Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2009

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl A. Richards, MBA

4. **Grant Contact Person's Telephone Number:** 412-641-8932

5. **Grant ME Number or SAP Number:** 4100047639

6. **Project Number and Title of Research Project:** Project 4 - Integration Study of the Target Genes of PPAR gamma in Human and Mouse Placenta

7. **Start and End Date of Research Project:** 01/01/2009 – 12/31/2009

8. **Name of Principal Investigator for the Research Project:** Tianjiao Chu, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 79,268.35

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Chu	Principal Investigator	40%	$43,558

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

As a continuation of the study of the PPAR gamma target genes in mouse placenta in this project, I plan to work with Dr Barak to analyze the qPCR experiment data of the genes identified in this project.

As a continuation of the study of the PPAR gamma target genes in human trophoblast in this project, I plan to work with Dr Sadovsky to study the relation among PPAR gamma activity, trophoblast differentiation, hypoxic placental injury, and fetal growth restriction through the analysis of microarray and qPCR data of human trophoblast cells and human placenta.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				

Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No___X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    With further refinement of the user interface, the data analysis procedures developed in this project will be available to other researchers at Magee Womens Research Institute to facilitate research involving similar data analysis tasks.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes_____ No____X_____

    If yes, please describe the collaborations:

    16(B) Did the research project result in commercial development of any research products?

    Yes_____ No____X_____

    If yes, please describe commercial development activities that resulted from the research project:

    16(C) Did the research lead to new involvement with the community?

    Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Peroxisome Proliferator Activated Receptor gamma (PPAR-gamma) is a transcription factor that plays important roles in many essential processes such as differentiation, metabolism, and inflammation. From the developmental biological point of view, PPAR-gamma is essential to placental development and function. The main objective of this project is to search for potential target genes of PPAR gamma in mouse and human placenta by studying how the change of the expression level or activity of PPAR gamma in human and mouse placenta could affect the expression level of other genes.

In this project, I analyzed the microarray gene expression data of mouse placenta and mouse and human trophoblast cells where the PPAR gamma activity was either reduced by knocking out the PPAR gamma gene or adding PPAR gamma inhibitor, or elevated by the induction of PPAR agonists. The data sets were generated by two researchers Drs. Sadovsky and Barak from this institute. I generated the lists of potential direct and indirect target genes for PPAR gamma in mouse placenta and human trophoblast. With the help of gene ontology analysis, the main functions and processes involving these potential target genes were revealed.

This project has three specific aims:

Aim 1: Identify mouse genes that are potential targets of PPAR gamma in placenta.

To identify the potential target genes of PPAR gamma in mouse placenta, I first compared the gene expression profile of PPAR gamma null mouse placenta with wild type mouse placenta and identify the genes down regulated in the PPAR gamma null placentas. The PPAR gamma wild type data contains 3 samples, each of which is a pool of 4 mouse placentas harvested at E9.5. The PPAR gamma null data also contains 3 samples, each of which is a pool of 4 mouse placentas harvested at E9.5 and is litter matched to a PPAR gamma wild type sample. Each sample was hybridized to an Affymetrix MOE430 v2.0 Chip.

The signal intensity of each probe set was summarized and normalized using the Robust Multi-chip Average (RMA) algorithm (Irizarry et al., 2003). In addition, the presence of the mRNA transcripts targeted by each probe set was tested using the MAS 5.0 algorithm developed by Affymetrix. To test if a gene is differentially expressed between wild type and PPAR gamma null placentas, I applied the MAANOVA algorithm, which is a modified analysis of variance algorithm (Cui et al., 2003). Using the MAANOVA algorithm, a modified F statistic was calculated based on the James-Stein shrinkage estimate of the within group variance of each probe set. Permutation algorithm was used to estimate the empirical distribution of the modified F statistic under the null hypothesis that each gene was identically distributed in both wild type and PPAR gamma null placentas. The p values of the modified F statistic under the null hypothesis then were calculated using the estimated empirical distribution. The probes that were reported to be “Absent” using the MAS 5.0 algorithm were excluded. Then the Storey’s method (Storey and Tibshirani 2003) was used to calculate the adjusted p values to control the false discovery rate. A threshold of 30% fold up regulation or 23% down regulation was adopted to ensure that the genes selected being at least moderately affected by the knock out of PPAR gamma. (Note that to say that a gene is 23% down regulated from wild type to PPAR gamma null is the same as to say that it is 30% up regulated from PPAR gamma null to wild type.)

When the false discovery rate was controlled at 5%, there were in total 790 probe sets differentially expressed between the PPAR gamma null placenta and wild type placenta. Removing the genes linked to the sex chromosomes, (these genes might be differentially expressed because of imbalance in the sex of the placenta samples), we got 745 probe sets targeting 633 genes that were differentially expressed, among them 395 genes were down regulated in the PPAR gamma null placenta, and 238 were up-regulated.

