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Whole Genome Microarray Analysis of Gene Expression in an Imprinting Center Deletion Mouse Model of Prader–Willi Syndrome

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Abstract

Prader–Willi syndrome (PWS) is caused by loss of paternally expressed genes in the 15q11-q13 region. To further characterize alterations in gene expression in this classical obesity syndrome we used whole genome microarrays to study a PWS mouse model resulting from a paternally derived imprinting center (IC) deletion (PWS IC deletion). These mice die generally within 2–3 days of life (reflective of failure to thrive in infants with PWS) and therefore, the analysis was performed on RNA extracted from the whole brain of PWS IC deletion mice and normal littermates at less than 24 hr after birth. Of more than 45,000 probes examined, 26,471 (59%) were detected for further analysis, and 69 had a significant change in expression of at least 1.5-fold and a false discovery rate (FDR) of 5%. Eight of the genes with differential expression were imprinted and from the PWS critical region (PWSCR). The three genes with the highest expression in the PWS IC mice were pro-opiomelanocortin (*Pomc*) and two transcripts of unknown function. *Pomc* knockout mice have been shown to develop obesity. Therefore, elevated *Pomc* RNA in PWS IC deletion neonatal mice may be an important genetic factor in the survival of these mice as it may affect eating behavior. Interestingly, *Mc5r*, a melanocortin receptor known to directly respond to *Pomc* expression changes, was upregulated as well. *Mc5r* is known to be involved with thermoregulation which is reportedly abnormal in PWS infants. These observations support a role for *Pomc* and the network of genes involved in regulating energy homeostasis in the early clinical findings of failure to thrive observed in PWS. Other notable patterns include three previously unstudied transcripts that are expressed only from the paternal allele under regulatory control of the IC and include *AK013560*, *BB3144814*, and *BB182944* (whose genes are located in the mouse PWSCR on chromosome 7B). As expected, all the known paternally expressed genes from the PWSCR had detection signals below the threshold in the PWS IC deletion mice but were clearly detectable in control littermates. Several of the genes in this study were further examined by quantitative reverse transcription-PCR (RT-PCR) to confirm their expression status. Further analysis of gene expression in these mice may lead to novel pathways affected in PWS. These results, along with other recent reports, suggest that the cumulative effect of modest changes in

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expression of many genes, especially genes involved in energy metabolism, contribute to the failure to thrive of infants with PWS.

Keywords

Prader–Willi syndrome (PWS); PWS IC deletion mouse; microarray gene expression; pro-opiomelano-cortin (*Pomc*); quantitative RT-PCR

INTRODUCTION

Prader–Willi syndrome (PWS) is a complex neurodevelopmental disorder characterized by a two stage clinical sequence. Infants with PWS have severe hypotonia, feeding difficulties, hypogonadism, small hands and feet, respiratory problems, and genital hypoplasia [Cassidy, 1997; Butler and Thompson, 2000]. At about 2–4 years of age, children with PWS develop an insatiable appetite resulting in the onset of severe obesity if food intake is not strictly controlled. Subjects with PWS have a reduced metabolic rate and abnormal body composition with reduced lean mass and higher fat mass, particularly subcutaneous rather than visceral fat [Talebizadeh and Butler, 2005]. In addition, PWS subjects have moderate cognitive impairment often accompanied by behavioral difficulties [Cassidy et al., 1997; Butler and Thompson, 2000; Bittel and Butler, 2005].

The chromosome 15q11-q13 region contains imprinted sequences that are differentially expressed depending on the parent of origin. PWS results from the absence of expression of imprinted genes (paternally expressed) located on chromosome 15q11-q13. Imprinted expression is coordinately controlled in *cis* by an imprinting center (IC) which regulates the establishment of parental specific allelic differences in DNA methylation, chromatin structure, and expression [Brannan and Bartolomei, 1999; Nicholls and Knepper, 2001; Bittel and Butler, 2005].

