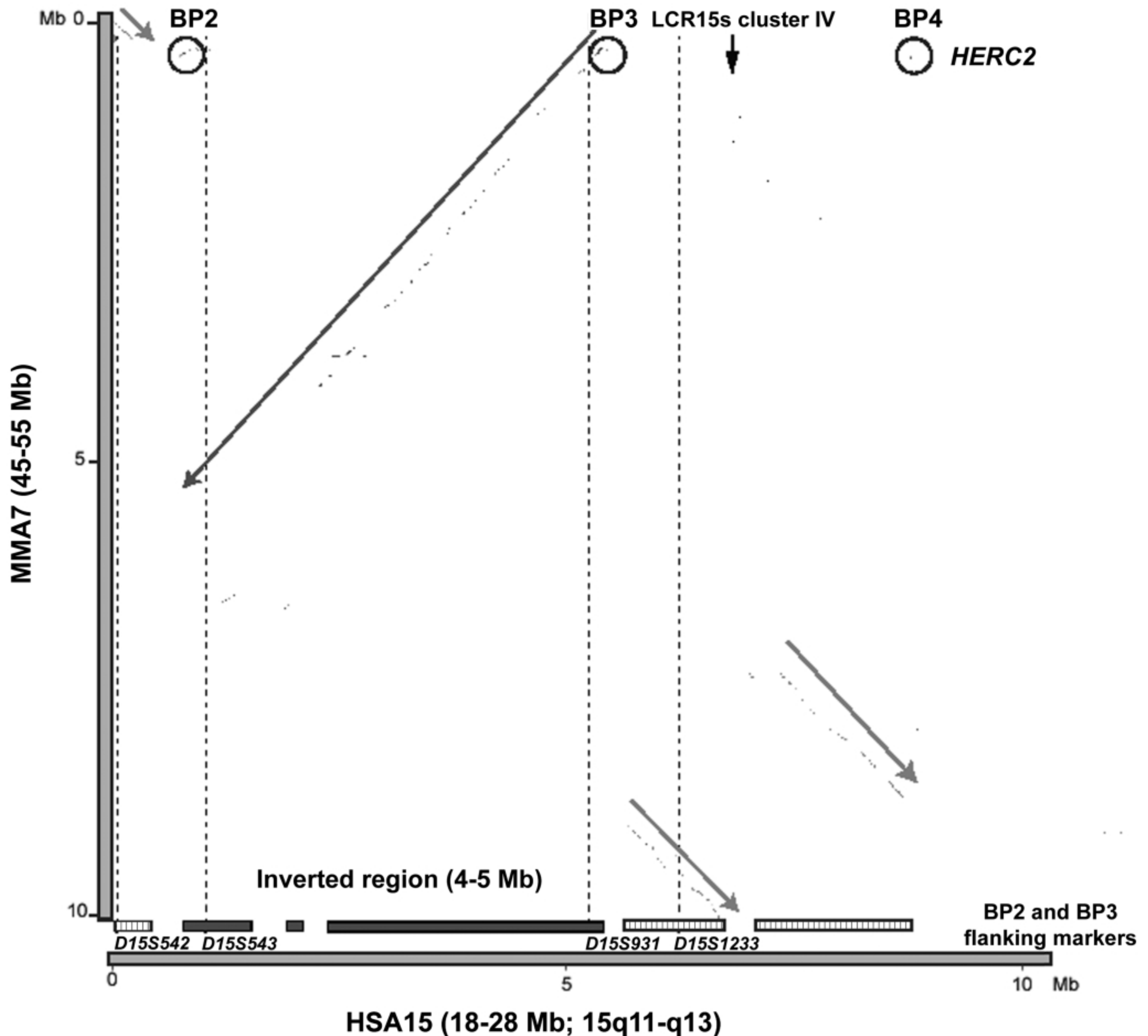


**Figure 4.** Dot plot alignment between human chromosome 15 (HSA15), region 15q11–q13 (18–28 Mb, build 30), and its syntenic counterpart on mouse chromosome 7 (MMA7; 45–55 Mb). Sloping arrows indicate the orientation of the alignments in direct or inverted regions. Large gaps within the alignment are due to the draft status of the genome sequences and the filtering of alignments. *HERC2* sequence similarities are shown by circles and reveal BP2, BP3 and BP4 segmental duplications. Marker positions flanking BP2 and BP3 segmental duplications are shown as vertical dashed lines. The LCR15 cluster IV (13) that maps between two genomic blocks of different order in the mouse/human alignment is shown as a vertical arrow. Dark and light horizontal boxes are a representation of the mouse/human sequence alignment.

segmental duplications. In addition, other rearrangements between the mouse and human syntenic regions correlate with the BP and LCR15 locations. These findings of inversions and rearrangements between mouse and human sequences flanked by segmental duplications are similar to those detected in the WBS region (20,21).

