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Transcriptome interrogation of human myometrium identifies differentially expressed sense-antisense pairs of protein-coding and long non-coding RNA genes in spontaneous labor at term

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Abstract

Objective—The mechanisms responsible for normal and abnormal parturition are poorly understood. Myometrial activation leading to regular uterine contractions is a key component of labor. Dysfunctional labor (arrest of dilatation and/or descent) is a leading indication for cesarean delivery. Compelling evidence suggests that most of these disorders are functional in nature, and not the result of cephalopelvic disproportion. The methodology and the datasets afforded by the post-genomic era provide novel opportunities to understand and target gene functions in these disorders. In 2012, the ENCODE Consortium elucidated the extraordinary abundance and functional complexity of long non-coding RNA genes in the human genome. The purpose of the study was to identify differentially expressed long non-coding RNA genes in human myometrium in women in spontaneous labor at term.

Materials and Methods—Myometrium was obtained from women undergoing cesarean deliveries who were not in labor (n=19) and women in spontaneous labor at term (n=20). RNA was extracted and profiled using an Illumina® microarray platform. The analysis of the protein coding genes from this study has been previously reported. Here, we have used computational

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approaches to bound the extent of long non-coding RNA representation on this platform, and to identify co-differentially expressed and correlated pairs of long non-coding RNA genes and protein-coding genes sharing the same genomic loci.

Results—Upon considering more than 18,498 distinct lncRNA genes compiled nonredundantly from public experimental data sources, and interrogating 2,634 that matched Illumina microarray probes, we identified co-differential expression and correlation at two genomic loci that contain coding-lncRNA gene pairs: SOCS2-AK054607 and LMCD1-NR_024065 in women in spontaneous labor at term. This co-differential expression and correlation was validated by qRT-PCR, an independent experimental method. Intriguingly, one of the two lncRNA genes differentially expressed in term labor had a key genomic structure element, a splice site that lacked evolutionary conservation beyond primates.

Conclusions—We provide for the first time evidence for coordinated differential expression and correlation of cis-encoded antisense lncRNAs and protein-coding genes with known, as well as novel roles in pregnancy in the myometrium of women in spontaneous labor at term.

Keywords

parturition; preterm birth; genomics; pregnancy; lncRNA

Introduction

While the process of labor is vital to the survival of viviparous species, its physiology and pathology remain incompletely understood. Dysfunctional labor (arrest of dilatation and/or descent) is often an indication for cesarean delivery [1–7], which is being performed in approximately one of every three pregnant women in the United States [8,9]. Elucidation of the mechanism responsible for the spontaneous onset of labor is considered essential for developing strategies to prevent and treat labor disorders at term, and also for the prevention of spontaneous preterm birth, the leading cause of perinatal morbidity and mortality worldwide [10–15].

High-throughput post-genomic biology has already resulted in numerous new treatments, drug candidates, and novel actionable therapeutics for a wide range of human diseases, [16,17] although techniques such as genomics, transcriptomics, and proteomics have been used to gain insight into the molecular basis of parturition in myometrium, its impact remains minimal [18–30]. To harness the promise of this new knowledge and develop diagnostics and transform rational therapeutics for the great obstetrical syndromes, we have undertaken an unbiased whole transcriptome approach to the expression of known and novel gene classes in the myometrium. Long non-coding RNA (lncRNA) has been reviewed in the past decade, upon completion of the Human Genome Project, to be the most frequent, prevalent, and abundantly expressed novel class of human genes [31–45].

This study has focused on the myometrium because uterine contractility is a key feature of spontaneous labor at term. We have already established a beach-head in this field by studying the protein encoding genes and the regulatory networks therein within the framework of the myometrium at term [26]. Moreover, we have characterized these changes

in the most important and common labor disorders, arrest of descent [46] and arrest of dilatation [47].

Materials and Methods

Study Design

This is a cross-sectional study designed to compare the expression of long non-coding RNAs in the myometrium of women not in labor at term vs. those in labor at term. We have previously reported the original analysis of the protein-coding genes using microarrays [26].

Briefly, myometrium was obtained from women undergoing cesarean section at term (≥37 weeks) in the following groups: 1) women not in labor (n=20); and 2) those in spontaneous labor (n=19). Labor was diagnosed in the presence of spontaneous regular uterine contractions occurring at a minimum frequency of 2 every 10 minutes with cervical change that required hospital admission. Women in the not in labor group underwent cesarean section due to fetus in non-cephalic presentation, previous uterine surgery, non-reassuring fetal status, classical cesarean section, or an elective cesarean section with no more than one previous cesarean section. Only women who delivered an appropriate for gestational age neonates (AGA) were included. Patients with clinical or histological chorioamnionitis, those undergoing induction of labor, and those who underwent cesarean section for arrest of dilatation or arrest of descent were excluded. Histologic chorioamnionitis was diagnosed using previously described criteria [48,49]. Clinical chorioamnionitis was diagnosed using the criteria proposed by Gibbs et al [50]. An AGA neonate was defined as a birthweight between the 10th and 90th percentile for the gestational age at birth [51].

Eligible patients were enrolled at Hutzel Women's Hospital (Detroit, MI, USA). All women provided written informed consent prior to the collection of myometrial samples. The collection and utilization of the samples for research purposes was approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS, Bethesda, Maryland), and the Human Investigation Committee of Wayne State University (Detroit, MI, USA).

Sample collection—Myometrial tissue samples were collected during cesarean section following delivery of the placenta from the midpoint of the superior aspect of the uterine incision using Metzenbaum scissors and measured approximately 1.0cm^3. Tissues were snap-frozen in liquid nitrogen, and were kept at −80°C until use.

RNA isolation and microarray analysis of RNA expression—The methods for RNA isolation and microarray data generation have been reported previously [26]. Briefly, we used the Illumina® HumanHT-12 v3 expression microarray platform (Illumina®, San Diego, CA, USA) to assess the expression levels in each individual specimen following the manufacturer's instructions.