Absence of paternally expressed genes is due to a paternally derived deletion of the 15q11-q13 region in about 70% of PWS subjects, maternal disomy 15 in approximately 25%, and an imprinting defect in 2–3% [Bittel and Butler, 2005]. The 15q11-q13 region contains about 4 million base pairs of DNA and as many as 50 genes/transcripts. To date, 10 genes have been located in this region that have been shown to be paternally expressed [Bittel and Butler, 2005; Stefan et al., 2005]. In addition, at least two genes in this region are maternally expressed in some tissues (*UBE3A*, *ATP10C*) and loss of expression of these genes causes Angelman syndrome, an entirely different clinical syndrome [Meguro et al., 2001a,b]. Analysis of gene expression in the 15q11-q13 region has identified several candidate genes which may play a role in PWS, including *SNURF-SNRPN*, *NDN*, *MAGEL2*, *MKRN3*, and one or more members of the *snoRNA* genes in the 15q11-q13 region; but the molecular basis of the PWS phenotype remains poorly understood [Nicholls, 1999; Lee et al., 2000, 2005; Hanel and Wevrick, 2001; Meguro et al., 2001a,b; Gallagher et al., 2002; Bittel et al., 2003, 2005; Ren et al., 2003].

Many of the genes in the 15q11-q13 region appear to have either RNA or protein processing functions, suggesting a wider role for these sequences in controlling gene expression. For

example, the multi-cistronic *SNURF-SNRPN-snoRNA* locus codes for two nuclear localized proteins, SMN and SNURF, and multiple C/D small nucleolar RNAs (snoRNAs). SMN is a spliceosomal protein involved in RNA splicing [Gray et al., 1999a,b] and SNURF is rich in arginine residues suggesting that it may bind RNA but its exact function remains unknown [Gray et al., 1999a,b]. The function of the snoRNAs is unclear, but they likely play a role in mRNA splicing or methylation [Cavaille et al., 2000; de los Santos et al., 2000; Runte et al., 2001]. *MKRN3* encodes a putative ribonucleoprotein, *NDN*, a MAGE protein, may act as a transcriptional repressor [Matsumoto et al., 2001; Ren et al., 2003; Lee et al., 2005] and the related MAGE gene, *MAGEL2*, encodes protein that is widely expressed in the central nervous system with unknown function [Boccaccio et al., 1999; Lee et al., 2000].

Although the 15q11-q13 defect underlies the PWS phenotype, the genes within the region do not appear to be directly responsible for the complex phenotype. Rather, it seems likely that the PWS phenotype results from dysregulation of multiple interconnected neurological and metabolic pathways. We have previously used custom made microarrays to examine the expression patterns of the genes within and nearby the 15q11-q13 region [Bittel et al., 2003, 2005] in human lymphoblastoid cells. Our results indicated several genes/transcripts in the region had parent of origin allelic bias in expression.

The functional and regulatory mechanisms of this region have been conserved in mice. Postnatal survival of mice with a paternally derived deletion of the PWS critical region (PWSCR) caused by a fortuitous transgenic insert fail to survive beyond a few days, referred to here as TgPWS mice [Gabriel et al., 1999]. The TgPWS mice have been shown to have decreased levels of agouti-related protein (Agrp) and increased levels of pro-opiomelanocortin (*Pomc*) [Ge et al., 2002]. In addition, RNA from whole brain of this TgPWS mouse model analyzed by microarrays and quantitative reverse transcription-PCR (RT-PCR) revealed loss of expression of imprinted genes in the PWSCR as expected [Stefan et al., 2005]. Surprisingly, no other large changes in gene expression were identified. However, several genes located in close proximity to each other on mouse chromosome 18 had a modest increase in expression [Stefan et al., 2005]. The authors concluded that these genes were upregulated as a result of haploinsufficiency of a biallelically expressed (non-imprinted) gene(s) because upregulation occurred regardless of the parent of origin of the deletion.

