Int J Mol Epidemiol Genet 2011;2(1):78-94
www.ijmeg.org /ISSN1948-1756/IJMEG1011002

Original Article

Maternal peripheral blood gene expression in early pregnancy and preeclampsia

Daniel A. Enquobahrie[1,2,3], Chunfang Qiu[1], Seid Y. Muhie[4], Michelle A. Williams[1,3]

[1]Center for Perinatal Studies, Swedish Medical Center, Seattle, WA, USA; [2]Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA; [3]Department of Epidemiology, University of Washington, Seattle, WA, USA; [4]Walter Reed Army Institute of Research, Silver Spring, MD, USA.

Received November 3, 2010; accepted December 28, 2010; Epub December 29, 2010; published January 1, 2011

Abstract: We investigated associations of early pregnancy maternal peripheral blood gene expression with preeclampsia. In a nested case control study, gene expression of peripheral blood, collected at 16weeks of gestation on average from 16 women destined to develop preeclampsia and 16 women who had normotensive pregnancies was profiled using Affymetrix GeneChip Arrays. Fold change and Student's T-test analyses were used to compare differential gene expression across the groups. Functions and functional relationships as well as common regulatory sequences of differentially expressed genes were investigated. Genes participating in abnormal placentation (e.g COL1A1), immune/inflammation response (e.g. IKBKB) and cellular development (including cell cycle) (e.g. RB1) were differentially expressed in early pregnancy peripheral blood in preeclampsia. We identified transcription factors (i.e. Sp1, MAZ and MZF1) that may account for co-expression of differentially expressed genes. Preeclampsia is associated with differential gene expression in early pregnancy peripheral blood.

Keywords: preeclampsia; early pregnancy; gene; expression

Introduction

The pathogenesis of preeclampsia, a pregnancy-related vascular disorder, is a complex process that has been associated with angiogenesis, immune dysfunction, inflammation and oxidative stress [1-3]. While preeclampsia is a disorder of the second half of pregnancy, accumulating evidence supports the multi-stage developmental phases of preeclampsia that start early in pregnancy [1-3]. For instance, immune sensitivity and abnormal placentation in early pregnancy contribute to placental hypoxemia which promotes diffuse inflammation, oxidative stress and endothelial dysfunction later in pregnancy [1-4]. However, significant gaps in knowledge persist on preeclampsia related events and risk factors in early pregnancy that is critical for prevention and early detection of disease [5].

Increasingly, gene expression studies are being used to investigate pathophysiologic processes underlying preeclampsia [5]. Several investigators, including our team, have conducted gene expression profiling of preeclamptic placenta after delivery [6-9]. Although results from these studies provide new insights about preeclampsia pathophysiology, inferences are limited by critical questions concerning temporal relationships between gene expression profiles, onset of the clinical disorder, and its management. Few gene expression studies investigating preeclampsia were conducted in early pregnancy [10-12] and even fewer were conducted using early pregnancy peripheral blood [12], a tissue that may reflect local and systemic pathophysiological changes associated with preeclampsia.

Taking into account the potential significance of this research area, in 2003, we expanded an on-going pregnancy cohort study by prospectively collecting and storing peripheral blood samples in Paxgene™ Blood RNA tubes for gene expression studies. In this report, we describe findings of a nested case control study that investigated early pregnancy maternal peripheral blood gene expressions among 16 women destined to develop preeclampsia and 16 women who had normotensive pregnancies. We also compared similarities and differences between preeclampsia related underlying pathomechanisms in early and late pregnancy using gene expression profiles of peripheral blood (early pregnancy) and placenta (at-delivery), respectively.

Materials and methods

Study population

This nested case control study was conducted using information collected from participants of the Omega study (1996-2007), a prospective study designed to examine risk factors of pregnancy complications. Participants were recruited from women who initiate prenatal care before 20 weeks gestation at Swedish Medical Center (SMC) affiliated clinics. Ineligibility criteria included < 18 years of age, not speaking or reading English, not planning to carry the pregnancy to term, and/or not planning to deliver at SMC. The study for this report was conducted among selected preeclampsia cases (N=16) and controls (N=16) from Omega cohort members enrolled during the period of July 2003 to May 2007. During this interval, > 80% of approached women consented to participate in the study and > 95% of enrolled participants were followed through pregnancy completion.

Preeclampsia cases were selected using the then current 1996 ACOG guidelines when both pregnancy-induced hypertension (PIH) and proteinuria were present. PIH was defined as a sustained (≥2 measures 6 hrs apart) blood pressure (Bp) elevation (>140/90 mmHg) after 20 weeks of gestation or a sustained 15-mm Hg rise in diastolic Bp or a 30-mm Hg rise in systolic Bp above 1st trimester values. Proteinuria was defined as a sustained (≥2 measures 4 hrs apart) presence of elevated protein in the urine (>30 mg/dL or >1+on a urine dipstick). Controls were selected among women who had normotensive pregnancies uncomplicated by proteinuria or gestational diabetes. Women who were multiparous or had history of chronic hypertension and/or pre-gestational diabetes as well as women with non-singleton pregnancies were excluded. The Institutional Review Board of the SMC approved study protocols. All participants provided written informed consent.