Data analysis for long non-coding RNA—The goal of the analysis was to use a commercially-available microarray platform to interrogate the expression of putative long non-coding RNA genes that we had determined to be represented by this commercial

platform (Illumina®). All microarray probes were mapped to the collection of lncRNAs that we describe in the next paragraph, and then pairs of lncRNAs and neighbor or overlapping protein-coding genes were identified such that a coding gene and an lncRNA gene were co-differentially expressed in the same locus (definition: the distance between the nearest pair of lncRNA gene and protein-coding gene boundaries had to be 10,000 bases or less) and correlated based on the microarray expression.

Description of the lncRNA dataset—To construct a non-redundant (a single reference transcript per gene) set, we considered at least 1 base pair overlap in the entire genomic span (including exons and introns along the hg19 human genome reference assembly coordinates) among all transcripts located on the same strand in the same locus. We assembled 18,498 experimentally supported [with full-length cDNA or manually-curated high-quality expressed sequence tag (EST) evidence], non-redundant (with respect to genomic position and orientation) lncRNA genes from multiple public sources: Gencode v3 [52] and v7 lncRNAs [38]; the human lncRNA catalog from our previous work [33]; NCBI Refseq non-coding transcripts; all human ESTs submitted to Genbank by RIKEN, Japan; manually annotated lncRNAs from human sense-antisense pairs [53,54]; and Broad Institute lincRNAs [35]. We used genomic positional overlap of UCSC all_mrna, all_est, and ref_all files from http://genome.ucsc.edu [55] as well as Gencode transcripts from www.gencodegenes.org [52,56] to define transcriptional unit boundaries according to the FANTOM3 definition of a transcriptional unit [57] along the hg19 assembly, in the UCSC Genome Brower (http://genome.ucsc.edu) [58], collapsing the cDNA/EST/Gencode/Broad transcript-to-genome alignments into genomic positionally nonredundant transcriptional units with one randomly selected reference transcript per transcriptional unit.

Mapping microarray probes to lncRNAs

The data measured by microarray probes that were not assigned by the manufacturer to genes having a valid Genbank identifier had been discarded in the original analysis. Here, we considered all probes on the microarray Human HT-12 V3 array. The 50 base pair sequence of the probes was used to match these against the human genome (hg19) using BLAT software [59]. The probes that had a perfect match (100%) were retained. The microarray probes alignments were then matched against the genomic coordinates of all human cDNAs from the “Genbank mRNA” track of the UCSC Human Genome Database [58], and hence, having a match between the Illumina® probes and the cDNAs from the UCSC database. The list of cDNAs from the UCSC database was intersected by complete name string matching with the list of cDNAs corresponding to lncRNAs described above.

Assessing differential expression of coding and non-coding RNAs

We used Illumina® BeadArray Reader to image the microarrays, and the BeadStudio Software V.3.4.0 to extract raw expression data from the array images. The raw probe intensity data were log (base 2) transformed and then quantile normalized [60] to make expression levels comparable among arrays. Probes with intensity above the background (p-value <0.1) in more than 5 of the 39 samples were retained for further analysis. A moderated *t*-test was used at probe level to test for differential expression between groups, and the resulting p-values were corrected using the false discovery rate (FDR) method [61] to obtain

q-values. Probes with a q-value<0.1 and fold change>1.25 were defined as differentially expressed.

Determining the significance of pairwise correlations between coding and non-coding genes

The correlation between lncRNA expression level on a $\log_2$ scale (denoted with Y) and the pair coding gene (denoted with X) was tested by fitting a linear model in which the response was Y, while the independent variables were X and the group variable and their interaction term ($Y=a+b\cdot X+c\cdot Group+ d\cdot X\cdot Group+ \eta$). Then, a second model was fit including only the group variable ($Y=a+ c\cdot Group + \eta$). By comparing the quality of the data fit between these two models using an F-test, we assessed whether the expression of the lncRNA (Y) was significantly correlated with the expression level of the coding gene (X) within a same-locus gene pair while allowing that the slopes (Y vs. X) be different between the two groups (not in labor vs. in labor). The correlation p-values obtained in this way were further adjusted for multiple tests, and significance was inferred using q-value <0.1. We defined "adjacent to or overlapping" as an lncRNA having a genomic distance of 10,000 base pairs or less from the nearest boundary of a protein-coding gene; antisense overlaps were considered as zero distance [62]. After identifying differentially-expressed lncRNAs that were significantly correlated with protein-coding genes adjacent to or overlapping the lncRNA genes on opposite strands, validation was undertaken using quantitative real-time reverse transcription PCR (qRT-PCR) (see below).

Validation of microarray results using qRT-PCR experiments

A subset of specimen for each group (spontaneous term labor: n=16; term not in labor: n=18) were obtained for qRT-PCR assay of a selected group of genes found to be differentially expressed by microarray analysis. Taqman®(Life Technologies, Inc., Foster City, CA) qRT-PCR analysis was performed as described elsewhere [62]. Taqman primers/probesets were: Hs00205871_m1 (LMCD1), Hs00705981_s1 (NR_024065), Hs04274293_g1 (SOCS2), Hs00919620_m1(AK054607). The Ct values were averaged over the 3 replicates per primer and sample, and $\Delta Ct=Ct_{target}-Ct_{reference}$ were computed for each sample. $-\Delta Ct$ values, surrogate for mRNA abundance in a given sample were compared between groups using a t-test. The correlation between the lncRNA and the coding gene within each gene pair was tested using the same model as described for the microarray data.

Results

The clinical and demographic characteristics of the population included in this study are displayed in Table 1.

We matched 2,995 Illumina® probes to 2,634 lncRNAs from our lncRNA dataset of 18,498 lncRNAs which are described in Materials and Methods. Of the 2,995 probes, 1,868 (62%) were expressed above background in the myometrium samples and were assigned to 1,692 unique lncRNAs. Of the 1,692 lncRNAs, 13 were differentially expressed between the myometrium of women not in labor and those in labor (fold change >1.25, q-value <0.1) (see Table S1). Of these 13 lncRNAs, seven were computationally identified as residing in

putative sense-antisense and neighbor-gene pairs, where they were co-differentially expressed (fold change >1.25, q-value <0.1) and correlated (q-value<0.1) with the corresponding coding genes (see Table S2). Those seven loci collapsed into only four that were genomically unique, due to probe redundancy. Our UCSC Genome Browser-based manual annotation of these four loci identified two *bona fide* lncRNA pairs, which we then attempted to validate by qRT-PCR. The microarray gene expression data for the two manually curated *bona fide* lncRNA-coding gene pairs were (AK054607 - SOCS2) and (NR_024065- LMCD1) are displayed in Figure 1. For the AK054607 - SOCS2 pair, we showed that both the lncRNA and the protein-coding gene were significantly down-regulated in term labor vs. non-labor myometrium (see Table 2). For the NR_024065-LMCD1 pair, we demonstrated that both the lncRNA and the protein-coding gene were significantly up-regulated in the myometrium of women in term labor compared to that of women not in labor at term (see Table 2).