Two other mouse models for PWS have been described including a model derived from uniparental maternal disomy [Cattanach et al., 1992] and an IC deletion model [Yang et al., 1998]. The maternal disomy mouse model dies within 2–7 days after birth due to failure to thrive. The mice with a targeted deletion of the IC that is paternally inherited (PWS IC deletion) also have failure to thrive and usually die within 2 days. The PWS IC deletion mice exhibit poor feeding, reduced activity, and small size. The PWS IC deletion mice were initially reported to lack expression of the imprinted genes, *Snurf-Snrpn*, *Magel2*, *Ndn*, *Mkrn3* and the paternally expressed snoRNAs [Yang et al., 1998]. However, in a later report it was noted that the PWS IC deletion mice had a low level of expression of both alleles of the imprinted genes within the PWSCR [Chamberlain et al., 2004]. Interestingly, there is strain-dependent survival of these mice which appears to be the result of strain-specific maternally inherited modifiers of survival. Herein, we describe transcriptome analysis of

RNA isolated from whole brains of PWS IC deletion mice [Yang et al., 1998] and compare the gene expression results with the reported findings seen in the TgPWS mouse model [Stefan et al., 2005].

MATERIALS AND METHODS

Production and Confirmation of Paternally Derived PWS IC Deletion Mice

PWS IC deletion mice were obtained from Dr. Camilynn Brannan at the University of Florida Medical Center [Yang et al., 1998]. The PWS IC deletion mice result from a targeted deletion of 35 kb which included exons 1 through 6 with part of exon 7 of the *Snrpn* gene and 16 kb of upstream sequence [Yang et al., 1998; Chamberlain et al., 2004]. A colony of C57BL/6J congenic PWS IC knockout mice was established and maintained by breeding female carriers of the PWS IC deletion with C57BL/6J male mice. Alternatively, paternally derived PWS IC deletion mice were produced by the mating of male PWS IC deletion carrier mice with C57BL/6J females. All mice for this study were maintained at the University of Missouri-Kansas City (UMKC) Lab Animal Center with animal usage guidelines and procedures approved by the UMKC Institutional Animal Care and Use Committee.

Genomic PCR was performed on DNA obtained from tail samples to identify mice carrying a maternal IC deletion which has no obvious phenotype. DNA extractions of tail clippings were performed as previously described [White et al., 2005]. Genotyping of the PWS IC deletion was accomplished using a forward primer (GTCACGTCCTGCACGACGCGAG) from the PGK-neo insert (used for the production of the deletion) and a reverse primer (CCGCATTTCAT-CATTCTCAGGCTC) from intron 7 of *Snrpn*. The product size visualized by 3% agarose gel electrophoresis was 423 base pairs. Wild-type alleles were identified by the amplification of a 218 base pair band using a forward primer from the *Snrpn* exon 7 (GGCATTGCTCGTGTGCCTCTTGC) and the same intron 5 reverse primer. Genomic PCR was completed using the Fail Safe PCR system (Epicentre Technologies, Madison, WI) using Fail Safe buffer D and the following thermal cycles: an initial denaturing at 94°C for 2 min and 35 cycles consisting of denaturing temperature of 94°C for 1 min, annealing temperature of 66°C for 1 min, and extension at 72°C for 1 min with a final extension of 72°C for 5 min.

Extraction of Whole Brain RNA for Microarray Analyses

Whole brains from normal and paternally derived PWS IC deletion mouse pups less than 24 hr old were collected, quick frozen in liquid nitrogen, and RNA extracted using the RNeasy Midi Kit (Qiagen, Valencia, CA) according to manufacturer's protocol. The quality of RNA was assessed by gel electrophoresis to determine the relative amounts of 28s and 18s ribosomal markers and by quantitation via spectrophotometer. All RNAs were found to have a 260/280 OD ratio of greater than 1.8. The concentration of RNA samples was adjusted to 1.0 μg/μl.

Analysis of Whole Genome Mouse Microarrays

Affymetrix GeneChip® Mouse Genome 430 2.0 Array which assays more than 45,000 transcripts was used to examine the mouse brain transcriptome. Target preparation, hybridization, and initial data collection were done at the microarray facility, University of Kansas Medical Center according to standard protocols (Affymetrix, Inc., Santa Clara, CA). GeneSpring software (Silicon Genetics, Agilent Technologies, Palo Alto, CA) was used for microarray data characterization and analysis as previously described [Bittel and Butler, 2005].