Data collection

Information on risk factors was collected using in-person interviews, blood collection and medical records abstraction. Following enrollment, in-person interviews were conducted to collect data on socio-demographic characteristics and reproductive and medical histories. At or near the time of in-person interviews (16 weeks of gestation on average), trained phlebotomists collected peripheral blood samples. PAXgene™ Blood RNA tubes (Qiagen Inc, Valencia, CA) [13] were used to collect blood samples for gene expression studies. After delivery, trained personnel abstracted maternal and infant medical records to ascertain pregnancy outcomes.

Total RNA extraction, target preparation and hybridization

The PAXgene Blood RNA Kit (Qiagen Inc., Valencia, CA) was used for extraction and purification of total RNA. Total RNA concentration was determined by UV absorbance at 260 nm (A_{260}) by direct measurement on a NanoDrop ND1000 spectrophotometer (ThermoFisher Scientific, Wilmington, DE). RNA purity was assessed by evaluating readings at 260 nm and 280 nm (A_{260}/A_{280}). All samples had A_{260}/A_{280} values > 2.0 indicating high level of purity. Samples were then kept in frozen storage at -80°C. All RNA samples, including reference RNAs, underwent quality control checks and were labeled using same standardized protocols.

RNA target preparations were conducted using guidelines of the NuGEN™ Ovation™ RNA Amplification System V2 (amplification) and the NuGEN™ FL-Ovation™ cDNA Biotin Module V2 (fragmentation and labeling) (NuGen Technologies Inc., San Carlos, CA). The resultant fragmented and labeled cDNA was added to the hybridization cocktail in accordance with the NuGEN and Affymetrix guidelines for hybridization onto Affymetrix Human Genome U133 Plus 2.0 GeneChip® Arrays (Affymetrix, Sunnyvale, CA). The arrays were washed and stained on the GeneChip® Fluidics Station 450 (Affymetrix, Sunnyvale, CA), before being inserted into the Affymetrix autoloader carousel and scanned using the GeneChip® Scanner 3000 (Affymetrix, Sunnyvale, CA). Data from each array was quantified using GeneChip® Operating Software (Affymetrix, Sunnyvale, CA).

GeneChip quality controls and normalization

GeneChip quality control procedures included the following. First, background values of GeneArray scanners calibrated to the new PMT setting (10% of maximum) were assessed for comparability. Second, GAPDH gene was used to assess RNA sample and assay quality. Third, controls on the GeneChip array (four *E.Coli* genes, bioB, bioC and bioD and the cre gene) were spiked into each sample to evaluate hybridization efficiency. Fourth, raw noise (Q value), a measure of pixel-to-pixel variation of probe cells due to operation-associated electrical noise, was evaluated. Fifth, PolyA control genes (dap, lys, phe, thr and trp genes from *B. subtilis*) were amplified and spiked into the RNA samples prior to amplification to serve as internal control genes. Finally, data were normalized using an error-weighted model based on Rosetta Resolver Error Models (Rosetta, Seattle, WA) [14].

Real time quantitative polymerase chain reaction (RT-qPCR) experiment

RT-qPCR experiment was conducted to confirm microarray based expression measures of selected genes. Initially, 1 μg total RNA was reverse transcribed using the Transcriptor first strand cDNA synthesis kit (Roche Applied Science, Indianapolis, IN). The qPCR reactions were performed using the Roche LightCycler 480® Probes kit and the LightCycler 480® instrument (Roche Applied Science, Indianapolis, IN). Pre-designed exon spanning Taqman® assays for each gene target were obtained from Applied Biosystems (Foster City, CA). Each individual assay was run on an individual 96-well plate in duplicate for each sample; and, 2 reverse transcription negative controls and 2 no template control wells were included with each assay. Individual reactions were characterized by the PCR cycle at which fluorescence first rises above threshold background fluorescence (the threshold cycle, Ct). ACTB and GAPDH genes, selected based on their non-variant gene expression across cases and controls in the microarray experiment, were used for normalization.

