ORIGINAL ARTICLE

Microarray analysis of gene/transcript expression in Prader-Willi syndrome: deletion versus UPD

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Background: Prader-Willi syndrome (PWS), the most common genetic cause of marked obesity, is caused by genomic imprinting and loss of expression of paternal genes in the 15q11-q13 region. There is a paucity of data examining simultaneous gene expression in this syndrome.
Methods: We generated cDNA microarrays representing 73 non-redundant genes/transcripts from the 15q11-q13 region, the majority within the PWS critical region and others distally on chromosome 15. We used our custom microarrays to compare gene expression from actively growing lymphoblastoid cell lines established from nine young adult males (six with PWS (three with deletion and three with UPD) and three controls).
Results: There was no evidence of expression of genes previously identified as paternally expressed in the PWS cell lines with either deletion or UPD. We detected no difference in expression of genes with known biallelic expression located outside the 15q11-q13 region in all cell lines studied. There was no difference in expression levels of biallelically expressed genes (for example, *OCA2*) from within 15q11-q13 when comparing UPD cell lines with controls. However, two genes previously identified as maternally expressed (*UBE3A* and *ATP10C*) showed a significant increase in expression in UPD cell lines compared with control and PWS deletion subjects. Several genes/transcripts (for example, *GABRA5*, *GABRB3*) had increased expression in UPD cell lines compared with deletion, but less than controls indicating paternal bias.
Conclusions: Our results suggest that differences in expression of candidate genes may contribute to phenotypic differences between PWS subjects with deletion or UPD and warrant further investigations.

Prader-Willi syndrome (PWS) is characterised by infantile hypotonia, feeding difficulties, hypogonadism, small hands and feet, mental deficiency, hyperphagia leading to obesity in early childhood, and a particular facial appearance.[1 2] The chromosome 15q11-q13 region is known to contain imprinted sequences that are differentially expressed depending on the parent of origin. Imprinted expression is coordinately controlled in *cis* by an imprinting centre (IC) which is functional in the germline and early postzygotic development. The IC regulates the establishment of parental specific allelic differences in DNA methylation, chromatin structure, and expression.[3 4]

A deletion of the paternal 15q11-q13 region is found in about 70% of PWS subjects, maternal disomy 15 or UPD in approximately 25%, and an imprinting mutation in 2-3%.[1] The 15q11-q13 region contains about 4 million base pairs of DNA and as many as 50-100 genes/transcripts. Recent evidence supports the existence of at least 30 presumed genes in the region.[5] To date, several genes have been located in this region and shown to be paternally expressed. The lack of expression of these genes causes PWS. In addition, at least two genes in this region are maternally expressed in some tissues (*UBE3A*, *ATP10C*).[6] Deletions in *UBE3A* have been shown to cause Angelman syndrome, an entirely different clinical syndrome.

Numerous transcripts have been identified within and nearby the PWS critical region, 15q11-q13.[7–9] Analysis of gene expression has identified several candidate genes which may play a role in PWS.[7] In particular, *SNRPN* is a prime candidate gene. Recent evidence has shown that the *SNRPN* locus is highly complex, composed of a very long variable precursor transcript with multiple functions. *SNURF*, *IPW*, *PAR5*, and multiple small nucleolar RNAs (snoRNAs) are also associated with the *SNRPN* locus.[10] The snoRNAs associated with the *SNRPN* locus (for example, HBII-85) have been proposed to confer most of the PWS phenotype.[11] However, this hypothesis remains to be verified. Regardless, it is still unclear how subtle changes in gene expression resulting from the loss of both imprinted genes and reduced expression of non-imprinted genes lead to the clinical manifestations associated with PWS.

Analyses of the genetic subtypes of PWS have shown a number of cognitive and behavioural differences between PWS subjects with deletions compared to those with UPD. We previously reported significantly higher verbal IQ scores in PWS subjects with UPD compared to subjects with deletions.[12] The UPD subgroup scored significantly higher than the deletion subgroup in four subcategories of verbal testing including information, arithmetic, vocabulary, and comprehension. Several reports have indicated that PWS subjects with UPD had less severe manifestations than subjects with deletions of specific maladaptive behaviours commonly associated with PWS.[1 2 13 14] These differences must be related to the underlying differences in gene expression of the genetic subtypes.