I further investigated the expression pattern of these 633 genes in RXR alpha knock out placenta. It is generally agreed that PPARs regulate the expression of the target genes through the heterodimer with RXR (Kliewer et al., 1992). Thus, the deficiency of RXR alpha should have similar effect on the placenta development as the deficiency of PPAR gamma. The RXR alpha wild type data contains 3 samples, each of which is a pool of 3 mouse placentas harvested at E9.5. The RXR alpha null data also contains 3 samples, each of which is a pool of 3 mouse placentas harvested at E9.5 and is litter matched to a RXR alpha wild type sample. Each sample is hybridized to an Affymetrix MOE430 v2.0 Chip.

Thee RXR alpha knock out experiment data was analyzed in the same way as the PPAR gamma knock out data, except that the analysis was limited mainly to the 745 probe sets that were identified in the analysis of the PPAR gamma knock out data. With the false discovery rate controlled at 5%, 150 genes, represented by 180 probes sets, were down regulated at least 23% in RXR alpha null placenta, and 89 genes, represented by 111 probe sets, were up regulated at least 30% in RXR alpha null placenta. Moreover, except for one gene represented by a single probe set, all other probe sets down regulated in RXR alpha null placenta were also down regulated in PPAR gamma null placenta, and all probe sets up regulated in RXR alpha null placenta were also up regulated in PPAR gamma null placenta.

Next I investigated the expression pattern of the 633 genes (identified in the analysis of PPAR gamma null placenta data), in PPAR gamma knock out trophoblast cell lines treated with PPAR agonist rosiglitazone. The data set includes three lines of wild type trophoblast stem cells treated with rosiglitazone, the same three lines of wild type trophoblast stem cells used as control, and two lines of PPAR gamma null trophoblast stem cells treated with rosiglitazone. All three types of samples were harvested at 2 days and 4 days after the initiation of differentiation. The sample from each cell line at each time point is hybridized to an Affymetrix MOE430 v2.0 Chip.

The trophoblast cell line data were normalized and summarized in the same way as the PPAR gamma knock out placenta data. The genes differentially expressed between wild type trophoblast and PPAR gamma null trophoblast treated with rosiglitazone, as well as genes differentially expressed between wild type trophoblast treated with rosiglitazone and PPAR gamma null trophoblast treated with rosiglitazone, in the samples collected respectively after 2 days and 4 days of differentiation, were identified using the MAANOVA algorithm, with the p values adjusted by the Storey's method. However, the analysis of variance algorithm provided in the R package limma (Smyth 2005) was used to compare the wild type trophoblast cell lines against the wild type trophoblast treated with rosiglitazone, where the within group variance of a gene was estimated using the empirical Bayesian method. The reason was that Smyth's algorithm is more efficient when the samples from the two groups can be paired, as the wild type trophoblast cells with or without rosiglizatone in this study. The p values calculated using Smyth's algorithm were also adjusted by Storey's method to control the false discovery rate. The threshold of 30% up regulation or 23% down regulation was applied to all of the 633 genes.

Table 1 below summarizes the results of the analysis of the expression patterns of the 633 genes in the trophoblast cell line data. It is clear that most genes down regulated in the group where we would expect lower activity of PPAR gamma, either because of PPAR gamma knock out, or because of the lack of PPAR agonist, were also down regulated in PPAR gamma null placenta.

Similar pattern was also observed for genes up regulated in the group where we would expect lower activity of PPAR gamma.

Days of Differentiation	Group 1	Group 2	Down Regulated in PPAR Gamma Null (395)		Up Regulated in PPAR Gamma Null (238)	
			Down Regulated in Group 2	Up Regulated in Group 2	Down Regulated in Group 2	Up Regulated in Group 2
2	WT+	WT	37	28	2	40
2	WT	Null	6	0	0	0
2	WT+	Null	50	9	2	15
4	WT+	WT	63	19	3	34
4	WT	Null	85	4	10	18
4	WT+	Null	139	14	12	60

Table 1. Genes Differentially Expressed in the Trophoblast Cell Lines

To better visualize the results, I also plotted the trend of the gene expression fold change from 2 days of differentiation to 4 days of differentiation, as shown in Figure 1. From the plot, it is clear that, first, for most genes, its expression level were regulated in the same direction on both day 2 and day 4 of differentiation for all three comparisons; second, the expression fold change of most genes in all three comparisons are more significant on the day 4 of differentiation than on the day 2 of differentiation.