These pairs were tested by qRT-PCR in a subset of 34 of the 39 (see Table 1) samples used in the microarray analysis and their co-differential expression and correlation were confirmed (see Figure 2 and Table 2).

AK054607 – SOCS2 is an mRNA-lncRNA sense-antisense pair such that the two genes are divergently transcribed and the transcription start site of each gene is embedded in the first interim of the other gene, leading to an intronic divergent sense-antisense pair. This locus is characterized by substantial transcriptional complexity: both the sense protein-coding gene SOCS2 and the lncRNA gene AK054607 have multiple alternatively spliced isoforms evident in public cDNA data (see Figure 3a). The Illumina® probe corresponding to the lncRNA is in the constitutively expressed last exon of the lncRNA gene; therefore, it is genomically distant from the region of sense-antisense overlap and clearly allows specific profiling of this lncRNA gene. LMCD1- NR_024065- is a same-strand, neighbor gene mRNA-lncRNA sense-antisense pair. The single-exon lncRNA is genomically located downstream of the 3′ end of the mRNA. Multiple independent sources of experimental evidence (cDNA and EST datasets in the UCSC Genome Browser, as well as Broad Institute lincRNAs) indicate that the lncRNA is distinct from its upstream protein-coding neighbor, since there are no overlapping or bridging transcripts between the two.

Conservation assessment of validated antisense lncRNA transcription in spontaneous labor at term

Because lncRNAs do not have protein-coding sequences, evolutionary conversation of their gene structure can be analyzed using key features of the gene structure itself: promoters, splice sites, and polyadenylation signals, as reviewed by Lipovich L et al [34] and Johnsson et al [63]. Therefore, we compared the genomic sequence of this lncRNA's splice sites between humans and the 45 non-human vertebrates in the UCSC genome browser public multispecies sequence alignment [58]. We found that a canonical splice acceptor (-AG) of an AK054607 intron resides within a non conserved, primate-specific retroviral insertion in the human genome (Figure 3b). The insertion was a long terminal repeat inside an ERV1 element, which the multispecies alignment suggests originated in Old World monkeys after the prosimian split. NR_024065, on the contrary, is an unspliced gene that has its canonical

hexamer polyadenylation signals conserved all the way through marsupials and monotremes, attesting to an origin of the lncRNA before the emergence of placental mammals.

Discussion

Principal Findings of the Study

Here, for the first time, we have intersected a meta-collection of experimentally-supported lncRNA genes with a clinical microarray dataset from myometrium of women not in labor and those in labor. We have demonstrated co-differential expression at two genomic loci that contain coding-noncoding gene pairs. At one of these loci, the sense gene (SOCS2) has been implicated in reproductive disorders (recurrent spontaneous pregnancy loss [64] and fetal growth restriction [65]; whereas in the other locus, the protein-coding gene (LMCD1), although well-characterized as a heart muscle regulator [66,67], has no known association with human reproduction. Both genes were previously not understood to be relevant to or regulated by the lncRNA genes that reside at the same loci, and that previously lacked annotation. The key finding of this study is that two lncRNA genes, which are genomically antisense to those two known protein-coding genes, are co-differentially expressed with the protein coding genes in human spontaneous labor at term.

What are long non-coding RNAs?

The final draft of the human genome was released in 2003 [68]. In subsequent years, experimental analyses of mammalian transcriptome using high-throughput cDNA construction and next-generation sequencing by the ENCODE [69] and FANTOM Consortia [57] have led to a key unexpected finding of post-genomic biology: that approximately 70% of the human genome is transcribed [70] but only 2% corresponds to messenger RNA of protein-coding genes [42]. The term “junk DNA” had been coined to refer to the major part of the genome, the part that resides outside of the protein-coding genes [71,72]. This derogatory term – “junk DNA” has now been repudiated by substantial experimental evidence from multiple high-throughput projects (transcriptome sequencing), indicating that the majority of this fraction of the genome is transcribed into diverse and functional classes of non-coding RNA [36,73–77]. Although these classes include previously well-characterized classical housekeeping RNAs, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs) and piRNAs, which are constitutively expressed and play critical roles in protein biosynthesis [43] the majority of this transcriptional output consists of a new class of RNA, long non-coding RNA. In fact, the study of long non-coding RNAs is not new; rather, it is the ubiquity of lncRNA expression that has only recently been grasped [38]. The estimated number of long non-coding RNA genes in the human genome is more than 20,000 and has now surpassed the number of protein-coding genes [56]. Although only a fraction of these long non-coding RNAs has been mechanistically and functionally characterized in human cells and tissues, it is already evident that long non-coding RNAs have important, often essential, roles in normal cellular function (including but not limited to: proliferation, differentiation, pluripotency [78], apoptosis, and energy metabolism) [31,32,34,37,43,45,74,79–85], human development, and disease (i.e. epilepsy [62], cancer [42,63,86], Alzheimer’s disease [87], diabetes and obesity [88–90].

Why study long non-coding RNAs in parturition?

Despite the central importance of long non-coding RNA in cellular economy [38], there is a paucity of information about long non-coding RNA function in normal labor at term. We undertook this study to examine the extent to which known and novel long non-coding RNA may or may not be differentially expressed in this essential mammalian biological process. This is important, not only to gain an understanding of physiologic parturition, but also to leverage upon our new post-genomic understanding of long non-coding RNAs as an unprecedented window into preterm labor and delivery.