Our data suggest that the most common form of the AS deletion involving class II patients relies on misalignment between segmental duplications BP2 and BP3 flanking the 15q11–q13 region, due to an inversion of the targeted deleted region. Since the same type of BP2/3 deletion is present in PWS patients (7–9), it is expected that some deletions in these patients are mediated by the 15q11–q13 inversion described here, but arising from paternal chromosomes. Furthermore, we do not know at this stage if other rearrangements occur in the offspring of carriers of 15q11–q13 inversions, or which is the viability of embryos carrying 15q11–q13 rearrangements. The different order of segments in the mouse/human syntenic region distal to BP3 could provide useful information for the analysis of other rearrangements of chromosome 15q11–q14, specially those that have been described in cases of autism (22–24). In conclusion, the data presented here reinforce the

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

idea that genomic polymorphisms could provide susceptibility to *de novo* human chromosome rearrangements (4–6,25) and present further evidence of the role of segmental duplications in genomic variability.

## MATERIALS AND METHODS

### Patients

Patients with AS were diagnosed according to clinical criteria of the disorder and by analysis of deletions in the 15q11–q13 region. The studied sample consisted of six BP2/3 AS patients and their corresponding mothers, two BP1/3 AS patients and their corresponding mothers, seven UPD 15 mothers, three mothers of AS patients with UBE3A mutations, and 44 control subjects from the general population undergoing routine blood laboratory tests. Informed consent was obtained from the patients, their parents and control samples.

### FISH analysis

Patients with AS syndrome have been classified as deleted through metaphase FISH with specific commercial probes (Vysis). AS class I (BP1/3) patients were defined using FISH BAC probes RP11-25C1 and RP11-289D12 (GenBank accession nos. AC016204 and AC090764, respectively; Fig. 1A). Both clones map between BP1 and BP2 according to markers *NIB1540* or *D15S18* and *D15S542*, respectively (9). AS class II (BP2/3) patients were defined using FISH BAC probes RP11-494F2 and RP11-322N14 (GenBank accession nos. AC103750 and AC017046, respectively), which correspond to the *NDN* and *OCA2 loci* within the PWS/AS deleted region, respectively (14). The RP11-494F2 sequence maps at the proximal end and the RP11-322N14 sequence at the distal end of the BP2–BP3 region. The RP11-322N14 clone also contains known PWS/AS markers as *D15S931* and *D15S24*. To define the deletion as BP1/BP3 or BP2/BP3 we performed FISH experiments in all patients' metaphases using both RP11-289D12 and RP11-25C1 in separate experiments. The BP3 distal breakpoint was defined in all cases by dual-colour FISH with probes RP11-494F2 and RP11-25D17. All the mothers of AS patients were analysed for the 15q11–q13 inversion by both metaphase and interphase FISH. Probes RP11-494F2 and RP11-322N14 were used in dual FISH metaphase studies (26). The two probes have a physical distance of about 4 Mb. Thus, to limit the overlapping of the signals we used the Vysis nick translation kit designed for direct fluorescence labelling of DNA so to avoid signal amplification. While screening the slides we considered only those metaphases with distinct green and orange signals, discarding those with overlapping ones. No less than 30 metaphases were analysed in each case. We used two sets of three probes each to analyse cells in interphase. In order to avoid misinterpretation due to regional looping, probes of each set covered a region smaller (about 2 and 2.5 Mb, respectively) than that covered by the probes used for metaphase experiments (about 4 Mb). The two sets were RP11-494F2 and RP11-131I21 (GenBank accession no. AC009696) plus RP11-25C1 as a control probe, and RP11-20B10 (GenBank accession no. AC0022603) and RP1-322N14 plus RP11-25D17 (GenBank accession no. AC021360) as a control probe. Probes from both sets were used in triple-colour FISH (26). We examined no less than 40 nuclei for each set tested and we scored only those chromosomes where all three probes could be visualized in close alignment with each other. These experiments should have been able to detect inversions of BP1–BP3 and BP2–BP3 regions but not those involving the BP1–BP2 regions, which are difficult to detect with the actually available BACs. Probe and slide preparation, DNA hybridization, and analysis were performed using conventional methods. At least 20 cells per case were analysed by direct microscopic visualization and digital-imaging analysis. BAC DNA mini-preparations were labelled with SpectrumGreen-16dUTP or SpectrumOrange-16dUTP (Vysis) by standard nick-translation reaction and FISH protocol was performed according to supplier's instructions. Slides were studied under a fluorescence microscope equipped with the appropriate filter set.