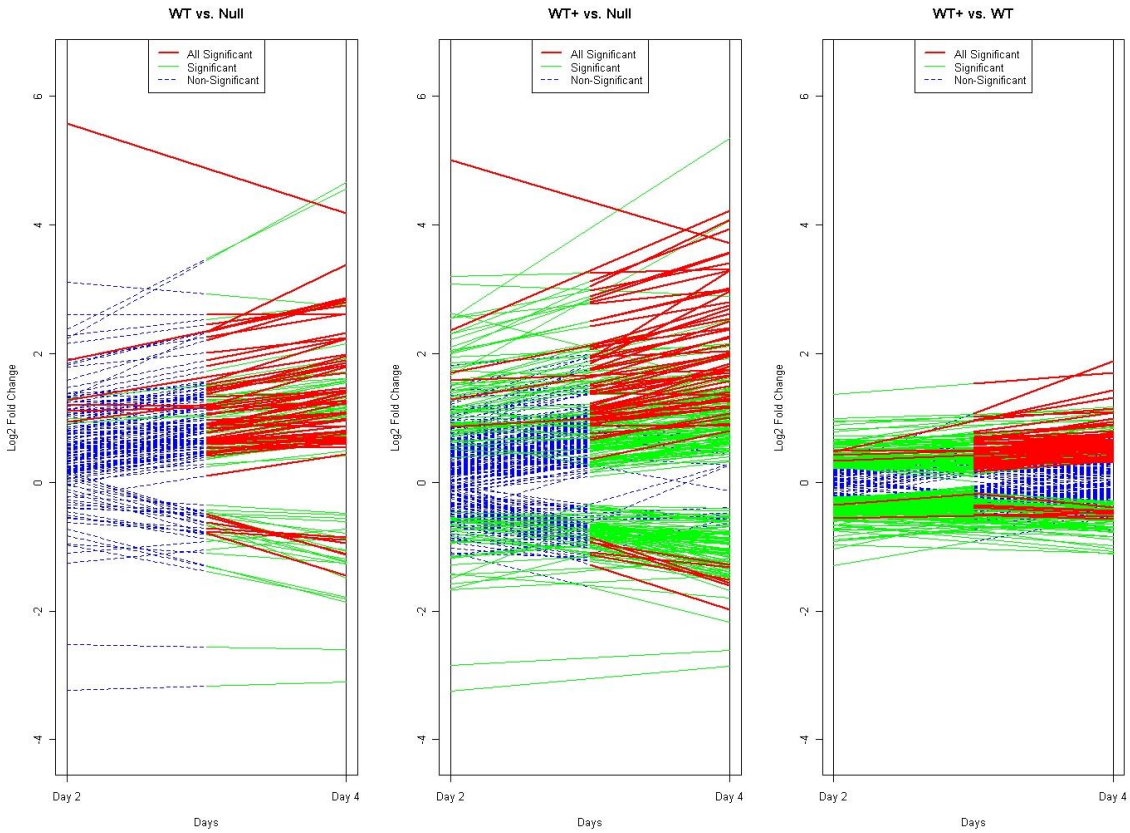

Figure 1 Gene Expression Changes on Day 2 and Day 4 of Differentiation. Each line represents a gene. Its two ends give the log 2 fold chang of the gene between two groups on day 2 and day 4 of differentiation, and its two segments represent the significance of the expression fold change on day 2 and day 4. Red line segment means the gene is significantly differentially expressed in all 3 comparisons on the correponding day of differentiation, green line segment means the gene is signficantly expressied in the current comparison, but not all. Blue dotted line means the gene is not significantly differentially expressed in the current comparison**.**

Combining the results of the analysis of the mouse PPAR gamma null placenta data, RXR alpha knock out placenta data, and PPAR gamma null trophoblast cell line data two lists of genes whose expression level are strongly affected by the PPAR gamma activity in mouse placenta were identified. The first list of genes consists of those always regulated in the same direction as PPAR gamma. More specifically, a gene is regulated in the same direction as PPAR gamma if:

- It is significantly down regulated in PPAR gamma knock out placenta with false discovery rate controlled at 5%,
- Its expression in PPAR gamma knock out placenta is down regulated by at least 23%,
- It is significantly down regulated in RXR alpha knock out placenta with false discovery rate controlled at 5%,
- Its expression in RXR alpha knock out placenta is down regulated by at least 23%,
- After 4 days of differentiation, it is significantly down regulated in PPAR gamma null trophoblast cells compared with wild type trophoblast cell lines treated with rosiglizatone, with false discovery rate controlled at 5%,

- After 4 days of differentiation, its expression in PPAR gamma null trophoblast cells is down regulated by at least 23% compared with wild type trophoblast cell lines treated with rosiglizatone,
- After 4 days of differentiation, it is either significantly down regulated in PPAR gamma null trophoblast cells compared with wild type trophoblast cell lines, or significantly down regulated in wild type trophoblast cells compared with wild type trophoblast cell lines treated with rosiglizatone, with false discovery rate controlled at 5%,
- After 4 days of differentiation, either its expression in PPAR gamma null trophoblast cells is down regulated by at least 23% compared with wild type trophoblast cell lines, or its expression in wild type trophoblast cells is down regulated by at least 23% compared with wild type trophoblast cells treated with rosiglizatone.
- After 4 days of differentiation, its expression is not up regulated in any type of trophoblast cells when compared with another type of trophoblast cells with lower PPAR gamma activity.