Strong evidence that two long non-coding RNA genes with putative cis-regulatory functions are differentially expressed in spontaneous labor at term

In this study, we have analyzed approximately 18,498 human long non-coding RNA genes using a computational approach that intersects their genomic coordinates with Illumina® microarray data. Of these, 2,634 were represented by probes on the commercial catalog Illumina® platform that we used. Upon computational enrichment for putative cis-regulatory long non-coding RNAs, meaning those at or near protein coding genes with potential to regulate those genes, we converged upon two bona fide mRNA-lncRNA gene pairs, one sense-antisense, and one neighbor-gene.

One of these pairs is comprised of an antisense lncRNA, AK054607, which overlaps the protein-coding gene SOCS2. There is compelling evidence that physiologic parturition is associated with cellular and molecular signatures of inflammation in the chorioamniotic membranes [28,91–96], myometrium [18,20,22,23,25–27,30,93,96–102], and cervix [19,21,24,93,96,103–107]. Indeed, the participation of chemokines and pro-inflammatory cytokines [108–126], eicosanoids [99,127–137] and lipooxygenase arachidonate products [138–140] in physiologic labor is well established. Blumensteinet al. reported the behavior of the suppression of cytokine signaling of molecules in spontaneous labor at term and found differential expression for SOCS1 and SOCS3 [141]. SOCS2 protein levels increased following labor [141]. Here we found that both SOCS2 and its antisense lncRNA encoded in the same locus were down regulated in the myometrium of women experiencing spontaneous labor at term vs. those not in labor. Our previous analyses of human sense-antisense pairs have found that in most pairs, the two transcripts expression levels change in the same direction in a given biological process (synergistic pairs), while in a minority of pairs, the sense and antisense levels changed in the opposite direction (reciprocal pairs) [142]. Here, both of the pairs that we found were in fact, synergistic, concordant with our earlier findings of Katayama et al. [142]. This represents the first evidence that a lncRNA in the same locus as SOCS2 is a putative novel regulator of SOCS2, because there is a vast amount of literature showing that antisense lncRNAs are not merely coexpressed with their sense partners, but directly regulate them, as recently reviewed [34,43,54,62,143].

The other lncRNA-mRNA pair identified and validated in our study is LMCD1-NR_024065. LMCD1 is well known for its regulatory function in the heart [67] and in smooth muscle [66], although it has never been previously connected to myometrium or the process of labor. For the first time herein, we establish a direct and significant connection of LMCD1 expression with spontaneous parturition at term. Moreover, we established that

NR_024065, a neighboring lncRNA in the LMCD1 locus, is significantly and synergistically up-regulated in concert with LMCD1 in term labor, strongly suggesting a regulatory function for this lncRNA. Unlike the SOCS2 locus where the protein coding gene and the lncRNA share a substantial sense-antisense overlap, LMCD1 and NR_024065 are neighbor genes, which share close genomic positional proximity, although their boundaries do not overlap. Emerging mechanisms of lncRNA function include epigenetic cis-regulation and co-regulation of protein-coding genes by genomic-neighbor lncRNAs [144,145]. In this study, we demonstrated that an lncRNA-mRNA sense-strand neighbor-gene pair is functionally relevant spontaneous parturition at term.

A window into the evolutionary complexity of the lncRNA transcriptome in human parturition

Unlike protein coding genes, which are highly conserved in evolution, a key and bewildering property of the lncRNAome is its lack of evolutionary conservation: one-third of human lncRNA are not conserved beyond primates [38], and both sequences and structures of other lncRNA are poorly conserved across metazoa [63]. Accordingly, we examined the extent of evolutionary conservation in the two lncRNA that we validated in this study, and we found that the genomic structure of the AK054607 lncRNA gene relies upon a primate-specific splice site, consistent with the reported human and primate exclusivity of thousands of lncRNAs. This has important implications for rodent models of labor, highlighting that key cis-regulatory lncRNAs that impact protein-coding functional genes may be altered or absent in those models.

The future of lncRNAs in reproductive biology

Little remains known about the role of lncRNAs in pregnancy. This year, an analysis of several pedigrees in a Dutch study converged on an autosomal recessive gene (HELLP), a lncRNA which is functional in trophoblasts and causes HELLP syndrome [146]. This lncRNA is expressed in extravillous trophoblast obtained from first trimester placentas, and its knockdown activated a large set of genes involved in the cell cycle. By blocking mutation sites, transcription of the lncRNA itself was upregulated and led to a reduction in trophoblast cell invasion [147]. While the disease causing mutation is detected in the fetus, the disease phenotype is diagnosed in the mother.

In contrast to the limited power of Mendelian pedigree approaches, our work has established a precedent for transcriptomic analysis of groupwise and case-control study designs to discover additional lncRNAs functional in the disorders of pregnancy. Future approaches in this field should assess lncRNA transcriptomes in additional diseases of pregnancy, as well as utilize the broad power and scope of published GWAS analysis of reproductive diseases to emerge with causative SNP variants in additional lncRNAs.

Conclusions

We have harnessed and leveraged upon the power of postgenomic transcriptome biology to set forth a precedent of clinically relevant discoveries of lncRNAs functionally relevant to parturition. We have canvassed computational and experimental data integration, as well as