Microarray analysis was performed using RNA from the brains of male mice with an IC deletion [Yang et al., 1998] and control littermates. The PWS mice on the C57BL/6J genetic background do not survive beyond 4 days so the brains were removed before the mice were 24 hr old. The brains of unaffected littermates were isolated at the same time.

Analysis and Statistics

The inclusion criteria for retention of probes for analysis required a “present” or “marginal” signal in at least three of the four microarrays done in each group (control or PWS IC deletion mice). In addition, within the PWS IC deletion group, all values for the gene had to be in the same direction (up or down) relative to the control group. Differences between mean gene expression values were evaluated using a Welsh *t*-test with Bonferroni correction without assuming equal variances and a false discovery rate (FDR) of 20% or less. We developed an a priori list of candidate genes associated with neurodevelopment using Ingenuity pathways analysis software (Ingenuity Systems, Inc., Redwood City, CA) which was used for small group analysis.

Quantitative RT-PCR

Quantitative RT-PCR was performed on a subset of genes/transcripts using a QuantiTect SYBR Green RT-PCR kit (Qiagen) according to the manufacturer’s directions. Total RNA was isolated from homogenized whole brain using Trizol reagent (Invitrogen, Inc., Carlsbad, CA) and evaluated by spectroscopy. An equal quantity of total RNA (500 ng) from each brain, together with gene-specific primers were added to a reaction mix containing all components necessary for reverse transcription and PCR (Superscript 3 Platinum SYBR Green 1 step quantitative RT-PCR kit with ROX, Invitrogen, Inc.). The reaction was carried out in an ABI 7000 system (Applied Biosystems, Foster City, CA) beginning with a 30 min step at 50°C to allow for reverse transcription, followed by 15 min at 95°C. PCR was followed for 45 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 PCR cycle threshold (C_T) was used to compare individual reactions. At least five replicates were performed on each sample for each gene. A dissociation curve was then generated for all reactions and reactions were run on agarose gels to verify the presence of a single band. Amplification of *GAPD* served as a control gene for each sample. Normalization of expression was performed as previously described [Bittel et al., 2006] by dividing the mean *GAPD* gene expression (C_T) value from each subject by the mean *GAPD* gene expression (C_T) value of one of the comparison subjects to produce a correction value. Each mean C_T value for the other genes was divided by the correction value to produce the

normalized value. The normalized C_T values were averaged to produce the mean C_T value for each gene analyzed.

RESULTS

IC Knockout Mice Versus Control Mice

We analyzed total RNA expression isolated from four paternally derived PWS IC deletion male mice and four control male littermates. The PWS IC deletion pups do not survive more than 4 days on the mouse genetic strain used in this study. Therefore, the brains were removed prior to 24 hr to prevent changes in gene expression due to loss of viability as done in a previous analysis of a different PWS mouse model [Stefan et al., 2005]. Of more than 45,000 probes on the microarrays, 26,471 (about 59%) met the inclusion criteria for detectable expression. A total of 1,135 probes had changed expression of at least 1.5-fold, either increased or decreased, in the PWS IC deletion mice compared to the control mice. Of the 1,135 probes with at least a 1.5-fold or greater change, 424 had a statistically significant difference in expression with a FDR set at 20%. This group of genes was assigned to functional categories by Ingenuity pathways analysis and the top 10 categories with the number of genes in that group are shown in Table I. However, only 69 probes were significant with an FDR of 5% (Fig. 1 and Table II). Eight of the genes with differential expression were imprinted and from the PWSCR, including three transcripts not previously analyzed. Only 61 genes with a significant change in expression were located outside the PWSCR. Furthermore, with the exception of the dramatically reduced expression of the paternally expressed genes in the PWSCR, no genes had differential expression of more than threefold except *Pomc*. Differential gene expression identified by microarray analysis was validated by quantitative RT-PCR for several genes of interest with the greatest change in expression and because of their functional relevance to the PWS phenotype. These genes included *Snrpn*, *Ndn*, *Pomc*, *Mc5r*, and three imprinted transcripts from the PWSCR which have not been previously studied (Table III).