The second list of genes consists of those always regulated in the opposite direction of PPAR gamma. More specifically, a gene is regulated in the opposite direction of PPAR gamma if:

- It is significantly up regulated in PPAR gamma knock out placenta with false discovery rate controlled at 5%,
- Its expression in PPAR gamma knock out placenta is up regulated by at least 30%,
- It is significantly up regulated in RXR alpha knock out placenta with false discovery rate controlled at 5%,
- Its expression in RXR alpha knock out placenta is up regulated by at least 30%,
- After 4 days of differentiation, it is significantly up regulated in PPAR gamma null trophoblast cells compared with wild type trophoblast cell lines treated with rosiglizatone, with false discovery rate controlled at 5%,
- After 4 days of differentiation, its expression in PPAR gamma null trophoblast cells is up regulated by at least 30% compared with wild type trophoblast cell lines treated with rosiglizatone,
- After 4 days of differentiation, it is either significantly up regulated in PPAR gamma null trophoblast cells compared with wild type trophoblast cell lines, or significantly up regulated in wild type trophoblast cells compared with wild type trophoblast cell lines treated with rosiglizatone, with false discovery rate controlled at 5%,
- After 4 days of differentiation, either its expression in PPAR gamma null trophoblast cells is up regulated by at least 30% compared with wild type trophoblast cell lines, or its expression in wild type trophoblast cells is up regulated by at least 30% compared with wild type trophoblast cells treated with rosiglizatone.
- After 4 days of differentiation, its expression is not down regulated in any type of trophoblast cells when compared with another type of trophoblast cells with lower PPAR gamma activity.

Table 2 gives the lists of genes regulated in the same/opposite direction of PPAR gamma in mouse placenta, and well as the human homologue of these genes. Further experimental data would be needed to establish the relation between PPAR gamma and these genes. However, given that PPAR gamma is a transcription factor, based on the expression pattern of these genes in various mouse placenta and trophoblast cell lines, it is reasonable to assume that some genes

regulated in the same direction as PPAR gamma could be direct target of PPAR gamma in mouse placenta, and many genes regulated in the opposite direction of PPAR gamma could be indirect target of PPAR gamma.

Potential PPAR gamma targets		Potential indirect PPAR gamma targets	
Mouse Gene	Human Homolog	Mouse Gene	Human Homolog
1110038D17Rik	C22orf13	Acpp	ACPP
4931406C07Rik	C11orf54	Akap12	AKAP12
6230427J02Rik	C3orf54	Atp2a3	ATP2A3
Acvrl1	ACVRL1	Clu	CLU
Adck5	ADCK5	Cmpk2	CMPK2
Als2cl	ALS2CL	Dppa5a	DPPA5
Arhgap18	ARHGAP18	Elovl7	ELOVL7
Btg2	BTG2	Fgfrl1	FGFRL1
Caskin1	CASKIN1	Folr1	FOLR1
Chst13	CHST13	Hbegf	HBEGF
Ctsr		L1td1	L1TD1
Dcakd	DCAKD	Pkdcc	SGK493
Eif1b	EIF1B	Lrrn4cl	LRRN4CL
Extl1	EXTL1	Pdzd2	PDZD2
Fam176a	FAM176A	Sh3rf2	SH3RF2
Fam83d		Swap70	SWAP70
Fn1	FN1	Tcfcp2l1	TFCP2L1
Grtp1		Tec	TEC
Gtlf3b	C17orf103		
Hrct1			
Hsd17b11	HSD17B11		
Hsd17b4	HSD17B4		
Hunk	HUNK		
Ippk	IPPK		
Ldhb	LDHB		
Lepr	LEPR		
Lifr	LIFR		
Limd2	LIMD2		
Megf9	MEGF9		
Mertk	MERTK		
Muc1	MUC1		
Nostrin	NOSTRIN		
Nt5c2	NT5C2		
Nt5e	NT5E		
Numb	NUMB		
Obsl1			
Pcx	PC		

Pex1	PEX1
Pex13	PEX13
Prl7d1	
Rarres2	RARRES2
Rdh12	RDH12
Retsat	RETSAT
Rnd2	RND2
Sgk2	SGK2
Slc45a4	SLC45A4
Slco3a1	SLCO3A1
Snx24	SNX24
Tgfb3	TGFB3
Tmem125	
Unc119	UNC119
Unc119b	UNC119B

Table 2. Potential direct and indirect targets of PPAR gamma in mouse placenta

Aim 2: Identify human genes that are potentially targets of PPAR gamma in placenta

To search for potential targets of PPAR gamma in human placenta, I first compared the gene expression profiles of human trophoblast stem cells versus trophoblast stem cells treated with PPAR agonists GW 7845 and troglitazone. The trophoblast samples were collected after 48 hours, either treated with one of the two PPAR agonists, or untreated. One sample from each condition was divided into 5 technical replicates, and each technical replicate is hybridized to a set of Affymetrix Human U95 A-E chips. As in the analysis of the mouse placenta and trophoblast cell data, the signal intensity of the probe sets in the Affymetrix arrays were normalized and summarized using the RMA algorithm. The MAANOVA algorithm was applied to test, for each gene, if it was differentially expressed between untreated trophoblast cells and trophoblast cells treated by GW 7845 or troglitazone. The Storey's method was used to calculate the adjusted p values to control the false discovery rate.