multiple independent validation approaches, to establish a new methodology that is capable of transforming our understanding of human reproduction through lncRNAomics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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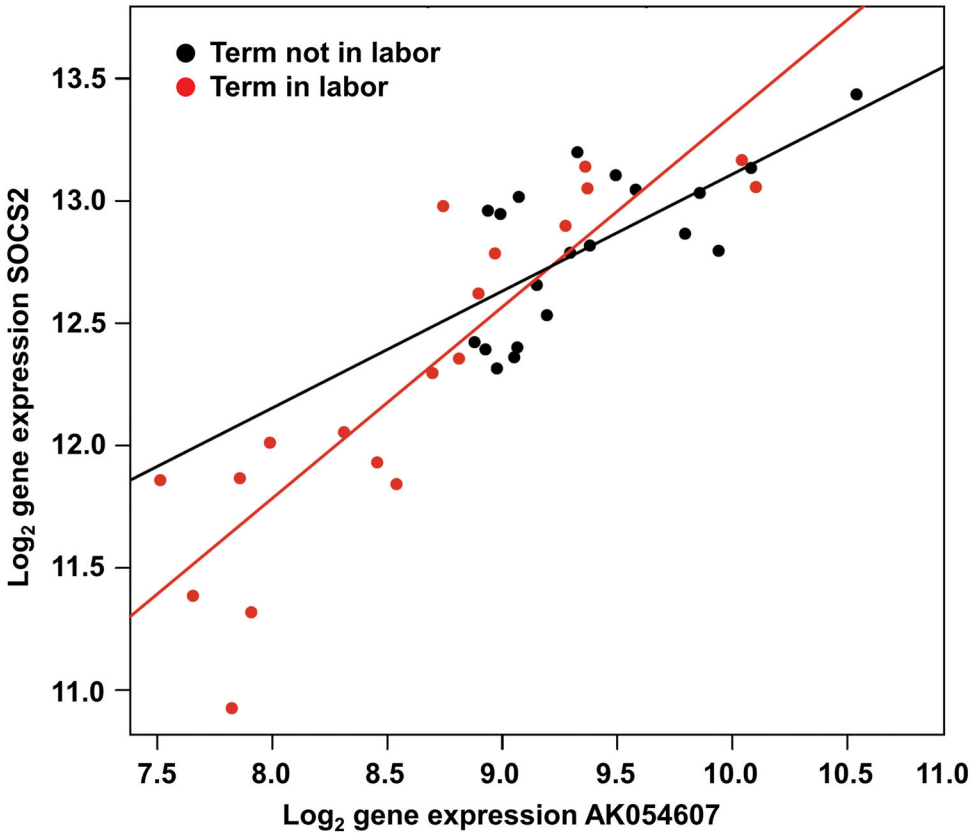


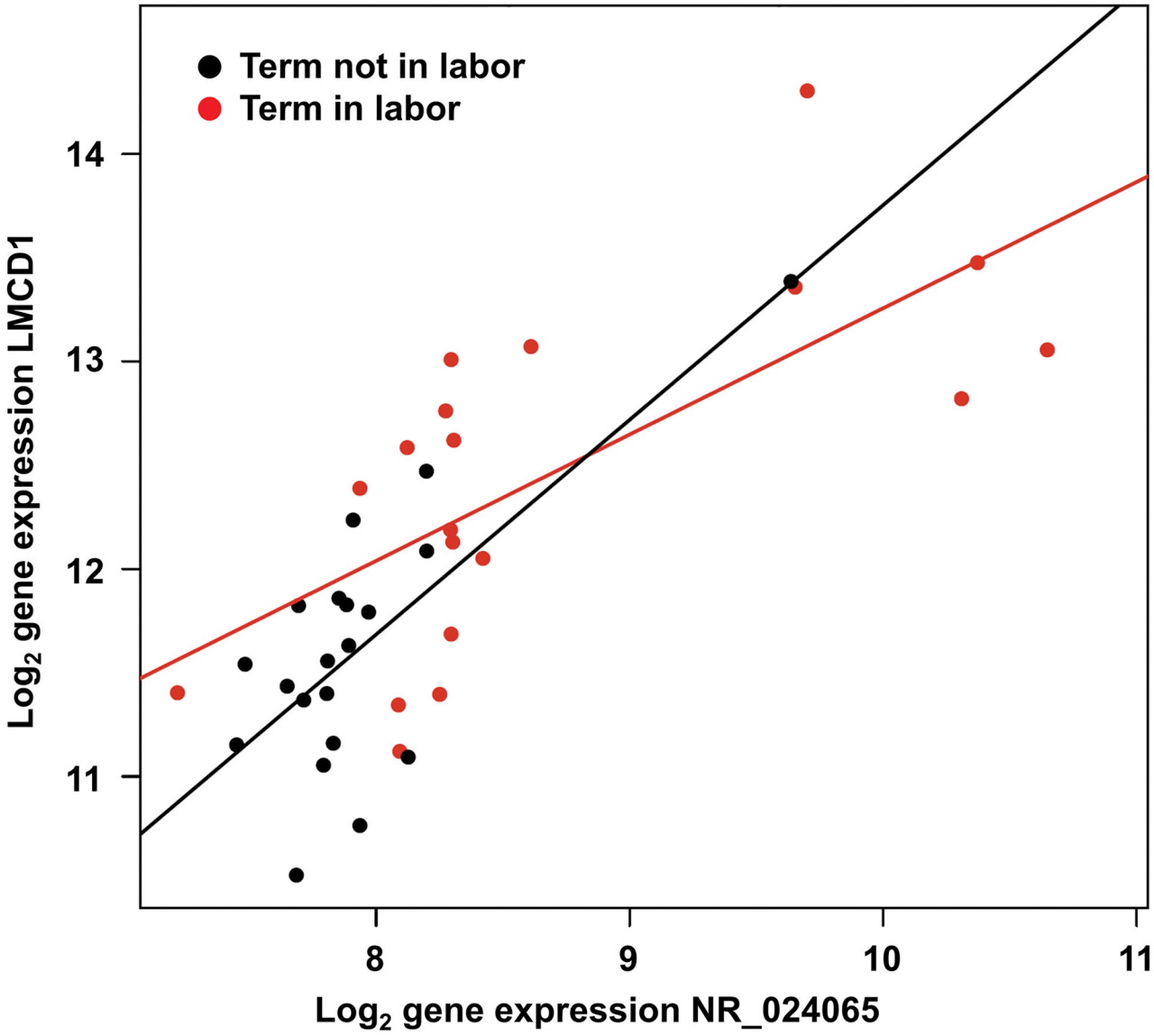
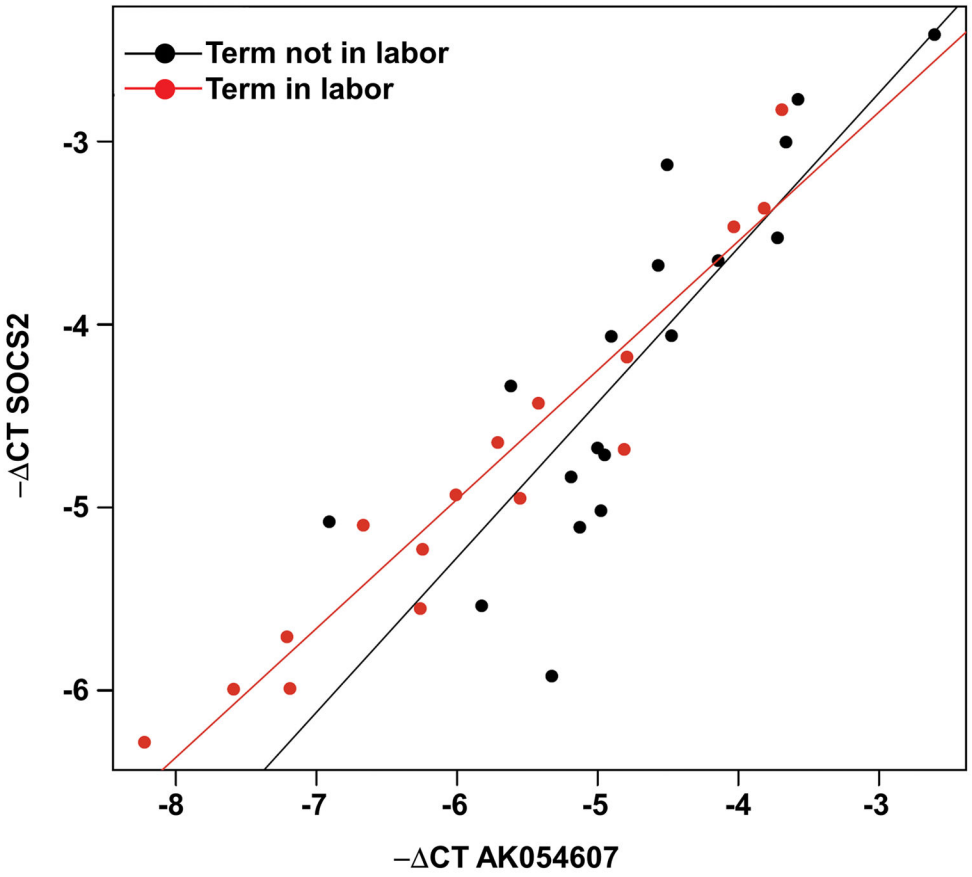