Genes on Chromosome 18B3

A small cluster of genes on chromosome 18B3 was reported to be upregulated by haploinsufficiency of the 7B region in the TgPWS mice [Stefan et al., 2005]. We examined all genes in this region on microarrays in our PWS IC deletion mice and could find no evidence of changed expression, even without correction for multiple sampling. However, this is perhaps an expected result since the lesion of the IC in our PWS IC deletion mice is significantly smaller than that in the TgPWS mice analyzed by Stefan et al. [2005]; therefore, providing support to their conclusion that the increased expression was the result of haploinsufficiency of a biallelically expressed gene(s). In addition, our mice were a different strain which may account for expression differences observed in our experiments.

DISCUSSION

Prader–Willi syndrome originates with the loss of expression of paternally expressed genes in the PWSCR, which directly or indirectly causes the dysregulation of genes that control neurodevelop-ment, metabolism, and behavior. While the underlying expression changes in

the PWSCR have been delineated, little is known about the global effects on gene expression that result from disruption of this region. Whole genome expression analysis together with validation by other approaches such as quantitative RT-PCR may begin to identify the downstream transcriptional effects of the PWS chromosome 15 disruption.

A previous microarray analysis of a mouse model found no significant changes in gene expression between TgPWS mice with a large paternally inherited deletion encompassing the PWSCR and normal littermates [Stefan et al., 2005]. We identified 61 genes (excluding paternally expressed genes within the PWSCR) which are significantly differentially expressed in the PWS IC deletion mice and control littermates. Our microarrays contained more transcripts than those used by Stefan et al. [2005], >45,000 compared to their ~12,000, which together with strain differences may account for differences in the two studies. It is also possible that the gene expression in PWS IC deletion mice is more dramatically altered in response to this lesion compared to the TgPWS mice used by Stefan et al. [2005].

We identified three transcripts with little or no expression in the brains of the PWS IC deletion mice which are localized to chromosome 7B, the PWSCR. These three transcripts BB314814, AK013560, and BB182944 are located at nucleotide positions 55,723,161, 56,944,766, and 57,277,109 on chromosome 7B, respectively, according to the UCSC genome browser (http://genome.ucsc.edu/). *Snrpn* is located at 55,730,763 and *Ndn* is located at 58,098,171. Although the region is conserved between human and mouse, there does not appear to be a sequence of high similarity to any of these three sequences within the human EST database. Thus, these three transcripts may represent the product of retrotransposition into the mouse PWSCR as described for *Peg12*(*Frat3*) which is conserved in both the mouse and human genomes [Chai et al., 2001].

In addition, we identified two genes with increased expression in the PWS IC deletion mice which are of particular interest, *Pomc* and *Mc5r*, both of which were validated by QRT-PCR along with *Snrpn*, *Ndn*, and the three imprinted transcripts (Table III). Products of the *Pomc* gene are known to be anorexigenic. Upregulation of this gene was reported in the brains of PWS deletion mice [Ge et al., 2002] and our data are in agreement with this observation. Ge et al. [2002] speculated that upregu-lation of *Pomc* might contribute to failure of the deletion mice to survive. Our observation that *Pomc* is upregulated in the PWS IC deletion mice further support that *Pomc* overexpression might contribute to the inability of the PWS model mice to survive. *Mc5r* is known to directly respond to upregulation of *Pomc* [Mountjoy et al., 1999]. *Mc5r* is known to be involved with thermoregulation which is reportedly abnormal in PWS infants [Williams et al., 1994; Ince et al., 2005]. *Mc5r* is required for stress-regulated synthesis of porphyrins by the Harderian gland and ACTH/MSH-regulated protein secretion by the lacri-mal gland [Chen et al., 1997]. These observations support a role for *Pomc* and its derived peptides in the early clinical findings of failure to thrive (Stage I) observed in PWS. Additional microarray gene expression studies with PWS IC deletion mice from different strains are needed to confirm these findings, as well as to identify other genes that may be impacted by this abnormality. Other strains of mice with a paternally derived PWS IC deletion are known to be viable [Chamberlain et al., 2004]. These mice should prove particularly interesting for comparison with the mice in our study to allow examination of older mice for observation of expression of *Pomc* in Stage I and possibly

during the hyperphagic Stage II and compare with *Pomc* expression in human subjects with PWS in Stage I and Stage II. These ongoing studies will identify additional genes and interacting networks that contribute to the PWS clinical presentation and may point the way toward improved therapeutic interventions.