### 15q11–q14 sequence analysis

The June 2002 (NCBI build 30) human chromosome 15 sequence was obtained through the UCSC (University of California, Santa Cruz) Human Genome Browser website (ftp://genome.cse.ucsc.edu/goldenPath/05apr2002/). Identification of segmental duplications by BLAST was as described (27). The chromosome 15 sequence was repeat-masked from highly repetitive elements and the masked sequence was compared against itself by chromosome-wide BLAST to detect intrachromosomal segmental duplications using BLAST2 setting with MegaBlast option on a local Unix server. A BLAST report table was generated using the *-D* command option. Results were subsequently parsed under the following criteria: BLAST results having $\geq$90% sequence identity, $\geq$80 bp in length, and with expected value $\leq e^{-30}$. All identical hits, including sub-optimal BLAST alignments recognized by multiple overlap alignments, as well as mirror hits (reverse coordinate alignments) from the BLAST results of the intrachromosomal set were removed. Alignment coordinates separated by neighbour distance less than 5 kb were joined together into modules to account for masked repetitive sequences, and only modules with size over 10 kb were kept for analysis in this study (28). Results generated from the detection of segmental duplications were subsequently converted into coordinate files as input for display using GenomePixelizer (29), obtained from www.atgc.org/GenomePixelizer/GenomePixelizer_Welcome.html.

Raw sequences from mouse (July 2002) and human (September 2002) were obtained from Ensembl sequence repository, masked with RepeatMasker (http://repeatmasker.genome.washington.edu/cgi-bin/RM2_req.pl) locally and aligned using MegaBlast. Resulting alignments were parsed using perl scripts based on bioperl modules. Graphical displays of the alignments were generated from the parsed data using several perl scripts.

### PFGE analysis

Probe 1, located near the *D15S542* marker, was obtained by PCR with primers 5′-cctgtggtctccttgacag-3′ and 5′-caaaacactct-gaaagcagtg-3′ designed on GenBank accession no. AC016446

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

(RP11-289D12); probe 2, located near the *D15S543* marker, was obtained by PCR with primers 5′-gaatccagcatggtgctcag-3′ and 5′-ccaggagacaacttggtttcc-3′ designed on GenBank accession no. AC073446 (RP11-757E13); probe 3, located near the *D15S931* marker, was obtained by PCR with primers 5′-caataatgggagggaggtcac-3′ and 5′-ctcattcaagcattcacctgt designed on GenBank accession no. AC017046 (RP11-322N14); probe 4, located near the *D15S1233* marker, was obtained by PCR with primers 5′-cccagcttccatgctgatg-3′ and 5′-gggtggagtgagaagtgtc-3′ designed on GenBank accession no. AC021360 (RP11-25D17). Prior to probe purification, the corresponding PCR probes were subcloned in the pGEM-T Easy vector (Promega). PFGE high-molecular DNA restrictions were performed using 80U of the corresponding enzyme during 24 h and electrophoresed by means of the CHEF Mapper XA system (Bio-Rad). Hybridization conditions were standard in 7% SDS and 0.5 M phosphate buffer, following stringency washes of 0.5–0.2 × SSC at 55–65°C. At least two completely independent analyses were performed for each restriction enzyme and probe.

## ACKNOWLEDGEMENTS

We would like to thank ORSA (Organizzazione sindrome di Angelman) for providing us with the blood samples of the mothers and AS patients. This work was supported by cofin01- and cofin02-MIUR (to O.Z.); the Italian Telethon Foundation (GP0247Y01 to O.Z.); the Ministero della Salute (to O.Z.); the Departament d'Universitats Recerca i Societat de la Informació (DURSI) (to X.E.); the Departament de Sanitat (to X.E.), the Marató de TV3 (014330 to X.E.); the Comision Interministerial de Ciencia y Tecnología (SAF2002-00799 to X.E.); and the Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Consumo (Red G03/184 to X.E.). X.E. is Senior Scientist of the Centre de Regulació Genòmica (CRG).