Most analyses of the gene expression patterns of the 15q11-q13 region have focused on identifying imprinted genes and transcripts. However, the imprinted sequences cannot by themselves account for the phenotypic differences observed between the UPD and deletion subtypes within PWS, since paternally expressed sequences would presumably be silent in both the deletion and UPD subtypes.

In this report, to improve our understanding of gene expression within or close to the 15q11-q13 region, we report our experience using a custom made cDNA microarray comprised mostly of sequences within the PWS critical region (PWSCR). This report represents the first application of microarray technology to examine the simultaneous expression of multiple genes/transcripts from genetic subtypes of a

Table 1 Seventy-three genes/transcripts from chromosome 15 spotted on our custom made microarray*

Proximal to D15S1035	SHGC-32610, MYLE, A002B45, NIB1540
D15S1035 - D15S122	ZNF127, WI-15987, NDN, MAGEL2, SGC44643, WI-15028,WI-13791, SNRPN, AA258222, R99003, PAR5, stSG12920, WI-6780, sts-N21972, WI-14946, IPW, PAR1, SHGC-308, WI-6654, Cda0jb12, WI-11918
D15S122- D15S156	UBE3A, WI-16777, ATP10C, GABRB3, GABRA5, GABRG3
D15S156-D15S165	OCA2, HERC2, WI-6527, sts-T53143, sts-H73492, A007E33, STSG3316, STSG10131, STSG15956, SGC33431, SGC35687
D15S165-D15S144	sts-N35472, SHGC-31211, WI-18157, Cda1if10, A006O15, stSG42878, D13638
D15S144-D15S118	WI-15231, SGC33497, SGC30306, SGC35611, A005N08, WI-14003, BCD2917
Distal to D15S118	WI-18493, 38449, R32464, WI-17962, H81848, Bda98d12, H66974, WI-15193, SHGC-30973, SHGC-13414, SHGC-17227, FIBRILLIN, Fb3a9, Cda01b10, UTE-9875, Bdac5a05, STSG4005

*Signals unable to amplify from lymphoblast or brain cDNA: WI-18351, HO2863, sts-H58001, STSG15842. Signals amplified from brain cDNA but not detected on array: GABRG3, A007E33, WI-16777, sts-N35112, sts-Y00757.

human condition resulting from haploinsufficiency and/or genomic imprinting.

SUBJECTS AND METHODS

Subjects

All subjects were matched for age, cognition, obesity status, and sex. Our study subjects included young adult males, six with PWS (three with 15q11-q13 deletion (mean age 28 years) and three with UPD (mean age 27 years)), and three non-syndromic comparison males with obesity of unknown cause (mean age 26 years). Chromosomal status was confirmed by FISH and microsatellite analysis using standard techniques in the PWS subjects.

Methods

Transcripts were chosen for analysis by searching the Unigene and UniSTS databanks. A total of 129 transcripts and genes were identified and subsequently reduced to 73 non-redundant sequences mapping to the 15q11-q13 region, the majority within the PWSCR, or more distally on chromosome 15. Four of these were mapped proximal to D15S1035, 21 were mapped between D15S1035 and D15S122 anchor markers, six between D15S122 and D15S156, 11 between D15S156 and D15S165, seven between D15S165 and D15S144, seven between D15S144 and D15S118, and 17 were distal to D15S118 (table 1). Where possible, longer sequences were identified and used to develop custom primers for PCR. PCR primers were chosen with the aid of MacVector V.7 software (Oxford Molecular, Oxford, UK). Because of the highly repetitive structure and complexity of the snoRNA loci, no specific probes designed for snoRNAs were included in this microarray.

Microarrays were generated by spotting cDNA resulting from PCR amplification of reverse transcribed mRNA isolated from actively growing lymphoblast cell cultures from a healthy, chromosomally normal, adult male. In those few genes/transcripts for which no message was detectable in lymphoblast cultures, adult brain cDNA was used to generate the probe for spotting (Stratagene, La Jolla, CA). Five probes derived from brain cDNA were spotted. Four additional EST or transcript sequences from the uniSTS databank could not be amplified from either lymphoblast or brain cDNA. PCR products were purified and electrophoresed to verify the presence of a single band of the correct size for each gene/transcript. The products were purified and resuspended in 50% DMSO. Each probe (cDNAs) was spotted five times onto CGAP slides (Corning, Corning, NY) using an Affymetrix 427 arrayer.