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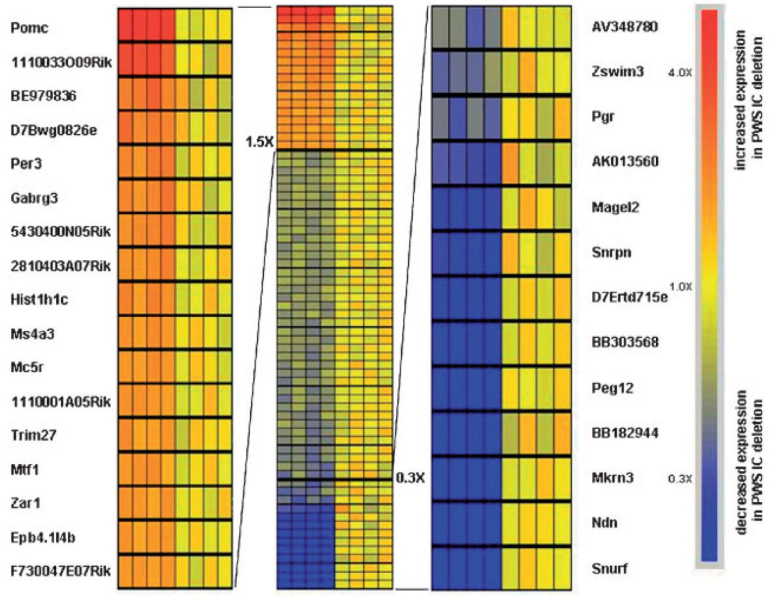

Fig. 1.

Probes with a significant difference in expression with a FDR of 5% in brain from PWS IC deletion mice compared to control littermates. There were 26,471 probes with a detectable signal and 1,135 had a difference in mean intensity of at least 1.5-fold between the two groups, of which 69 probes had a significant change of expression. The gene order is consistent with the order in Table II. The probes are arranged in order of descending gene expression ratio for PWS IC deletion mice relative to control littermates. The 17 probes with increased expression (1.5×) in PWS IC deletion mice are shown to the left. The 13 probes with lowest expression (0.3×) in the PWS IC deletion mice are shown to the right.

TABLE I

Top 10 Significant Functional Categories (Assigned by Ingenuity Pathways Analysis Software) of Probes With a Significant Difference in Expression With a False Discovery Rate of 20% in Brain From PWS Imprinting Center Deletion Mice Compared to Control Littermates

Category	Number of genes
Cancer	16
Cell death	15
Genetic disorder	12
Cellular development and organization	10
Molecular transport	10
Gene expression	9
Hemotological disease	8
Developmental disorder	6
Hepatic system development and function	3
Gastrointestinal disease	2

There were 474 genes with $P<0.05$ by Welsh *t*-test.

TABLE II

Probes With a Significant Difference in Expression With a False Discovery Rate of 5% in Brain From PWS Imprinting Center (IC) Deletion Mice Compared to Control Littermates