With false discovery rate controlled at 10%, 2918 probe sets were found to be differentially expressed either between trophoblast cell control and trophoblast cells treated with GW 7845, or between trophoblast cell control and trophoblast cells treated with troglitazone. Most of these probe sets (2658 out of 2918) behaved consistently in the sense that, if it is up/down regulated in trophoblast cells treated with GW 7845, it is also regulated in the same direction in trophoblast cells treated with troglitazone, and vice versa. This suggested that the two PPAR gamma ligands do have similar effects on the gene expression pattern of trophoblast cells, although the troglitazone may have a slightly stronger effect. For example, for the probe sets up regulated both in trophoblast cells treated with GW 7845 and in trophoblast cells treated with troglitazone, on average they were up regulated by 6.72% in trophoblast cells treated with GW 7845, and 7.65% in trophoblast cells treated with troglitazone. For the probe sets down regulated both in trophoblast cells treated with GW 7845 and in trophoblast cells treated with troglitazone, on average they were down regulated by 7.53% in trophoblast cells treated with GW 7845, and 8.50% in trophoblast cells treated with troglitazone.

To narrow the range of genes that might be targeted by PPAR gamma in human trophoblast cells, only those genes significantly regulated in the same direction in both trophoblast cells treated with GW 7845 and trophoblast cells treated with troglizaton (with false discveroy rate controlled at 10%) were selected. It turns out that more than half of the probe sets significantly differentially expressed in trophoblast cells treated with GW 7845 were also significantly regulated in the same direction in trophoblast cells treated with troglitazone, as the Venn diagram in **Figure 2** shows. Specifically, 55 genes, represented by 83 probe sets, up regulated significantly in both the trophoblast cells treated with GW 7845 and the trophoblast cells treated with troglitazone, and 94 genes, represented by 120 probe sets, down regulated significantly in both the trophoblast cells treated with GW 7845 and the trophoblast cells treated with troglitazone.

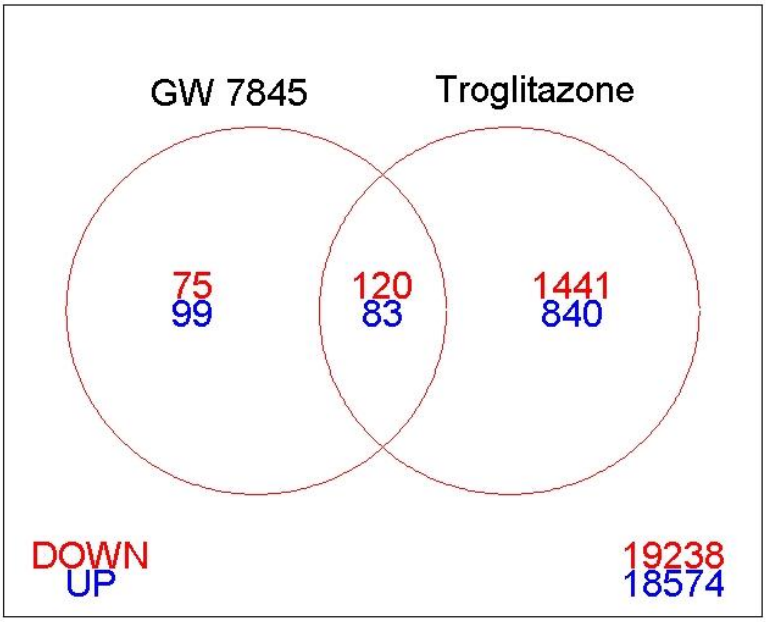

Figure 2. Probe sets up/down regulated in the presence of PPAR gamma ligands. There are in total 19238 probe sets down regulated by both GW 7845 and troglitazone, and 18574 probe sets up regulated by both GW 7845 and troglitazone. Numbers inside the circles are probe sets significantly up/down regulated, with false discovery rate controlled at 10%.

I further studied the expression pattern of the 149 genes, represented by 203 probes sets, in the trophoblast cells treated with PPAR gamma inhibitor p38. The human trophoblast samples were collected after 48 hours, either treated with PPAR inhibitor p38 or with no treatment. One sample from each condition is hybridized to a set of Affymetrix Human U95 A-E chips.