Target mRNA was isolated from actively growing lymphoblastoid cell cultures using a message maker kit (Gibco/BRL, Carlsbad, CA) from each of the nine adult males. Equal quantities of mRNA from lymphoblast cell cultures derived from the PWS and the obese comparison subjects were alternately labelled with fluorochromes Cy3 (green) and Cy5 (red) using a CyScribe reverse transcription labelling kit (Amersham, Piscataway, NY) and hybridisations were done in 3 × SSC at 65°C overnight in individual hybridisation chambers. The following day the slides were washed in 2 × SSC, 0.1% SDS, followed by four washes in 0.1 × SSC and one wash in distilled water with a final rinse in 100% ethanol. The slides were scanned with an Affymetrix 428 Slide Scanner and the data analysed with Jaguar V.2 software (Affymetrix, Santa Clara, CA).

In order to maximise the total number of comparisons, the arrays were performed in sets following a simple looping design as described by Churchill[15] (for example, set 1 compared control subjects to deletion subjects, set 2 compared control subjects to UPD subjects, and set 3 compared deletion subjects to UPD subjects, see table 2). This looping design for the total of nine subjects required the use of 18 custom made microarray slides. Within each set, each individual target was labelled with both Cy3 and Cy5 and compared to different subjects, respectively. Thus, each target was arrayed five times, replicated twice for each colour of dye and hybridised in four different combinations. The average signal intensity per subject was calculated from 20 replicate spots per gene/transcript for Cy3 and 20 replicate spots per gene/transcript for Cy5.

Quantitative reverse transcription-PCR (RT-PCR) was performed using a QuantiTect Sybr Green RT-PCR kit (Qiagen, Valencia, CA) according to the manufacturer's directions. Briefly, an equal quantity of total RNA from a representative individual subject from each group (control, UPD, deletion) and primers specific for the gene/transcript being quantified were added to a reaction mix containing all components necessary for reverse transcription and PCR. The reaction was carried out using an ABI 7000 system beginning with a 30 minute step at 50°C to allow for reverse transcription, followed by 15 minutes at 95°C. The PCR was performed for 45 to 50 cycles during which the intensity of the Sybr green fluorescence was measured at the extension step of each PCR cycle. The point at which the intensity level crossed the threshold (C_T, defined as the narrowest point between individual reactions in the logarithmic phase of the reaction) was used as recommended by the manufacturer's guidelines

Table 2 Microarray hybridisations between control and PWS subjects

	Label	Comparison strategy among subjects					
Set 1	Cy3	C1	D1	C2	D2	C3	D3
	Cy5	D1	C2	D2	C3	D3	C1
Set 2	Cy3	C1	U1	C2	U2	C3	U3
	Cy5	U1	C2	U2	C3	U3	C1
Set 3	Cy3	D1	U1	D2	U2	D3	U3
	Cy5	U1	D2	U2	D3	U3	D1

C = control (obese comparison) subject. D = PWS subject with 15q11-q13 deletion. U = PWS subject with UPD.

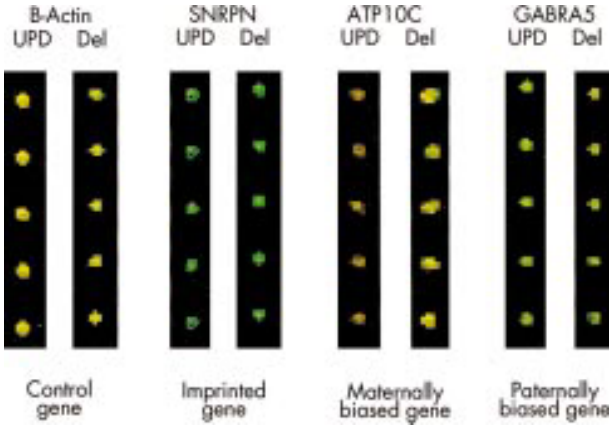

Figure 1 Five replicate hybridisation spots from the PWS custom cDNA array. Target sequences from PWS subjects resulting from UPD or 15q11-q13 deletion were labelled with Cy5 (red) and the target sequences from the controls were labelled with Cy3 (green).

to compare individual reactions. All samples were run at least six times. In addition, a quantitative RT-PCR was done using primers specific to *GAPDH*, a housekeeping gene, on all RNA samples. This allowed for normalisation of all samples relative to *GAPDH*.