## REFERENCES

1. Samonte, R.V. and Eichler, E.E. (2002) Segmental duplications and the evolution of the primate genome. *Nat. Rev. Genet.*, **3**, 65–72.
2. Stankiewicz, P. and Lupski, J.R. (2002) Molecular-evolutionary mechanisms for genomic disorders. *Curr. Opin. Genet. Dev.*, **12**, 312–319.
3. Emanuel, B.S. and Shaikh, T.H. (2001) Segmental duplications: an 'expanding' role in genomic instability and disease. *Nat. Rev. Genet.*, **2**, 791–800.
4. Giglio, S., Broman, K.W., Matsumoto, N., Calvari, V., Gimelli, G., Neumann, T., Ohashi, H., Voullaire, L., Larizza, D., Giorda, R. *et al.* (2001) Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. *Am. J. Hum. Genet.*, **68**, 874–883.
5. Giglio, S., Calvari, V., Gregato, G., Gimelli, G., Camanini, S., Giorda, R., Ragusa, A., Guerneri, S., Selicorni, A., Stumm, M. *et al.* (2002) Heterozygous submicroscopic inversions involving olfactory receptor–gene clusters mediate the recurrent t(4;8) (p16; p23) translocation. *Am. J. Hum. Genet.*, **71**, 276–285.
6. Osborne, L.R., Li, M., Pober, B., Chitayat, D., Bodurtha, J., Mandel, A., Costa, T., Grebe, T., Cox, S., Tsui, L.C. and Scherer, S.W. (2001) A 1.5 million-base pair inversion polymorphism in families with Williams-Beuren syndrome. *Nat. Genet.*, **29**, 321–325.
7. Khan, N.L. and Wood, N.W. (1999) Prader–Willi and Angelman syndromes: update on genetic mechanisms and diagnostic complexities. *Curr. Opin. Neurol.*, **12**, 149–154.
8. Kuwano, A., Mutirangura, A., Dittrich, B., Buiting, K., Horsthemke, B., Saitoh, S., Niikawa, N., Ledbetter, S.A., Greenberg, F., Chinault, A.C. *et al.* (1992) Molecular dissection of the Prader-Willi/Angelman syndrome region (15q11–13) by YAC cloning and FISH analysis. *Hum. Mol. Genet.*, **1**, 417–425.
9. Knoll, J.H., Nicholls, R.D., Magenis, R.E., Glatt, K., Graham, J.M. Jr., Kaplan, L. and Lalande, M. (1990) Angelman syndrome: three molecular classes identified with chromosome 15q11q13-specific DNA markers. *Am. J. Hum. Genet.*, **47**, 149–155.
10. Christian, S.L., Fantes, J.A., Mewborn, S.K., Huang, B. and Ledbetter, D.H. (1999) Large genomic duplicons map to sites of instability in the Prader–Willi/Angelman syndrome chromosome region (15q11–q13). *Hum. Mol. Genet.*, **8**, 1025–1037.
11. Amos-Landgraf, J.M., Ji, Y., Gottlieb, W., Depinet, T., Wandstrat, A.E., Cassidy, S.B., Driscoll, D.J., Rogan, P.K., Schwartz, S. and Nicholls, R.D. (1999) Chromosome breakage in the Prader–Willi and Angelman syndromes involves recombination between large, transcribed repeats at proximal and distal breakpoints. *Am. J. Hum. Genet.*, **65**, 370–386.
12. Goodman, M. (1999) The genomic record of Humankind's evolutionary roots. *Am. J. Hum. Genet.*, **64**, 31–39.
13. Pujana, M.A., Nadal, M., Guitart, M., Armengol, L., Gratacòs, M. and Estivill, X. (2002) Human chromosome 15q11–q14 regions of rearrangements contain clusters of LCR15 duplicons. *Eur. J. Hum. Genet.*, **10**, 26–35.
14. Christian, S.L., Bhatt, N.K., Martin, S.A., Sutcliffe, J.S., Kubota, T., Huang, B., Mutirangura, A., Chinault, A.C., Beaudet, A.L. and Ledbetter, D.H. (1998) Integrated YAC contig map of the Prader–Willi/Angelman region on chromosome 15q11–q13 with average STS spacing of 35 kb. *Genome Res.*, **8**, 146–157.
15. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
16. Kokkonen, H. and Leisti, J. (2000) An unexpected recurrence of Angelman syndrome suggestive of maternal germ-line mosaicism of del(15)(q11q13) in a Finnish family. *Hum. Genet.*, **107**, 83–85.
17. Mewborn, S.K., Miley, N.L., Fantes, J.A., Brown, R.L., Butler, M.G., Christian, S.L. and Ledbetter, D.H. (2002) Breakpoint junction fragments in Prader–Willi and Angelman syndrome (PWS/AS) deletion patients reveal variable breakpoints within large duplicons. *Am. J. Hum. Genet.*, **71** (Suppl.), 736.
18. Ritchie, R.J., Mattei, M.G. and Lalande, M. (1998) A large polymorphic repeat in the pericentromeric region of human chromosome 15q contains three partial gene duplications. *Hum. Mol. Genet.*, **7**, 1253–1260.
19. Riley, B., Williamson, M., Collier, D., Wilkie, H. and Makoff, A. (2002) A 3-Mb map of a large segmental duplication overlapping the alpha7-nicotinic acetylcholine receptor gene (*CHRNA7*) at human 15q13–q14. *Genomics*, **79**, 197–209.
20. Valero, M.C., de Luis, O., Cruces, J. and Perez Jurado, L.A. (2000) Fine-scale comparative mapping of the human 7q11.23 region and the orthologous region on mouse chromosome 5G: the low-copy repeats that flank the Williams–Beuren syndrome deletion arose at breakpoint sites of an evolutionary inversion(s). *Genomics*, **69**, 1–13.
21. DeSilva, U., Elnitski, L., Idol, J.R., Doyle, J.L., Gan, W., Thomas, J.W., Schwartz, S., Dietrich, N.L., Beckstrom-Sternberg, S.M., McDowell, J.C. *et al.* (2002) Generation and comparative analysis of approximately 3.3 Mb of mouse genomic sequence orthologous to the region of human chromosome 7q11.23 implicated in Williams syndrome. *Genome Res.*, **12**, 3–15.
22. Bundey, S., Hardy, C., Vickers. S., Kilpatrick, M.W. and Corbett, J.A. (1994) Duplication of the 15q11–13 region in a patient with autism, epilepsy and ataxia. *Dev. Med. Child. Neurol.*, **36**, 736–742.
23. Browne, C.E., Dennis, N.R., Maher, E., Long, F.L., Nicholson, J.C., Sillibourne, J. and Barber, J.C. (1997) Inherited interstitial duplications of proximal 15q: genotype–phenotype correlations. *Am. J. Hum. Genet.*, **61**, 1342–1352.
24. Cook, E.H. Jr., Lindgren, V., Leventhal, B.L., Courchesne, R., Lincoln, A., Shulman, C., Lord, C. and Courchesne, E. (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am. J. Hum. Genet.*, **60**, 928–934.
25. Jobling, M.A., Williams, G.A., Schiebel, G.A., Pandya, G.A., McElreavey, G.A., Salas, G.A., Rappold, G.A., Affara, N.A. and Tyler-Smith, C. (1998) A selective difference between human Y-chromosomal DNA haplotypes. *Curr. Biol.*, **8**, 1391–1394.