Because there is only a single sample of the trophoblast cells from the treated group and a single sample from the control, and there is no technical replicate, it is impossible to estimate the within-group variance of each gene based on the p38 data set. Therefore, we could not test directly, using the analysis of variance, if the expression level of a gene in the untreated

trophoblast sample is identical to the expression level of that gene in the trophoblast cells treated with p38. To solve this problem, the Affymetrix chips in the p38 experiment were normalized and summarized together with the Affymetrix chips from the previous experiment where trophoblast cells were treated with GW 7845 and troglitazone. Given that the samples in the p38 experiment are similar to the samples in the GW 7845/troglitazone experiment, and the Affymetrix chips from the two experiments were processed together using the RMA algorithm, it is reasonable to assume that the within group variance of the probe sets from the chips in the p38 experiment should be similar to that in the GW 7845/troglitazone experiment.

More precisely, suppose that a gene G is not differentially expressed between trophoblast cell control and trophoblast cells treated with p38, and that G is also not differentially expressed between trophoblast cell control and trophoblast cells treated with GW 7845 or troglitazone. Let D_{p38} be the difference in the expression level of gene G between a single trophoblast cell control sample and a single trophoblast cell sample treated with p38 in the p38 experiment. Let D_{GW7845} be the difference in the expression level of gene G between a single trophoblast cell control sample and a single trophoblast cell sample treated with GW 7845 in the GW 7845/troglitazone experiment. Then it is reasonable to assume that D_{p38} and D_{GW7845} are identically distributed. Furthermore, let $\bar{D}_{GW7845}$ be the difference in the average expression level of gene G between 5 replicates of a single trophoblast cell control sample and 5 replicates of a single trophoblast cell sample treated with GW 7845 in the GW 7845/troglitazone experiment, and $\hat{\sigma}^2$ be the within group variance of the expression level of G estimated from the GW 7845/troglitazone experiment, then the statistics $F_{p38} = D_{p38}{}^2 \big/ \left(2\hat{\sigma}^2\right)$ and $F_{GW7845} = \bar{D}_{GW7845}{}^2 \big/ \left(2\hat{\sigma}^2/5\right)$ should be identically distributed, and both have nominally a F distribution with (1, 12) degrees of freedom.

Based on the above analysis, I calculated the F_{p38} statistic for each of 203 probe sets identified in the analysis of the GW 7845/troglitazone experiment, with the estimation of $\hat{\sigma}^2$ also borrowed from the GW 7845/troglitazone experiment. The p values of the F_{p38} statistics were derived from the empirical distribution of the F_{GW7845} statistics under the null hypothesis that the genes are not differentially expressed between trophoblast control and trophoblast cells treated with GW 7845/troglitazone. With the false discovery rate controlled at 10%, I found 20 genes, represented by 26 probe sets, out of the 55 genes up regulated in trophoblast cells treated with GW 7845 or troglitazone, were down regulated in trophoblast cells treated with p38; and 23 genes, represented by 27 probe sets, out of the 94 genes down regulated in trophoblast cells treated with GW 7845 or troglitazone, were up regulated in trophoblast cells treated with p38.

I then investigated the expression pattern of the 43 genes identified from the analysis of the p38 experiment in trophoblast cells treated with another PPAR gamma ligand GW 1929. The human trophoblast samples were collected after 48 hours, either treated with GW 1929 or with no treatment. One sample from each condition is hybridized to an Affymetrix Human U95 AV2 chip. Just as the p38 experiment, in the GW 1929 experiment, there is only a single sample of the trophoblast cells from the treated group and a single sample from the control, and there is no

technical replicate. The same procedure used in the analysis of the p38 experiment was used to analyze the GW 1929 data, with the estimation of within group variance of each gene, as well as the empirical distribution of the modified F statistics, borrowed from the GW 7845/troglitazone experiment. Because the GW 1929 data consists of only Affymetrix U95 AV2 chips, not all genes identified in the analysis of p38 experiment were present in the GW 1929 data. With false discovery rate controlled at 10%, out of the 10 genes up that were regulated in the presence of GW 7845 and troglitazone, down regulated in the presence of p38, and targeted by U95 AV2, 8 of them were also significantly up regulated in the presence of GW 1929; out of the 10 genes that were down regulated in the presence of GW 7845 and troglitazone, up regulated in the presence of p38, and targeted by U95 AV2, 3 of them were also significantly down regulated in the presence of GW 1929.