Analysis

Multiple cDNAs with no hybridising signal including several plant genes, as well as artificial sequences from the Lucidea kit (Amersham, Piscataway, NY) were spotted in order to define the threshold of detection. Our threshold was defined as the average signal intensity from the negative probes plus two standard deviations. Signal intensities below the threshold were designated not detectable. The slides were normalised to account for slide to slide variability and differences in dye intensity using the calibration probes from the Lucidea kit. Average signal intensities for each sequence were determined for each subject and these were used to generate the mean signal intensity and standard error for the group. Statistically significant differences in expression levels of genes in the three groups were determined by independent *t* tests.

RESULTS

Representative replicate spots for several genes are shown in fig 1. The target sequences from PWS subjects (either UPD or deletion) were labelled in fig 1 with Cy5 (red) and the target sequences from the obese control subjects were labelled with Cy3 (green). Fig 1 is a visual presentation of the similarity in replicated spot intensities seen within the arrays. In general, when normalised, spot intensities for each probe were similar across arrays as represented by the standard errors shown in fig 2. Therefore in the above examples, control or biallelic expressed genes produce equal quantities of mRNA and when hybridised will produce a yellow signal indicating equal intensity of the red and green colours when comparing PWS subjects to control subjects. Imprinted genes (maternally imprinted, paternally expressed) are transcribed only from the control sample producing a green colour when comparing PWS subjects to control subjects. Maternally biased genes expressed preferentially from the maternal allele show a propensity towards the red colour when comparing PWS subjects with UPD to control subjects. Paternally biased genes expressed preferentially from the paternal allele show an increased propensity towards the green colour when comparing PWS subjects to control subjects.

The custom array used for our analyses contained several genes both within (for example, *OCA2*) and outside (for example, *GAPDH*, B-actin, and fibrillin) the PWSCR and known to be biallelically expressed. The expression levels of these genes were all very similar in the individual subjects and the groups examined (fig 2A). In addition, the PWSCR is known to contain a large number of imprinted genes/transcripts which express only from the paternal allele.[8 9] For the most part, we could not detect any expression from these sequences in the deletion or UPD subjects but clear signals were obtained from the comparison subjects (fig 2B). Three of the imprinted sequences, WI-15028, WI-15987, and sts-N21972, had signal intensities in the UPD cell cultures which were slightly above the threshold of detection which was defined as the average signal intensity from the negative probes plus two standard deviations. In addition, WI-15987 had a detectable signal in the deletion cell cultures. This may be because of non-specific cross hybridisation which we were unable to reduce given the constraints of array hybridisation and washing.

Fourteen of the genes/transcripts examined produced signal intensities from the PWS cell lines which were inconsistent with equal expression from both alleles (biallelic) or expression from the paternal allele (maternally imprinted) (fig 2C). The genes/transcripts with unusual expression patterns (confirmed by quantitative RT-PCR) can be divided into four groups. The first group had significantly less expression in cell cultures derived from UPD subjects than either of the other two groups (fig 2C, first panel). These three transcripts were all located outside the PWSCR suggesting that the expression from the maternal allele is reduced relative to the paternal allele. The second group contained genes/transcripts whereby expression from deletion subjects was less than half that of the controls and expression from the UPD cell lines was greater than the deletion lines but significantly less than the controls (fig 2C, second panel). This suggests paternal bias in the allelic expression pattern of these genes/transcripts. The third group contained two transcripts from outside the PWSCR in which the intensity levels from the deletion cell cultures were significantly greater than either the control or UPD cell cultures (fig 2C, third panel). The fourth group contained genes/transcripts with intensity levels significantly higher in the UPD cell cultures, suggesting that expression was exclusively, or at least primarily, from the maternal allele (fig 2C, fourth panel). Two of the genes in the fourth group, *UBE3A* and *ATP10C*, have been shown to be maternally expressed in some tissues.[6]

Several of the genes were chosen for validation by quantitative RT-PCR. Fig 3 shows a representative example of *GABRB3* and a single set of curves generated by real time PCR using an ABI 7000 system. Table 3 shows C_T values generated from at least six replicated quantitative RT-PCR reactions for each subject. The calculated fold change in expression is also shown. The expression data of all sequences were in agreement with the microarray data. Only a few significant differences identified with microarray were not observed with quantitative RT-PCR analysis (for example, WI-6527).