Statistical analysis

Analysis was conducted on normalized and log-transformed data. Fold change (FC) expression differences (absolute FC ≥ 1.5) and Student's T-test (two sample, unequal variances) p-values (<0.05) were used to identify differentially expressed genes across the two groups (cases and controls). Two-Dimensional hierarchical clustering, using Cluster and TreeView softwares [16], and Principle components analysis (PCA) techniques were used to evaluate whether differentially expressed genes cluster arrays into groups (case and control groups) [15]. Functions and functional relationships between differentially expressed genes were investigated using Ingenuity Pathway Analysis (IPA), as described before (Ingenuity, Redwood City, CA) [7]. Gene-enrichment of networks (network score) based on a modified Fisher's exact test, measured in IPA, was used to rank biological significance of gene function networks in relation to preeclampsia. In the confirmatory RT-qPCR experiment, we used fold change analysis and Student's T-test to compare whether results were consistent with those obtained from microarray experiments. Common regulatory sequences for the differentially expressed genes as well as their cognate regulators (transcription factors (TFs)) were searched using ConTra (conserved transcription factor binding sites, TFBs) and MAPPER [17]. Finally, using GeneGO pathway analysis tools (GeneGO Inc., St Joseph, MI), we compared gene ontology (GO) processes represented by differentially expressed genes in maternal early pregnancy peripheral blood in the current study with differentially expressed genes in preeclamptic placenta we reported before [7].

Results

Selected study population characteristics are summarized in **Table 1**. Mean age of preeclampsia cases and normotensive controls were 35.1 and 32.1 years, respectively. Maternal whole blood samples were collected from participants at 16 weeks of gestation, on average. Preeclampsia cases had higher pre-gestational BMI compared with controls.

Of the total >38,500 genes represented by ~47,400 probe sets on the GeneChip, 247 genes (<1%) represented by 356 probes that met the following criteria were up (N=86) or down (N=161) regulated in preeclampsia cases compared to controls; Student's T-test p-value < 0.05 and absolute fold change > 1.5 (**Table 2 and 3**). These differentially expressed genes included genes involved in abnormal placentation (e.g. COL1A1 and NRTK2) and immune response/inflammation (e.g. CLEC12B and IKBKB). The range of fold change differences in

Table 1 Characteristics of study population

Characteristics	Preeclampsia cases (N=16)	Normotensive controls (N=16)
GA at blood collection, weeks*	16.2 (1.7)	16.2 (2.5)
Maternal Age, years*	35.1 (5.3)	32.1 (4.4)
20-34 years	8 (50.0)	13 (81.3)
35 and above years	8 (50.0)	3 (13.7)
Maternal Race/Ethnicity		
White	14 (87.5)	13 (81.3)
African American	2 (12.5)	1 (6.3)
Other	0 (0.0)	2 (12.5)
Pre-gestational BMI, kg/m^2*	29.6 (11.9)	23.8 (6.2)
<20	2 (12.5)	1 (6.3)
20-24.99	7 (43.8)	12 (75.0)
25-29.99	5 (31.3)	2 (12.5)
≥30	2 (12.5)	1 (6.3)
Smoked in pregnancy	0 (0.0)	1 (6.3)
Family history of chronic hypertension	10 (62.5)	6 (37.5)
Family history of diabetes mellitus	3 (18.8)	1 (6.3)
Gestational diabetes	2 (12.5)	0 (0.0)

*Mean (standard deviation), otherwise n (%). Abbreviations: GA: gestational age, BMI: body mass index; kg/m^2: kilogram/meter^2

expression between preeclampsia cases and controls was -5.40 (DKFZp666G057) to 2.78 (TMEM176B). In hierarchical clustering, based on expressions measured by probes representing differentially expressed genes, all but three preeclampsia cases and all but one normotensive controls clustered in to the two main cluster groups of cases and controls, respectively (**Figure 1**). Similarly, in PCA, we demonstrated that preeclampsia cases and controls can be classified into two groups using expressions measured by probes representing differentially expressed genes (**Figure 2**).

We further evaluated functions and functional relationships of differentially expressed genes. In IPA, 12 networks with network scores > 3 were over represented by differentially expressed genes. These networks are involved in cellular development (particularly of the hematological system), cell signaling, cell cycle regulation, metabolism (lipid, vitamin, carbohydrates and nucleic acids), inflammation and cellular response (**Table 4**). In particular, the RB1_ E2F1 cell cycle pathway that regulates cellular development (e.g in the hematological system) was significantly over represented (**Figure 3**).

In the qRT-PCR experiment to confirm microarray-based measurements conducted on selected differentially expressed genes (of CLEC family of genes or functionally related genes), similar direction of fold change differences (and for some, similar size of fold change differences) between preeclampsia cases and controls were observed for most genes (6/8, 75%) (**Table 5**). However, most of the p-values in Student's T-test comparisons were not statistically significant.

In the promoter analysis of common regulatory sequences (motifs) of differentially expressed genes, binding sites of transcription factors Sp1 (specificity protein 1), MAZ (MYC associated zinc finger protein) and MZF1 (myeloid zinc finger 1) were identified (**Figure 4**).