The results of the analysis of the GW 7845/troglitazone experiment data, p38 experiment data, and GW 1929 experiment data, were combined to generate two list of genes, as shown in Table 3. The first list of genes were significantly up regulated in the presence of GW 7845, troglitazone, and GW 1929, but significantly down regulated in the presence of p38. The second list of genes were significantly down regulated in the presence of GW 7845, troglitazone, and GW 1929, but significantly up regulated in the presence of p38. Like the lists generated by the analysis of the mouse data, further experimental data would be needed to establish whether these genes are true targets of PPAR gamma. Nevertheless, based on their expression profile in trophoblast cells treated with various PPAR gamma ligands and inhibitor, it is reasonable to assume that some genes in the first list could be direct target of PPAR gamma in human trophoblast cells, and many genes in the second list could be indirect target of PPAR gamma.

Potential direct target of PPAR gamma in human trophoblast	Potential indirect target of PPAR gamma in human trophoblast
SLC20A1	LGALS1
CEBPA	FOLR1
ABCG2	CCL2
HMOX1	C1QTNF6
DGKZ	DOCK8
ARL4C	SCD5
CRH	KIAA1432
SLC7A8	ADAMTS1
ANGPTL4	RCN1
TUBB3	HS6ST2
LIMCH1	ITGB6
STARD10	C15orf48
BGN	DDAH1
CDYL	TRPM7
FAM100B	TMTC4
PRDX6	NOVA1
GPR157	
CXorf40A	

Table 3 Genes regulated in the presence of PPAR gamma ligands and inhibitor

Aim 3: Identify homologous potential target genes of PPAR gamma discovered in both the mouse placenta and the human placenta.

Comparing the human homologue of the list of potential targets of PPAR gamma in mouse placenta with the list of potential targets of PPAR gamma in human trophoblast cells, only a single gene, FOLR1, was listed as potential indirect target in both mouse placenta and human placenta. That is, FOLR1 was always significantly regulated in the opposite direction of PPAR gamma, in both human trophoblast and mouse placenta.

The gene ontology analysis was performed to find out the main functions of the potential direct and indirect target genes identified in mouse placenta and human trophoblast cells. Using the FuncAssociate program (Berriz et al., 2009), I found that, with false discovery rate controlled at 5%, the following attributes were significantly over represented in the potential direct targets of PPAR gamma in mouse placenta: peroxisome localization; microtubule-based peroxisome localization; pyruvate carboxylase activity; L-lactate dehydrogenase activity; anaerobic glycolysis; lactate dehydrogenase activity; biotin binding; ligase activity, forming carbon-carbon bonds; gluconeogenesis; glycolysis; glucose metabolic process; oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor; oxidoreductase activity, acting on CH-OH group of donors; hexose metabolic process; monosaccharide metabolic process; cellular carbohydrate metabolic process. In particular, the first two attributes, peroxisome localization and microtubule-based peroxisome localization, were overrepresented by more than 1000 fold. Only one attribute, cellular_component, was significantly underrepresented in the potential direct targets of PPAR gamma in mouse placenta. Many of these overrepresented attributes are closely related to the function of PPAR gamma, suggesting that the list of potential direct target of PPAR gamma in mouse placenta may contain some true target.

With false discovery rate controlled at 10%, 3 closely related attributes were significantly over represented in the potential indirect targets of PPAR gamma in mouse placenta: somatic recombination of immunoglobulin genes during immune response; somatic diversification of immunoglobulins during immune response; isotype switching.
However, for the two lists of potential direct and indirect targets of PPAR gamma in human trophoblast, no attribute was found to be significantly over or under represented.

Summary

Through the statistical analysis of the gene expression patterns of mouse placenta and human trophoblast cells where PPAR gamma activity was manipulated, I produced lists of genes that could potentially be the direct or indirect targets of PPAR gamma in mouse placenta and human trophoblast. These lists could serve as a starting point for further in-depth experimental and computational study of the function of PPAR gamma in human and mouse placenta. The gene ontology analysis suggested that the list of potential direct targets of PPAR gamma in mouse placenta were particularly promising.

References:
Irizarry, RA, Hobbs, B, Collin, F, Beazer-Barclay, YD, Antonellis, KJ, Scherf, U, Speed, TP. "Exploration, Normalization, and Summaries of High Density Oligonucleotide Array Probe Level Data." *Biostatistics*, 4(2): 249-264, 2003

Cui XQ, Hwang G, Qiu J, Blades N, and Churchill GA. "Improved Statistical Tests for Dierential Gene Expression by Shrinking Variance Components." *Biostatistics*, 6(1):59-75, 2005.

Storey JD and Tibshirani R. "Statistical significance for genome-wide studies." *PNAS*, 100: 9440-9445, 2003

Kliewer SA, Umesono K, Noonan DJ, Heyman RA, and Evans RM, "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, 358(6389), pp. 771–774, 1992.