DISCUSSION

The regulatory mechanisms which control gene expression in the 15q11-q13 region are complex and not clearly understood. Procedures used to examine gene expression in this region previously have been primarily non-quantitative. We have used a custom cDNA microarray to analyse and compare gene expression in lymphoblastoid cells from young adult male subjects with PWS with either 15q11-q13 deletion or UPD and obese comparison males. This has allowed a semiquantitative analysis of expression of the genes/transcripts located in or close to the PWSCR.

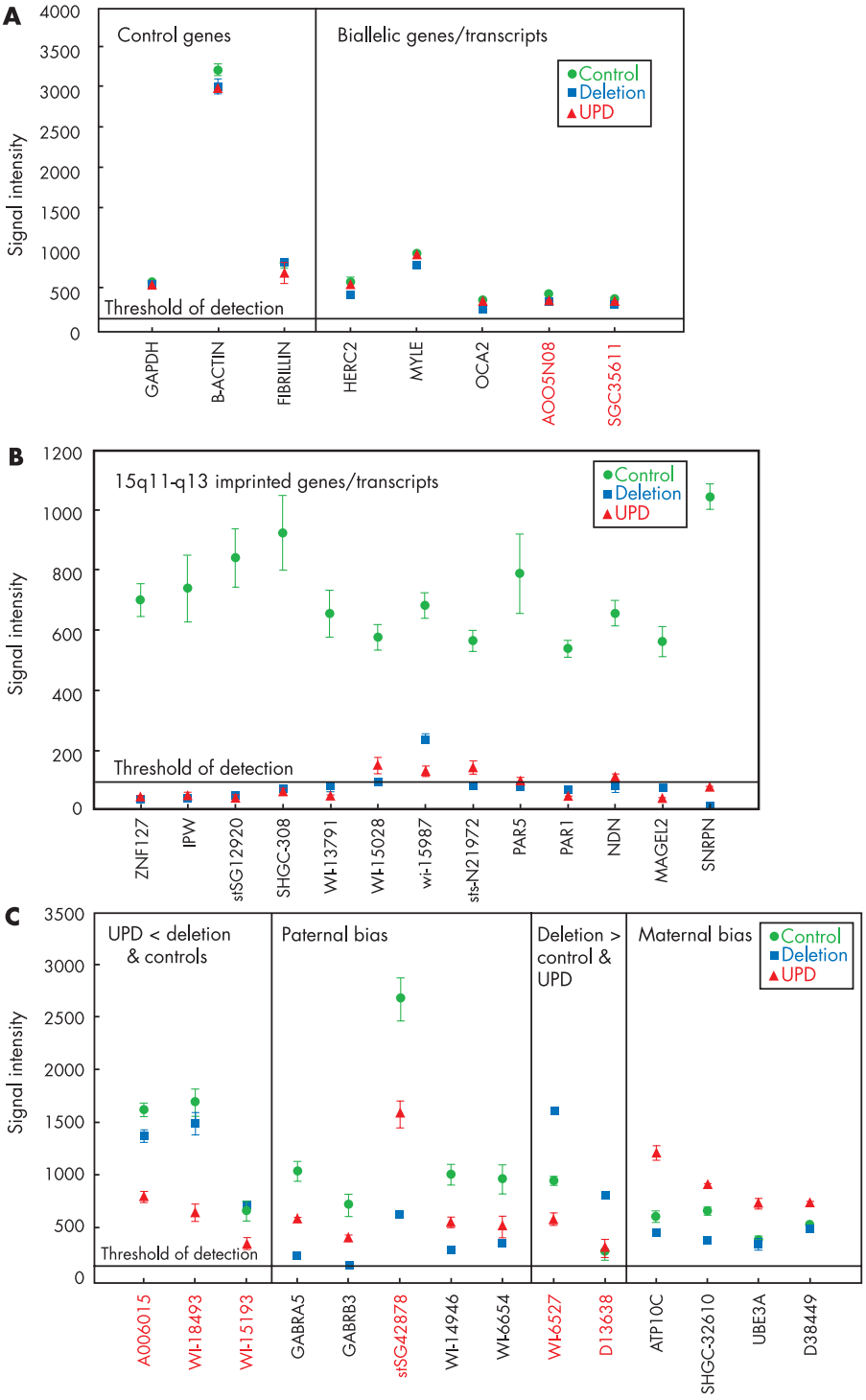

Figure 2 Comparative expression levels of genes/transcripts. Data represent the normalised mean (SE). Each probe was replicated five times on each of four arrays, two labelled with Cy3 and two labelled with Cy5. (A) Control and biallelic genes/transcripts. (B) Maternally imprinted and paternally expressed genes/transcripts from 15q11-q13. (C) Genes/transcripts with variant expression levels. Red lettering indicates sequences that map outside the PWS critical region.