Results of GO comparisons of preeclampsia

Table 2. Selected* list of differentially expressed genes

Gene Symbol	Gene Name	FC*	P-value*
Down regulated genes			
DKFZp666G057	hypothetical protein DKFZp666G057	-5.40	0.0245
HSD17B12	Hydroxysteroid (17-beta) dehydrogenase 12	-3.39	0.0121
PLEKHG2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2	-2.79	0.0157
COL5A3	collagen, type V, alpha 3	-2.69	0.0432
LOC400581	GRB2-related adaptor protein-like	-2.64	0.0037
ACCN2	amiloride-sensitive cation channel 2, neuronal	-2.38	0.0078
GTSF1L	gametocyte specific factor 1-like	-2.35	0.0033
CLEC12B	C-type lectin domain family 12, member B	-2.33	0.0010
COL1A1	collagen, type I, alpha 1	-2.26	0.0466
ZNF496	zinc finger protein 496	-2.21	0.0003
VN1R1	vomeronasal 1 receptor 1	-2.13	0.0006
PTPRM	protein tyrosine phosphatase, receptor type, M	-2.05	0.0012
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	-2.02	0.0124
PTPRM	protein tyrosine phosphatase, receptor type, M	-2.02	0.0003
Up regulated genes			
NTRK2	neurotrophic tyrosine kinase, receptor, type 2	2.01	0.0185
LOC728806	Similar to N-ethylmaleimide-sensitive factor	2.10	0.0081
MSL-1	Male-specific lethal-1 homolog	2.10	0.0018
VEPH1	ventricular zone expressed PH domain homolog 1 (zebrafish)	2.24	0.0107
B9D1	B9 protein domain 1	2.41	0.0106
MGC50559	hypothetical protein MGC50559	2.41	0.0493
RAB6A	RAB6A, member RAS oncogene family	2.50	0.0159
NALCN	sodium leak channel, non-selective	2.67	0.0022
PTPRD	protein tyrosine phosphatase, receptor type, D	2.67	0.0106
TMEM176B	Transmembrane protein 176B	2.78	0.0046

**Selected (absolute fold change > 2.0 [FC] and Student's T test p-value [p-value] < o.05) list of differentially expressed genes.

related differentially expressed genes in the current experiment with preeclampsia related differentially expressed genes in placenta at-delivery, reported before [7], are presented in **Figure 5**. GO processes of cell proliferation, response to hypoxia and smooth muscle contraction were over represented in preeclamptic placenta while GO processes of vasculature (blood vessel) development were over represented in early pregnancy peripheral blood among women who later developed preeclampsia.

Discussion

We demonstrated that preeclampsia is associated with differential gene expression in early pregnancy maternal peripheral blood. Genes participating in abnormal placentation (e.g COL1A1), immune/inflammation response (e.g. IKBKB) and cellular development (including cell cycle) (e.g. RB1) were differentially expressed. We identified transcription factors (e.g. Sp1, MAZ and MZF1) that may account for co-expression of differentially expressed genes. Comparison of preeclampsia related gene expression profiles of early pregnancy peripheral blood and placenta (at-delivery) suggest gestational age and tissue specific differences in pathophysiological processes (vasculature development versus hypoxia response, respectively) involved in preeclampsia.

Previous studies have investigated peripheral blood gene expression in relation to preeclampsia [10, 12, 18-20]. Okazaki et al reported up-regulation of pregnancy specific beta-1 glycoprotein and trophoblast glycoprotein in peripheral blood of women with preeclampsia at 38-