Gene	Ratio[a]	*P*-value	Genbank	Map	Description
Pomc	14.4	0.001	AV173741	12 A1.1	Pro-opiomelanocortin-alpha
1110033O09Rik	3.74	0.001	AW259452	7 D2	RIKEN cDNA 1110033O09 gene
Transcript	2.25	0.004	BE979836	8 A2	Transcribed sequences
D7Bwg0826e	2.14	0.004	R74675	7 B3	RIKEN cDNA 2600013E07 gene
Per3	1.96	0.005	AW553065	4 E2	Period homolog 3 (Drosophila)
Gabrg3	1.92	0.005	NM_008074	7 B4	Gamma-aminobutyric acid (GABA-A) receptor, subunit gamma 3
5430400N05Rik	1.89	0.002	AK017247	7 D1	RIKEN cDNA 5430400N05 gene
2810403A07Rik	1.78	0.002	AK020778	3 F1	RIKEN cDNA 2810403A07 gene
Hist1h1c	1.77	0.007	NM_015786	13 A3.1	Histone 1, H1c
Ms4a3	1.61	0.002	NM_133246	19 A	Membrane-spanning 4-domains, subfamily A, member 3
Mc5r	1.59	0.003	NM_013596	18 E2	Melanocortin 5 receptor
1110001A05Rik	1.57	0.001	AK009464	3 H1	ENH1 homolog [Mus musculus]
Trim27	1.55	0.004	BB401251	13 A3.1	Tripartite motif protein 27
Mtf1	1.53	0.008	AK012676	4 D2.2	Metal response element binding transcription factor 1
Zar1	1.53	0.004	BG071693	5 C3.2	Zygote arrest 1
Epb4.1l4b	1.52	0.003	NM_019427	4 B3	erythrocyte protein band 4.1-like 4b
F730047E07Rik	1.51	0.000	BG071041	4 A3	RIKEN cDNA F730047E07 gene
Kalrn	0.67	0.006	BB662566	16 B3	RIKEN cDNA E530005C20 gene
Transcript	0.66	0.007	BB272510	4 A5	BB272510 RIKEN mRNA sequence.
AI854408	0.66	0.004	AV274318	10 C2	Expressed sequence AI854408
5830457O10Rik	0.66	0.003	BC023107	15 A1	RIKEN cDNA 5830457O10 gene
Ankrd27	0.66	0.003	BB401190	7 B1	Similar to VPS9-ankyrin repeat-containing protein (LOC384745), mRNA
Transcript	0.66	0.003	BC027567	12 D1	Clone IMAGE:3472070, mRNA
Msh2	0.65	0.001	NM_008628	17 E4	mutS homolog 2 (E. coli)
Dmn	0.65	0.001	AI594683	7 C	Desmuslin
Nlk	0.65	0.005	NM_008702	11 B5	Nemo like kinase
Synj2bp	0.65	0.002	NM_025292	12 D1	Synaptojanin 2 binding protein

Gene	Ratio[a]	*P*-value	Genbank	Map	Description
Transcript	0.65	0.009	AV300514	9 A3	Transcribed sequences
Cdc37l	0.65	0.006	BE952207	19 C1	cell division cycle 37 homolog (S. cerevisiae)-like
Rab3d	0.64	0.002	BB349707	9 A3	RAB3D, member RAS oncogene family
Fbxo18	0.64	0.005	AF184275	2 A1	F-box only protein 18
Tardbp	0.64	0.003	BM935796	2 E2	TAR DNA binding protein
Transcript	0.63	0.003	BB428304	9 A2	Cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1
Qk	0.63	0.007	AW060288	17 A1	Quaking
C230094A16Rik	0.63	0.005	BC024726	11 A3.3	RIKEN cDNA C230094A16 gene
1700013G20Rik	0.62	0.008	AK005950	12 A1.1	RIKEN cDNA 1700013G20 gene
2410015B03Rik	0.62	0.004	BC005637	18 B2	RIKEN cDNA 2410015B03 gene
Ankhd1	0.62	0.008	BM243710	18 B2	RIKEN cDNA 9130019P20 gene
D330050P16Rik	0.61	0.006	BF140677	17 E2	RIKEN cDNA A230048G03 gene
1810063B05Rik	0.61	0.002	BG975168	8 E2	RIKEN cDNA 1810063B05 gene
AW210570	0.61	0.007	AK004129	2 F1	Expressed sequence AW210570
Transcript	0.60	0.005	AV308954	1 E2.1	AV308954 RIKEN mRNA sequence.
D130027M04Rik	0.60	0.003	AW412503	3 F2.1	Hypothetical protein D130027M04
Fgd4	0.60	0.008	AF402611	16 A1	FYVE, RhoGEF and PH domain containing 4
6430604M11Rik	0.60	0.008	BB188841	6 B1	RIKEN full-length clone:A330051C14
Transcript	0.58	0.005	BB745175	19 C1	RIKEN cDNA clone:A430010E21
Trip4	0.58	0.004	AV350958	9 C	Thyroid hormone receptor interactor 4
Fancg	0.57	0.007	BG072083	4 A5	Fanconi anemia, complementation group G
C030039L03Rik	0.57	0.008	BB359532	7 A3	RIKEN cDNA clone C030039L03
Cx3cr1	0.57	0.007	BC012653	9 F4	Chemokine (C-X3-C) receptor 1
Pnma2	0.55	0.005	BG072348	14 D1	Paraneoplastic antigen MA2
2610528B01Rik	0.54	0.002	AK012160	4 D3	RIKEN cDNA clone:2610528B01
Chordc1	0.52	0.002	NM_025844	9 A2	Cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1
2810021B07Rik	0.52	0.005	AK021189	13 A2	RIKEN cDNA 2810021B07 gene
C030032C09Rik	0.52	0.005	BB080832	10 C2	RIKEN cDNA C030032C09 gene
D430028G21Rik	0.48	0.006	BC025825	2 F1	RIKEN cDNA D430028G21 gene
Transcript	0.47	0.006	AV348780	2 H4	Transcribed sequences
Zswim3	0.46	0.006	AK014904	2 H3	Zinc finger, SWIM domain containing 3