For the most part, sequences from outside the PWSCR produced signal intensities that were similar in each of the three groups (see fig 2A for representative examples). Genes and transcripts previously identified as imprinted produced no detectable signal in the cell lines from PWS patients (fig 2B) with the exception of WI-15028, WI-15987, and N21972 which were slightly above the threshold in PWS subjects, probably as a result of cross hybridisation.

The remaining genes and transcripts can be divided into four categories (fig 2C). First, three transcripts had significantly less expression in the UPD cell lines than in either the deletion or control cell lines (fig 2C, panel 1). These were all located outside the PWSCR and may indicate that these transcripts have paternal bias in their expression pattern. Since the expression from the UPD cell lines was reduced relative to both the control and deletion lines, the expression from the

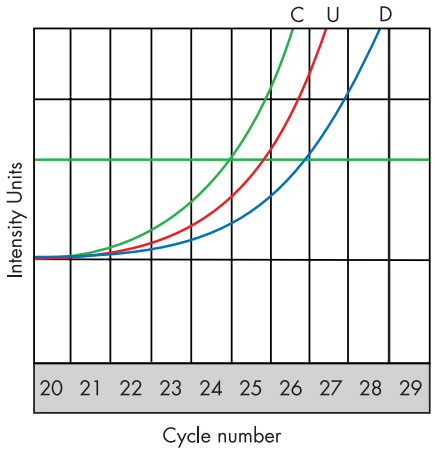

Figure 3 Representative quantitative RT-PCR using primers specific for *GABRB3*. Total RNA was extracted from actively growing lymphoblast cultures and equal quantities were used in a QuantiTect all in one RT-PCR reaction using Sybr green fluorescence to quantify the amplicon. The C_T or threshold PCR cycle line was set at the narrowest portion of the logarithmic phase of amplification. The cycle difference between the control (24.7 cycles) and UPD (25.4 cycles) was 0.7 cycles which corresponds to a fold change in expression of −1.63 compared to the control (lower expression in UPD). For the deletion subject the cycle was 26.1 which represents a difference of 1.4 cycles compared to the control and corresponds to a −2.64 fold change in expression compared with the control (lower expression in deletion). Both the deletion and UPD subjects had significantly less expression than the control; however, the deletion subject differed from the control by more than one cycle, which indicated a greater than two-fold reduction in expression from the deletion subject and represented less expression in the deletion subject compared with the UPD subject. C = Control, U = UPD, D = deletion.

maternal allele must be significantly less than the expression from the paternal allele. If confirmed, this observation suggests that a mechanism exists for regulating allele specific expression which does not require the presence of the paternal imprinting centre.

The second group of transcripts contained several sequences from within the PWSCR which had significantly less than half the control level of expression in the deletion lines (fig 2C, panel 2). Furthermore, signal intensities were significantly less in the UPD cell cultures compared to the control cell cultures. Taken together, these data suggest paternal bias in the expression of these sequences. When confirmed, these sequences express at higher levels from the paternal allele than the maternal allele. This suggests that the imprinting mechanism may play a role regulating quantitative allele specific expression. Interestingly, this group of genes includes the GABA receptor subunit genes where a decreased expression was seen in *GABRB3* in both the deletion and UPD subjects compared to controls in the microarray and quantitative RT-PCR studies (for example, −2.64 fold change in deletion compared with controls and −1.63 for UPD compared with controls). Although deletion and UPD subjects showed reduced expression compared with controls in both the microarray and quantitative RT-PCR studies, as well as biallelic expression, there appeared to be less expression from the maternal allele indicating paternal bias.

GABA is an amino acid neurotransmitter that is widely distributed throughout the central nervous system. It has been estimated that up to 40% of the neurones in brain and spinal cord use GABA as their neurotransmitter making it, quantitatively at least, the most important inhibitory neurotransmitter in brain.[16] GABAergic mechanisms have been implicated in a number of symptoms associated with PWS including hunger,[17 18] compulsivity,[19 20] metabolism,[21] and visual perception and memory.[22 23] GABAergic mechanisms may have a direct effect on these characteristics or there could be indirect effects owing to feedback from other GABA influenced systems.