Table 3. List of differentially expressed genes

Gene Symbol	Gene Name	FC*	P-value*
DKFZp666G057	hypothetical protein DKFZp666G057	-5.4	0.0245
HSD17B12	Hydroxysteroid (17-beta) dehydrogenase 12	-3.39	0.0121
PLEKHG2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2	-2.79	0.0157
COL5A3	collagen, type V, alpha 3	-2.69	0.0432
LOC400581	GRB2-related adaptor protein-like	-2.64	0.0037
ACCN2	amiloride-sensitive cation channel 2, neuronal	-2.38	0.0078
GTSF1L	gametocyte specific factor 1-like	-2.35	0.0033
CLEC12B	C-type lectin domain family 12, member B	-2.33	0.0010
COL1A1	collagen, type I, alpha 1	-2.26	0.0466
ZNF496	zinc finger protein 496	-2.21	0.0003
VN1R1	vomeronasal 1 receptor 1	-2.13	0.0006
PTPRM	protein tyrosine phosphatase, receptor type, M	-2.05	0.0012
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	-2.02	0.0124
PLXNA1	plexin A1	-1.99	0.0040
LTK	leukocyte tyrosine kinase	-1.96	0.0268
ULK4	unc-51-like kinase 4 (C. elegans)	-1.96	0.0160
AGRN	agrin	-1.94	0.0293
GIMAP5	GTPase, IMAP family member 5	-1.93	0.0027
RAB40A	RAB40A, member RAS oncogene family	-1.93	0.0187
CLEC12A	C-type lectin domain family 12, member A	-1.92	0.0023
DKFZP761N09121	hypothetical protein DKFZp761N09121	-1.9	0.0050
RAB3IP	RAB3A interacting protein (rabin3)	-1.89	0.0002
MUC5B	mucin 5B, oligomeric mucus/gel-forming	-1.88	0.0019
PICK1	protein interacting with PRKCA 1	-1.88	0.0282
FAM70A	family with sequence similarity 70, member A	-1.87	0.0275
DUSP2	dual specificity phosphatase 2	-1.86	0.0000
LOC161527	promyelocytic leukemia	-1.86	0.0209
PTGDS	prostaglandin D2 synthase 21kDa (brain)	-1.85	0.0004
MLZE	melanoma-derived leucine zipper, extra-nuclear factor	-1.83	0.0018
UACA	uveal autoantigen with coiled-coil domains and ankyrin repeats	-1.83	0.0481
MTHFSD	Methenyltetrahydrofolate synthetase domain containing	-1.82	0.0484
TTC28	tetratricopeptide repeat domain 28	-1.82	0.0011
LRRC23	leucine rich repeat containing 23	-1.81	0.0094
CA3	carbonic anhydrase III, muscle specific	-1.8	0.0155
CENTG2	centaurin, gamma 2	-1.8	0.0003
GPR4	G protein-coupled receptor 4	-1.8	0.0011
LDB2	LIM domain binding 2	-1.8	0.0204
SOX15	SRY (sex determining region Y)-box 15	-1.8	0.0436
GIPC3	GIPC PDZ domain containing family, member 3	-1.79	0.0189
LYPD3	LY6/PLAUR domain containing 3	-1.79	0.0081
DSTN	Destrin (actin depolymerizing factor)	-1.78	0.0015
KLHDC4	Kelch domain containing 4	-1.78	0.0176
PCOLCE	procollagen C-endopeptidase enhancer	-1.77	0.0132
ZNF542	zinc finger protein 542	-1.77	0.0418
FLJ44606	hypothetical gene supported by AK126569	-1.75	0.0093
FAM120AOS	family with sequence similarity 120A opposite strand	-1.74	0.0191
HEL308	DNA helicase HEL308	-1.74	0.0057
HLCS	holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase)	-1.74	0.0044
TMEM46	transmembrane protein 46	-1.74	0.0064
TRIM47	tripartite motif-containing 47	-1.74	0.0119
CASP10	caspase 10, apoptosis-related cysteine peptidase	-1.73	0.0124
CEL	carboxyl ester lipase (bile salt-stimulated lipase)	-1.73	0.0300
GPRASP2	G protein-coupled receptor associated sorting protein 2	-1.73	0.0018
RDH16	retinol dehydrogenase 16 (all-trans)	-1.73	0.0262
DHCR7	7-dehydrocholesterol reductase	-1.72	0.0033
FN1	fibronectin 1	-1.72	0.0145
PQLC3	PQ loop repeat containing 3	-1.72	0.0219