Smyth SK, "Limma: linear models for microarray data," in *Bioinformatics and Computational Biology Solutions using R and Bioconductor*, Gentleman R, Carey V, Dudoit S, Irizarry R, Huber W (eds.), Springer, New York, pages 397–420, 2005

Berriz GF, Beaver JE, Cenik C, Tasan M, and Roth FP. "Next generation software for functional trend analysis," *Bioinformatics* 25: 3043-3044. 2009

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__X_____ No_____

If yes, please describe your plans:

I plan to submit at least one paper on PPAR gamma target genes in mouse placenta in collaboration with Dr. Barak in 2010.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X__

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME Tianjiao Chu, PhD	POSITION TITLE Assistant Professor of OBGYN and Reproductive Sciences
eRA COMMONS USER NAME	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Shandong University, Jinan, China	BA	1989	Chinese Lang. & Lit
University of Toledo, Toledo, OH	MA	1997	Philosophy
Carnegie Mellon University, Pittsburgh, PA	MS	2001	Data Mining
Carnegie Mellon University, Pittsburgh, PA	PhD	2003	Logic & Computation

A. Personal statement

My research interests have been mainly in the developing the statistical models and computational algorithms for the analysis of biological/medical datasets. In the past few years, I have worked closely with biologists and medical researchers at the Magee Womens Research Institute, in studies focusing on the mechanisms of CpG methylation, miRNA expression and mRNA transcription in placenta.

B. Positions and employment

2003-2005 Research Scientist, Institute for Human and Machine Cognition, Univ. of West Florida
2005-2007 Research Scientist, JustSystems Evans Research, Pittsburgh, PA
2007- Assistant Professor, Dept. of OBGYN and Reproductive Sciences, University of Pittsburgh

Honors

2008-2009 Program committee member of the 24th and 25th Conferences on Uncertainty in Artificial Intelligence

C. Selected publications –

Refereed articles

1. **Chu T**, Glymour C, Scheines R, Spirtes P. A statistical problem for inference to regulatory structure from associations of gene expression measurement with microarrays. *Bioinformatics* 2003;19:1147-52.
2. Ning W, **Chu T**, Li CJ, Choi A, Peters D. Genome Wide Analysis of the Endothelial Transcriptome under Short-Term Chronic Hypoxia. *Physiol Genomics* 2004;18:70-8.
3. Burgansky-Eliash Z, Wollstein G, **Chu T**, Ramsey JD, Glymour C, Noecker RJ, Ishikawa H, Schuman JS. Optical coherence tomography machine learning classifiers for glaucoma detection: a preliminary study. *Invest Ophthalmol Vis Sci* 2005;46:4147-52. PMC1941765
4. Peters DG, Kudla DM, DeLoia JA, **Chu TJ**, Fairfull L, Edwards RP, Ferrell RE. Comparative gene expression analysis of ovarian carcinoma and normal ovarian epithelium by serial analysis of gene expression. *Cancer Epidemiol Biomarkers Prev* 2005;14:1717-23.
5. Peters DG, Ning W, **Chu TJ**, Li CJ, Choi AMK. Comparative SAGE analysis of the response to hypoxia in human pulmonary and aortic endothelial cells. *Physiol Genomics* 2006;26:99-108.
6. **Chu T.** Limitations of statistical learning from gene expression data. *Interface 2004: Computing Science and Statistics* 2008;36:266-85.
7. **Chu TJ**, Peters DG. Serial analysis of the vascular endothelial transcriptome under

static and shear stress conditions. *Physiol Genomics* 2008;34:185-92.

8. **Chu T**, Glymour C. Search for additive nonlinear time series causal models. *Journal of Machine Learning Research* 2008;9:967-91.
9. **Chu T**, Bunce K, Hogge WA, Peters DG. Statistical model for whole genome sequencing and its application to minimally invasive diagnosis of fetal genetic disease. *Bioinformatics* 2009;15;25:1244-50.
10. Hermann BP, Sukhwani M, Simorangkir DR, **Chu T**, Plant TM, Orwig KE. Molecular dissection of the male germ cell lineage identifies putative spermatogonial stem cells in rhesus macaques. *Hum Reprod* 2009;24:1704-16. PMC2698327
11. **Chu T**, Burke B, Bunce K, Surti U, Allen Hogge W, Peters DG. A microarray-based approach for the identification of epigenetic biomarkers for the noninvasive diagnosis of fetal disease. *Prenat Diagn* 2009 Nov;29(11):1020-30.
12. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. Mir-205 Silences MED1 in Hypoxic Primary Human Trophoblasts. *FASEB* 2010; Epub ahead of print.

Books and book chapters

1. Wimberly F, Danks D, Glymour C, **Chu, T.** Problems for Structure Learning: Aggregation and Computational Complexity. In: *Computational Methods in Gene Regulatory Networks*. Das, Welch, Caragea, Hsu (ed.). IGI Global, in press.

C. Research support

PA Department of Health (PI: T. Chu) 01/01/09–12/31/09
(Research Formula Funds)
Integration Study of the Target Genes of PPAR gamma in Human and Mouse Placenta
The goal of this project is to identify target genes of PPAR gamma in human and mouse placenta through analysis of microarray data.