The five subunits that comprise an individual GABA-A receptor are drawn from a family of 18 proteins, each of which is a distinct gene product.[24] These subunits are divided into six classes, α, β, γ, δ, ρ, and ϵ, based on their homologies. There are six α subunits ($\alpha_{1\text{-}6}$), four β subunits ($\beta_{1\text{-}4}$), four γ subunits ($\gamma_{1\text{-}4}$), two ρ subunits ($\rho_{1\text{-}2}$), one δ subunit, and one ϵ subunit. Given the number of possible pentameric combinations, including homomers that can be drawn from these 18 subunits, thousands of distinct GABA-A receptor subtypes are possible. However, only a dozen or so have been identified in mammalian central nervous system, with most of these containing a combination of α, β, and γ subunits in various stoichiometries.[25] The most prevalent GABA-A receptor in brain is a pentamer composed of α_1, β_2, and γ_2 subunits.

Reports suggesting that GABA and GABA-A receptors play a critical role in central nervous system development[26–28] are of

Table 3 Quantitative RT-PCR validation of selected genes/transcripts studied using microarrays

Gene/transcript		Control	Deletion	UPD
WI-15193	CT† (SD)	27.8 (0.15)	28.1 (0.88)	28.7 (0.01)**
(U<D&C)	Fold change‡		-1.23	-1.87
WI-18493	CT (SD)	27.1 (0.15)	27.0 (0.19)	27.9 (0.39)**
(U<D&C)	Fold change		+1.07	-1.87
GABRB3	CT (SD)	24.7 (0.20)	26.1 (0.38)**	25.4 (0.36)**
(Paternal bias)	Fold change		-2.64	-1.63
WI-6527	CT (SD)	37.8 (0.50)	37.3 (0.14)	38.2 (0.45)
(D>C & U)	Fold change		+1.41	-1.32
SHGC-32610	CT (SD)	27.4 (0.19)	27.9 (0.24)**	26.3 (0.35)**
(Maternal bias)	Fold change		-1.41	+2.14
D38449	CT (SD)	32.5 (0.51)	32.6 (0.21)	29.9 (0.48)***
(Maternal bias)	Fold change		-1.07	+6.06

†CT is the threshold PCR cycle which represents the cycle at which the fluorescence signal exceeds a baseline level. The threshold baseline is set at the narrowest point in the linear part of the amplification curve following recommended guidelines. The numbers represent an average (SD) calculated from six replicate RT-PCR reactions per subject.
‡Fold change in expression equals $2^{(X\text{-}C)}$, where X = the CT from the Deletion or UPD and C = the CT from the Control.
*Significant difference compared to the control, p<0.05.
**Significant difference compared to the control, p<0.01.
***Significant difference compared to the control, p<0.001.
C = Control comparison subject, D = PWS subject with deletion, U = PWS subject with UPD.

particular relevance to the present finding. Changes in subunit expression and, therefore, the composition of GABA-A receptors, appears to be essential for proper neurogenesis. Notably, brain α_5 and γ_3 subunit gene expression diminishes during development, suggesting that these proteins themselves, or as components of GABA-A receptors, may regulate neuronal development and the establishment of central nervous system pathways.[29] The concept of abnormal fetal brain development in PWS was proposed as early as 1977.[30] Our data suggest that even though the GABA receptors do not appear to be expressed solely from the paternal allele there may be a greater than expected reduction in expression from these genes, even in cases of UPD. Presumably, even a modest decrease in the synthesis of these proteins could have a significant effect on brain development, with permanent consequences on central nervous system function. This speculation is supported by the finding that the β_3 subunit is preferentially expressed in the hypothalamus and that a reduction in its production during development could lead to obesity and hypogonadism, hallmarks of PWS.[31] Whereas β_3 subunit proteins are found mainly in the hypothalamus, the γ_3 subunit message tends to be localised in the thalamus and α_5 message is expressed predominately in the hippocampus.[32] Thus, a decline in the production of one or more of these subunits could account, at least in part, for the somatic and behavioural abnormalities associated with PWS.