Figure 1.

Correlation and co-differential expression of mRNA-lncRNA sense-antisense pairs based on microarray data. (A) Log (base 2) microarray expression of SOCS2 gene (probed by Illumina® probe ILMN_2131861) are plotted against the expression of lncRNA AK054607 (probed by Illumina® probe ILMN_1699188). Each sample is represented by one filled circle (black: term not in labor; red: term in labor), while the solid lines give the linear fit to the data in each group separately. (B) Log (base 2) microarray expression of LMCD1 gene (probed by Illumina® probe ILMN_1754969) are plotted against the expression of lncRNA NR_024065 (probed by Illumina® probe ILMN_2052135). Each sample is represented by one filled circle (black: term not in labor; red: term in labor), while the solid lines give the linear fit to the data in each group separately.



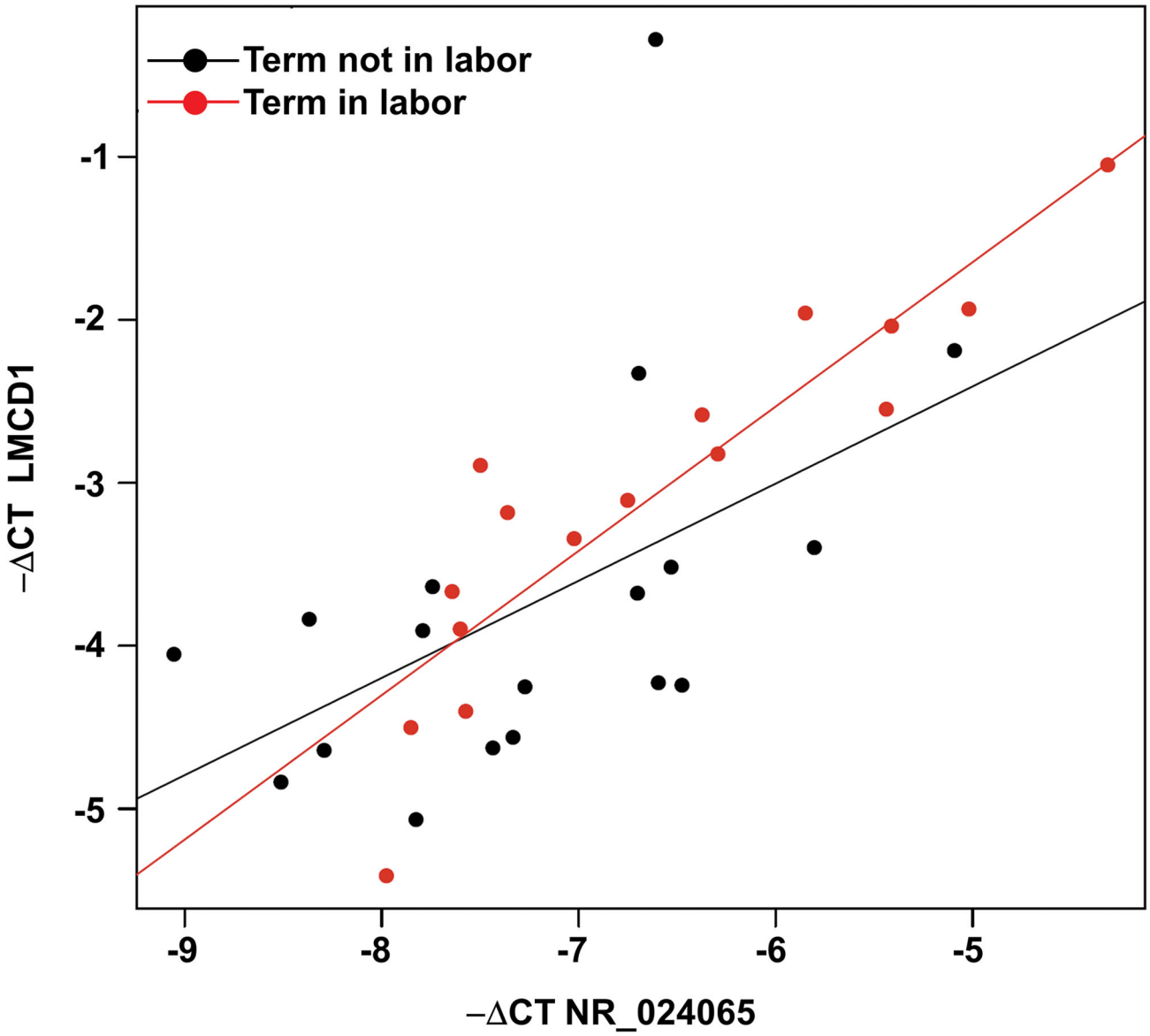
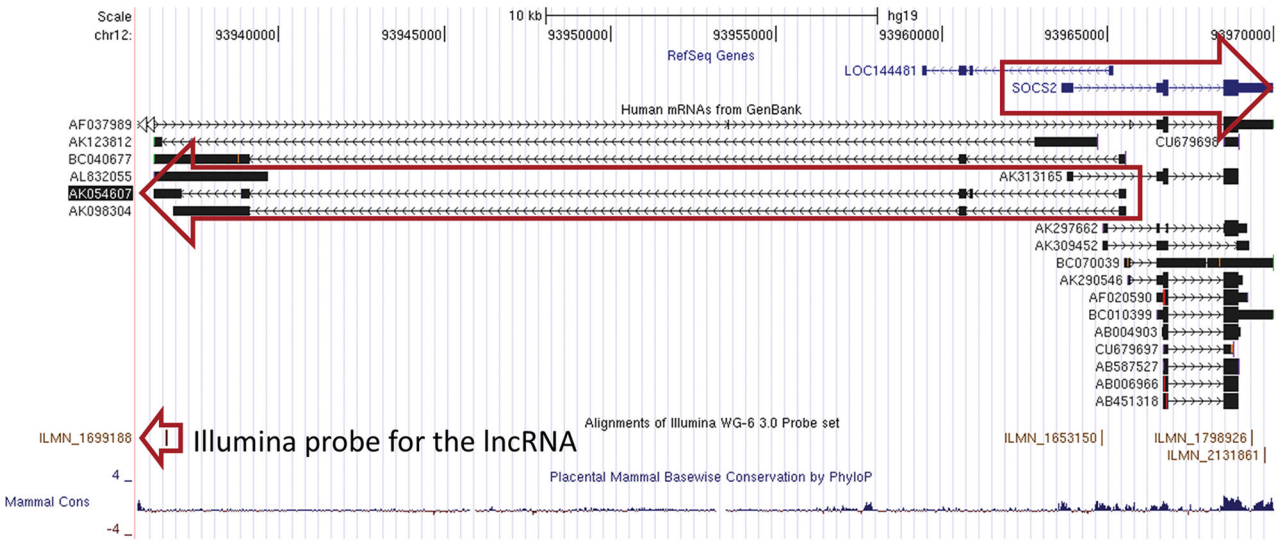
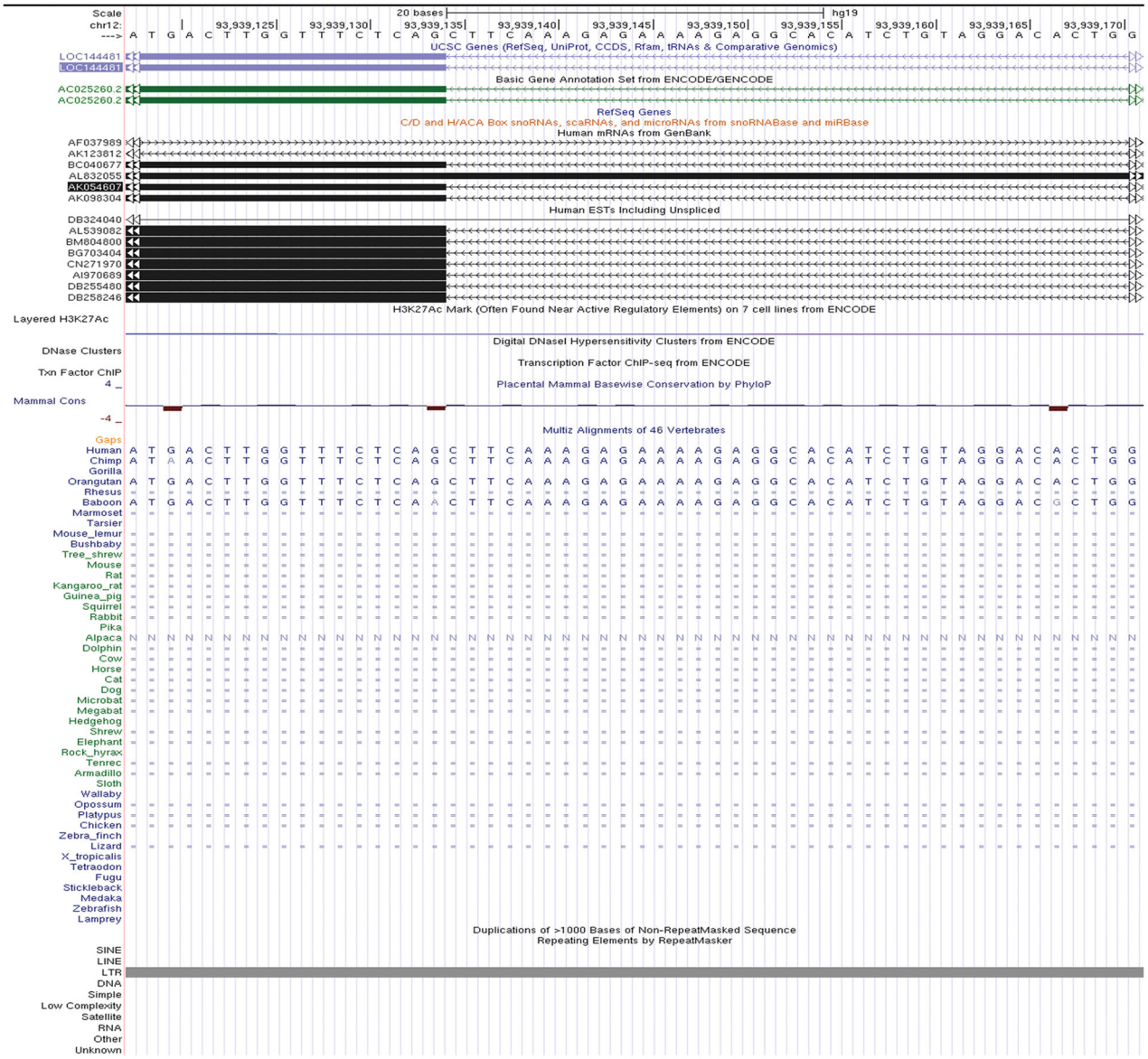


Figure 2.