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018

26. Bonaglia, M.C., Giorda, R., Poggi, G., Raggi, M.E., Rossi, E., Baroncini, A., Giglio, S., Borgatti, R. and Zuffardi, O. (2000) Inverted duplications are recurrent rearrangements always associated with a distal deletion: description of a new case involving 2q. *Eur. J. Hum. Genet.*, **8**, 597–603.
27. Estivill, X., Cheung, J., Pujana, M.A., Nakabayashi, K., Scherer, S.W. and Tsui, L.C. (2002) Chromosomal regions containing high-density and ambiguous-mapped single nucleotide polymorphisms (SNPs) correlate with segmental duplications in the human genome. *Hum. Mol. Genet.*, **11**, 1987–1995.
28. Cheung, J., Estivill, X., Razi, K., MacDonald, J.R., Lau, K., Tsui, L.-C. and Scherer, S.W. Genome-wide detection of segmental duplications and assembly errors in the human genome sequence. *Genome Biol.*, in press.
29. Kozik, A., Kochetkova, E. and Michelmore, R. (2002) GenomePixelizer—a visualization program for comparative genomics within and between species. *Bioinformatics*, **18**, 335–336.

Downloaded from https://academic.oup.com/hmg/article-abstract/12/8/849/645988
by guest
on 29 July 2018