USP18	ubiquitin specific peptidase 18	-1.71	0.0485
LOC150837	hypothetical protein LOC150837	-1.7	0.0311
TMEM177	transmembrane protein 177	-1.7	0.0084
LOC283859	hypothetical protein LOC283859	-1.69	0.0039
TAPBP	TAP binding protein (tapasin)	-1.69	0.0481
LOC283666	hypothetical protein LOC283666	-1.68	0.0059
HEYL	hairy/enhancer-of-split related with YRPW motif-like	-1.67	0.0283
SERHL	serine hydrolase-like	-1.67	0.0073
ZCCHC2	zinc finger, CCHC domain containing 2	-1.67	0.0118
ACOT4	acyl-CoA thioesterase 4	-1.66	0.0020
C10orf58	chromosome 10 open reading frame 58	-1.66	0.0079
CXCR6	chemokine (C-X-C motif) receptor 6	-1.66	0.0017
MKL2	MKL/myocardin-like 2	-1.66	0.0356
OSGIN1	oxidative stress induced growth inhibitor 1	-1.66	0.0496
ZNF804A	zinc finger protein 804A	-1.66	0.0091
ARL3	ADP-ribosylation factor-like 3	-1.65	0.0011
GPA33	glycoprotein A33 (transmembrane)	-1.65	0.0215
LOC751071	hypothetical protein LOC751071	-1.65	0.0043
RNF157	CDNA FLJ36181 fis, clone TESTI2026794	-1.65	0.0176
SF3A2	splicing factor 3a, subunit 2, 66kDa	-1.65	0.0075
7A5	putative binding protein 7a5	-1.64	0.0016
PTPN20A	protein tyrosine phosphatase, non-receptor type 20B	-1.64	0.0396
HEMK1	HemK methyltransferase family member 1	-1.63	0.0053
PMS2L4	postmeiotic segregation increased 2-like 4	-1.63	0.0252
TDRKH	tudor and KH domain containing	-1.63	0.0028
CD248	CD248 molecule, endosialin	-1.62	0.0186
FLJ35934	FLJ35934 protein	-1.62	0.0322
OIP5	Opa interacting protein 5	-1.62	0.0125
C5orf20	chromosome 5 open reading frame 20	-1.61	0.0312
FLJ45224	FLJ45224 protein	-1.61	0.0083
HDAC5	histone deacetylase 5	-1.61	0.0031
PDZD4	PDZ domain containing 4	-1.61	0.0231
ATG9B	ATG9 autophagy related 9 homolog B (S. cerevisiae)	-1.6	0.0183
CASKIN2	CASK interacting protein 2	-1.6	0.0006
FBXO15	F-box protein 15	-1.6	0.0250
FLJ37512	similar to Contactin-associated protein-like 3 precursor (Cell recognition molecule Caspr3)	-1.6	0.0307
GRAMD1B	GRAM domain containing 1B	-1.6	0.0112
LOC25845	hypothetical LOC25845	-1.6	0.0208
LOC791120	zinc finger protein 783	-1.6	0.0180
LRRC56	leucine rich repeat containing 56	-1.6	0.0102
ZNF10	zinc finger protein 10	-1.6	0.0210
C3orf39	chromosome 3 open reading frame 39	-1.59	0.0016
CYP4V2	Cytochrome P450, family 4, subfamily V, polypeptide 2	-1.59	0.0027
HEY2	hairy/enhancer-of-split related with YRPW motif 2	-1.59	0.0168
PMS1	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)	-1.59	0.0192
TMEM132E	transmembrane protein 132E	-1.59	0.0224
TRIM46	tripartite motif-containing 46	-1.59	0.0301
CACNB1	calcium channel, voltage-dependent, beta 1 subunit	-1.58	0.0096
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	-1.58	0.0085
ANKS6	ankyrin repeat and sterile alpha motif domain containing 6	-1.57	0.0279
BNIPL	BCL2/adenovirus E1B 19kD interacting protein like	-1.57	0.0137
JAG2	jagged 2	-1.57	0.0007
KPTN	kaptin (actin binding protein)	-1.57	0.0245
ME3	malic enzyme 3, NADP(+)-dependent, mitochondrial	-1.57	0.0172
MPI	mannose phosphate isomerase	-1.57	0.0098
RNF12	Ring finger protein 12	-1.57	0.0122
CLEC11A	C-type lectin domain family 11, member A	-1.56	0.0059
COL23A1	collagen, type XXIII, alpha 1	-1.56	0.0188
DOCK4	dedicator of cytokinesis 4	-1.56	0.0169
GARNL3	GTPase activating Rap/RanGAP domain-like 3	-1.56	0.0018
SPHK2	sphingosine kinase 2	-1.56	0.0170
TMEM117	transmembrane protein 117	-1.56	0.0128