Gene	Ratio[a]	*P*-value	Genbank	Map	Description
Pgr	0.41	0.003	BB428874	9 A1	Progesterone receptor
Transcript	0.26	0.002	AK013560	7 B5	Musculus adult male hippocampus, RIKEN cDNA clone:2900019J01.
Magel2	0.11	0.000	NM_013779	7 B5	Melanoma antigen, family L, 2
Snrpn	0.08	0.004	AK010671	7 B5	Small nuclear ribonucleoprotein N
D7Ertd715e	0.07	0.000	BB314814	7 B5	Adult male corpora quadrigemina, RIKEN cDNA clone:B230361E09
Transcript	0.04	0.002	BB303568	12qB3	Adult male corpora quadrigemina, RIKEN cDNA clone:B230105C16
Peg12	0.02	0.000	NM_013788	7 B5	Paternally expressed 12
Transcript	0.02	0.002	BB182944	7 B5	Transcribed sequences
Mkm3	0.01	0.000	NM_011746	7 B5	Makorin, ring finger protein, 3
Ndn	0.01	0.000	AW743020	7 B5	Necdin
Snurf	0.01	0.000	NM_033174	7 B5	Small nuclear ribonucleoprotein N

The shaded areas are sequences which map to the mouse PWS critical region on chromosome 7B.

There were 26,471 probes with a detectable signal and 1,135 had a difference in mean intensity of at least 1.5-fold between the two groups, of which 69 probes had a significant change of expression. The gene order is consistent with the order in Figure 1. The probes are arranged in order of descending gene expression ratio for PWS IC deletion mice relative to control littermates.

[a]The ratio is based on the mean gene expression value of the PWS IC deletion mice relative to the control littermates.

TABLE III

Quantitative RT-PCR Mean C_T (+/−SD) Values and Fold Change[a] of Selected Probes From the Gene Expression Microarrays Comparing Gene Expression in Brain of Normal Littermates to PWS IC Deletion Mice

Group	*Pomc*[*]	*Mc5r*[**]	AK013560	BB314814	BB182944
Control littermates	21.4 (0.5)	24.7 (0.3)	22.5 (0.9)	38.2 (0.7)	32.5 (0.7)
PWS IC deletion	18.1 (0.6), 9.9-fold change	23.6 (0.5), 2.1-fold change	nd	nd	nd

Significantly different by *t*-test.

nd, not detected.

[a]The C_T was set at the narrowest portion of the logarithmic phase of amplification in the quantitative RT-PCR reaction. Fold change = $2^{|\text{Control } C_T - \text{PWS IC } C_T|}$ (e.g., $2^{|21.4-18.1|} = 2^{3.3}$ = 9.9-fold change).

[*]P=0.002.

[**]P=0.019.