In our third group, two transcripts from outside the PWSCR expressed at higher levels in the deletion than in either UPD or controls (fig 2C, panel 3). This may also be a consequence of chromatin perturbation resulting from the deletion. Fourth, four sequences, including *UBE3A* and *ATP10C*, appeared to have greater expression in the UPD lines than in either the control or deletion lines (fig 2C, panel 4) indicating maternal bias of expression. *UBE3A* and *ATP10* are known to be maternally expressed, although *UBE3A* only in the brain.[3 6 33] Our data are in agreement with a previous report suggesting that SGC32610 may be maternally biased in its expression pattern.[9] Our data further suggest that, although expression from the paternal allele is detectable, a significantly greater proportion of the RNA is produced by the maternal allele.

Our data may suggest that the expression of genes and transcripts in and around the PWSCR is influenced by chromatin structure and context, as well as the imprinting centre. The dynamic interactions suggested by the microarray data reinforce the observations of the complex nature of expression in the 15q11-q13 region. Finally, we recognise that the targets applied to our microarray were isolated from lymphoblastoid cell cultures and gene expression in cell culture may not be in complete concordance with gene expression in brain tissue. Nevertheless, our results suggest differences in expression of candidate genes which may contribute to differences observed between PWS subjects with deletions and UPD. These sequences warrant further investigation to determine what contribution they make to the phenotype of persons with Prader-Willi syndrome.

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ECHO

New germline *p53* mutation turns up multiple colon tumours

Please visit the Journal of Medical Genetics website [www.jmedgenet.com] for link to this full article.

Japanese researchers have suggested that searches for rogue genes in multiple colon cancer should be broadened to include the *p53* mutation. This follows their discovery of a new *p53* germline mutation in a patient with multiple primary colon cancers.

The case was of a 73 year old man with one advanced colon carcinoma and five polyps in an area including the caecum and ascending colon. Histological analysis confirmed an advanced carcinoma, two early carcinomas, and three adenomas.

The advanced carcinoma yielded a novel germline *p53* mutation of GCC (Ala)→GTC (Val) at codon 189, plus a somatic mutation at codon 245, somatic *APC* mutations, and a somatic *K-ras-2* mutation on genetic analysis. The early carcinomas and adenomas yielded the germline *p53* mutation and somatic *APC* mutations resulting in stop sequences. One early carcinoma had a somatic *K-ras-2* mutation.

The case came from a series of 15 patients without germline mutations in the *APC* gene or DNA mismatch repair genes who had multiple primary colorectal cancers and colorectal polyps without microsatellite instability. Tissue samples from the tumours and adjacent healthy tissue were taken from resected colon for histological and genetic analysis.

The researchers looked for germline *p53* mutations because these occur with tumours in multiple organs in Li-Fraumeni syndrome, though it was not known if they might occur with multiple colon cancers. Whether this new germline mutation proves to be pathogenic or a rare polymorphism remains to be determined.

▲ *Gut* 2003;**52**:304–306.

ECHO

Rare eye condition maps within *NNO1* locus for nanophthalmia

Please visit the Journal of Medical Genetics website [www.jmedgenet.com] for link to this full article.

A new lead to understanding normal eye development has emerged from a molecular genetic study mapping the disease locus of a rare complex optical syndrome to a region within the locus for nanophthalmia

The study was confined to three generations of one English family, six of whom were affected with an autosomal dominant condition (MRCS) in which microcornea, progressive rod-cone dystrophy, cataract and posterior staphyloma segregate together.

The affected phenotype was consistent with nanophthalmia, plus other characteristics and suggested that the disease locus might occur on chromosome 11. Genetic testing with microsatellite markers associated with autosomal dominant nanophthalmia excluded *CMIC* and *CHX10* loci on chromosome 14q and *NNO2* on chromosome 15, but linkage results suggested a 5.0 cM genetic interval within the *NNO1* locus as the most likely site.

Six affected members and three unaffected members of one family were tested from 11 members who agreed to participate. Each had comprehensive medical and ophthalmological examinations and gave venous blood for DNA amplification and genotyping with microsatellite markers associated with nanophthalmia by a positional candidate gene approach.

Autosomal dominant nanophthalmia has been assigned a locus at *NNO1* between chromosome 11p12 and 11q13 by a previous linkage study in one family. So it seemed a useful candidate to test for mutation in a family with autosomal dominant MRCS. The researchers are seeking to confirm their results by testing more affected families and to screen for other candidate genes in this region.

▲ *British Journal of Ophthalmology* 2003;**87**:197–202.