Correlation and co-differential expression of mRNA-lncRNA sense-antisense pairs based on qRT-PCR data. (A) The −ΔCT values, surrogate for log (base 2) expression of SOCS2 gene (probed by Taqman primer Hs0091962_m1) are plotted against the −ΔCT values of lncRNA AK054607 (probed by Taqman primer Hs04274293_g1). (B) −ΔCT values of LMCD1 gene (probed by Taqman primer Hs00205871) are plotted against the −ΔCT values of lncRNA NR_024065 (probed by Taqman primer Hs00705981_s1). Each sample is represented by one filled circle (black: term not in labor; red: term in labor), while the solid lines give the linear fit to the data in each group separately.





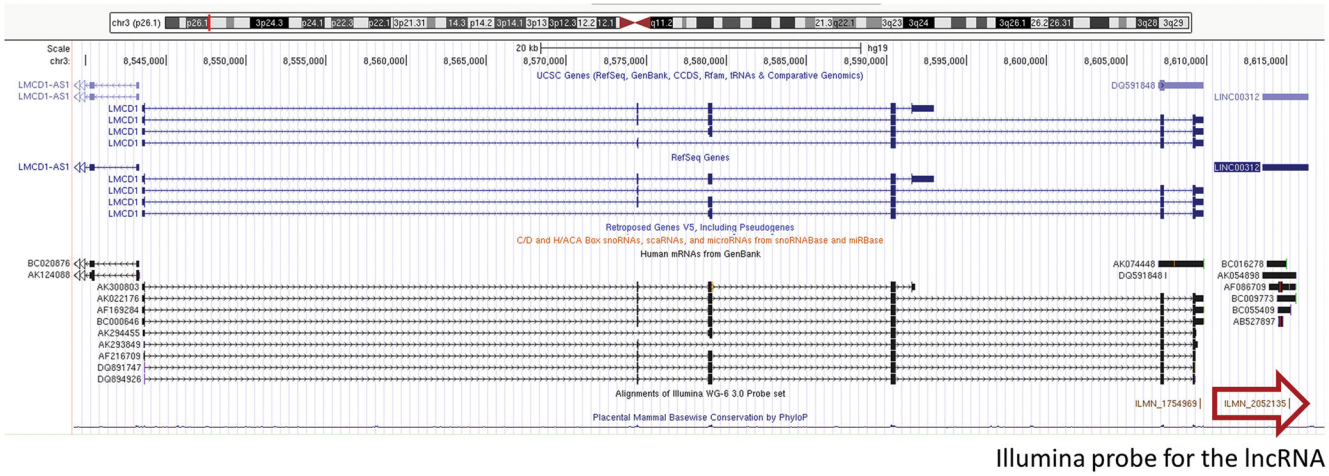


Figure 3.

Genomic structure and evolutionary conservation of mRNA-lncRNA sense-antisense pairs. (A) Sense-antisense transcriptional units and cognate Illumina® probe positions at the SOCS2-AK054607 locus. Arrows indicate: the differentially-expressed lncRNA probe (bottom), and genomic boundaries of the antisense and sense transcriptional units (middle and top, respectively). (B) Multi-species sequence alignment of a splice donor in an intron of AK054607. The spliced side is on the antisense strand, corresponding to sense strand nucleotides CT. Obtained from the UCSC Genome Browser. (C) The LMCD1 transcriptional unit and its downstream sense-strand neighbor, lncRNA NR_024065 (synonym: LINC00312). Arrow indicates the differentially-expressed Illumina® probe corresponding to this lncRNA.

Table 1

Demographic and clinical characteristics of the study groups

	Term not in labor Microarray (n=20)	Term labor Microarray (n=19)	Term not in labor qRT-PCR (n=18)	Term labor qRT-PCR (n=16)	P-value
Maternal age (years)	33 (21–39)	27 (19–39)	33 (21–39)	28 (19–40)	NS
African-American ethnicity	75 (15/20)	74 (14/19)	72 (13/18)	81 (13/16)	NS
BMI (kg/m2)	28 (18.2–47.2)	28.7 (22.1–54.6)	28.6 (18.2–47.3)	31.6 (24.7–54.6)	NS
Parity	1 (0–4)	0 (0–5)	1 (0–5)	1 (0–5)	NS
Gestational age at delivery (weeks)	38.7 (37–41.9)	39.3 (37–41.3)	39.1 (36.6–41.9)	38.3 (36.1–41.9)	NS
Birthweight (g)	3070 (2545–3805)	3150 (2570–3740)	3065 (2425–4120)	3165 (2375–3790)	NS

Values are expressed as percentage (number/total) or median (range) BMI: body mass index; NS: not significant at α=0.05.

Table 2

Co-differential expression and correlation of lncRNA and coding gene pair based on microarray and qRT-PCR data

	lncRNA: Differential Expression				Coding gene: Differential Expression				Correlation lncRNA - coding gene	
Analysis	**Symbol**	**FC**	**q-value**	**Direction**	**Symbol**	**FC**	**q-value**	**Direction**	**ρ**	**q-value**
Microarray	AK054607	1.7	0.017	Down in TL	SOCS2	1.4	0.039	Down in TL	0.86	<0.0001
qRT-PCR	AK054607	2.1	0.005	Down in TL	SOCS2	1.6	0.036	Down in TL	0.90	<0.0001
Microarray	NR_024065	1.7	0.069	Up in TL	LMCD1	1.8	0.003	Up in TL	0.76	<0.0001
qRT-PCR	NR_024065	1.5	0.055	Up in TL	LMCD1	1.6	0.052	Up in TL	0.73	<0.0001

FC: Fold Change; ρ: correlation coefficient; TL: term in labor.