ZNF668	zinc finger protein 668	-1.56	0.0325
CARD11	caspase recruitment domain family, member 11	-1.55	0.0076
CHD9	chromodomain helicase DNA binding protein 9	-1.55	0.0428
COL6A1	collagen, type VI, alpha 1	-1.55	0.0209
H2AFY	H2A histone family, member Y	-1.55	0.0459
IGSF11	immunoglobulin superfamily, member 11	-1.55	0.0236
TCF19	transcription factor 19 (SC1)	-1.55	0.005
UBQLNL	ubiquilin-like	-1.55	0.0101
C5orf42	chromosome 5 open reading frame 42	-1.54	0.0055
IFRG15	interferon responsive gene 15	-1.54	0.0142
LPHN1	latrophilin 1	-1.54	0.0052
MGC15705	hypothetical protein MGC15705	-1.54	0.0451
NRCAM	neuronal cell adhesion molecule	-1.54	0.0055
PTCH1	patched homolog 1 (Drosophila)	-1.54	0.019
RASGRP3	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	-1.54	0.0029
RUNX2	runt-related transcription factor 2	-1.54	0.0089
USP43	ubiquitin specific peptidase 43	-1.54	0.0057
CSNK1E	casein kinase 1, epsilon	-1.53	0.0191
CST4	cystatin S	-1.53	0.0195
DKFZP434C153	DKFZP434C153 protein	-1.53	0.0022
EDIL3	EGF-like repeats and discoidin I-like domains 3	-1.53	0.0341
FGFR4	fibroblast growth factor receptor 4	-1.53	0.0189
LOC388963	similar to short-chain dehydrogenase/reductase 1	-1.53	0.0094
NOS3	nitric oxide synthase 3 (endothelial cell)	-1.53	0.0032
C15orf50	chromosome 15 open reading frame 50	-1.52	0.0179
C2orf40	chromosome 2 open reading frame 40	-1.52	0.0257
FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	-1.52	0.0131
GATA3	GATA binding protein 3	-1.52	0.0022
PRR6	Proline rich 6	-1.52	0.0314
SPON1	spondin 1, extracellular matrix protein	-1.52	0.0025
CACHD1	cache domain containing 1	-1.51	0.0404
MCOLN3	mucolipin 3	-1.51	0.0115
PCDH7	protocadherin 7	-1.51	0.0042
PPP1R13L	protein phosphatase 1, regulatory (inhibitor) subunit 13 like	-1.51	0.0134
SLC24A1	solute carrier family 24 (sodium/potassium/calcium exchanger), member 1	-1.51	0.0332
SNRP70	small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen)	-1.51	0.0392
ATP13A4	ATPase type 13A4	-1.5	0.0369
DCST2	DC-STAMP domain containing 2	-1.5	0.0173
DMPK	dystrophia myotonica-protein kinase	-1.5	0.0204
INADL	InaD-like (Drosophila)	-1.5	0.0394
SNAPC4	small nuclear RNA activating complex, polypeptide 4, 190kDa	-1.5	0.0075
TMEM182	transmembrane protein 182	-1.5	0.0346
GOLIM4	golgi integral membrane protein 4	1.5	0.0372
GOLM1	golgi membrane protein 1	1.5	0.0253
TGM4	transglutaminase 4 (prostate)	1.5	0.0011
ADAM3A	ADAM metallopeptidase domain 3A (cyritestin 1)	1.51	0.0185
BCAS1	breast carcinoma amplified sequence 1	1.51	0.0066
HIST1H2BG	histone cluster 1, H2bg	1.51	0.0206
MGC13005	hypothetical protein MGC13005	1.51	0.0056
KBTBD2	kelch repeat and BTB (POZ) domain containing 2	1.52	0.0034
MUC20	Mucin 20, cell surface associated	1.52	0.0142
E2F1	E2F transcription factor 1	1.53	0.0431
CENPN	centromere protein N	1.54	0.0259
HUS1B	HUS1 checkpoint homolog b (S. pombe)	1.54	0.0096
MIPOL1	mirror-image polydactyly 1	1.54	0.0192
LAPTM4B	lysosomal associated protein transmembrane 4 beta	1.55	0.0211
LOC728142	hypothetical protein LOC728142	1.55	0.0059
PAP2D	phosphatidic acid phosphatase type 2	1.56	0.0227
SAMD5	sterile alpha motif domain containing 5	1.56	0.0492
WNK3	WNK lysine deficient protein kinase 3	1.56	0.0234
PAPPA	pregnancy-associated plasma protein A, pappalysin 1	1.57	0.0023

TMOD2	tropomodulin 2 (neuronal)	1.57	0.0392
CDH10	cadherin 10, type 2 (T2-cadherin)	1.58	0.0316
GAS2L3	growth arrest-specific 2 like 3	1.58	0.0409
HIPK1	homeodomain interacting protein kinase 1	1.58	0.0336
LOC151877	hypothetical protein LOC151877	1.58	0.0136
LONRF2	LON peptidase N-terminal domain and ring finger 2	1.58	0.0412
CTD-2248C21.2	G antigen 1	1.59	0.0415
AK7	adenylate kinase 7	1.61	0.0114
TFRC	transferrin receptor (p90, CD71)	1.61	0.0081
TMC1	transmembrane channel-like 1	1.61	0.0216
ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.62	0.0052
HIST1H4E	histone cluster 1, H4e	1.62	0.0038
PAK7	p21(CDKN1A)-activated kinase 7	1.62	0.0326
EGLN3	egl nine homolog 3 (C. elegans)	1.63	0.0009
XYLB	xylulokinase homolog (H. influenzae)	1.63	0.0219
CDC20B	Cell division cycle 20 homolog B (S. cerevisiae)	1.64	0.0428
RB1	retinoblastoma 1 (including osteosarcoma)	1.64	0.0001
SPAG4L	sperm associated antigen 4-like	1.64	0.0176
ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	1.65	0.0201
TSPAN17	tetraspanin 17	1.65	0.0146
CHD7	chromodomain helicase DNA binding protein 7	1.66	0.0204
IGSF3	immunoglobulin superfamily, member 3	1.66	0.0187
KIAA0644	KIAA0644 gene product	1.66	0.0167
DDX54	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54	1.67	0.0069
LTB4DH	leukotriene B4 12-hydroxydehydrogenase	1.67	0.0416
DCBLD2	discoidin, CUB and LCCL domain containing 2	1.68	0.01
KRT33A	keratin 33A	1.68	0.0312
PSG4	pregnancy specific beta-1-glycoprotein 4	1.68	0.0371
KIAA0746	KIAA0746 protein	1.69	0.0034
MFAP5	microfibrillar associated protein 5	1.71	0.0238
OR2B2	olfactory receptor, family 2, subfamily B, member 2	1.71	0.0457
SCN3B	sodium channel, voltage-gated, type III, beta	1.71	0.0493
LOC283194	hypothetical protein LOC283194	1.72	0.0499
SHC4	SHC (Src homology 2 domain containing) family, member 4	1.72	0.0146
SRGAP2P1	SLIT-ROBO Rho GTPase activating protein 2 pseudogene 1	1.72	0.0454
TAT	Tyrosine aminotransferase	1.72	0.0366
KRT25	keratin 25	1.73	0.0084
ATP13A3	ATPase type 13A3	1.74	0.0463
TTC7A	Tetratricopeptide repeat domain 7A	1.74	0.0178
EMCN	endomucin	1.75	0.0254
TMEM63A	Transmembrane protein 63A	1.75	0.0300
EVI1	Ecotropic viral integration site 1	1.77	0.0365
KCNB2	potassium voltage-gated channel, Shab-related subfamily, member 2	1.77	0.0333
FLJ14959	hypothetical protein FLJ14959	1.78	0.0072
UNC119B	Unc-119 homolog B (C. elegans)	1.78	0.0352
PHTF2	putative homeodomain transcription factor 2	1.79	0.0159
UTP11L	UTP11-like, U3 small nucleolar ribonucleoprotein, (yeast)	1.8	0.0496
DEFB107A	defensin, beta 107A	1.81	0.0363
LRP6	low density lipoprotein receptor-related protein 6	1.85	0.0161
RNF32	ring finger protein 32	1.86	0.0377
KIAA2022	KIAA2022	1.87	0.0241
ANKRD30B	ankyrin repeat domain 30B	1.88	0.0446
EDN1	endothelin 1	1.91	0.0163
RNF150	ring finger protein 150	1.91	0.0197
MMP25	matrix metallopeptidase 25	1.95	0.0191
PDE1A	phosphodiesterase 1A, calmodulin-dependent	1.96	0.0244
OR5H1	olfactory receptor, family 5, subfamily H, member 1	1.98	0.0429
NTRK2	neurotrophic tyrosine kinase, receptor, type 2	2.01	0.0185
LOC728806	Similar to N-ethylmaleimide-sensitive factor	2.1	0.0081
MSL-1	Male-specific lethal-1 homolog	2.1	0.0018
VEPH1	ventricular zone expressed PH domain homolog 1 (zebrafish)	2.24	0.0107
B9D1	B9 protein domain 1	2.41	0.0106

MGC50559	hypothetical protein MGC50559	2.41	0.0493
RAB6A	RAB6A, member RAS oncogene family	2.5	0.0159
NALCN	sodium leak channel, non-selective	2.67	0.0022
PTPRD	protein tyrosine phosphatase, receptor type, D	2.67	0.0106
TMEM176B	Transmembrane protein 176B	2.78	0.0046

**List of differentially expressed genes in fold change (FC) and Students' T-test (p-value) analyses.

Table 4. Gene networks overrepresented by differentially expressed genes

#	Genes in Network*	Score	Focus genes	Functions
1	26s Proteasome, **ARHGEF12, CARD11, CASP10**, Caspase, Cdc2, **CLEC11A**, Cyclin A, **DUSP2**, E2f, **E2F1, EGLN3, FGFR1**, Filamin, Hdac, **HDAC5**, Histone h3, Histone h4, **MECOM**, Mek, NFkB (complex), **OIP5, OSGIN1**, Pi3-kinase, **PML**, **PPP1R13L**, Ras, Rb, **RB1, RORA, RUNX2**, Shc, **SNRNP70, TMOD2**, Vegf	32	19	Cellular Development, Hematological System Development and Function, Hematopoiesis
2	**CA3**, CD28, **CENPN, COL23A1, CSGALNACT1, CSNK1E**, CSNK1G2, dihydrotestosterone, **EPS15**, GRB2, **HEYL, KCNB2, LRP6, LRRC23**, NAA38, ONECUT1, **PMS1**, PPP1R3D, PPP2R1A, PRNP, RDH5, **RDH16**, REPS1, RNF20, RPS28, SGIP1, **SLC24A1**, SMAD3, SOST, SVIL, **TGM4**, TMF1, UGT2B11, UGT2B15, UGT2B@	22	14	Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism
3	**AGAP1**, Alp, Ap1, **COL5A3**, Collagen type I, Collagen(s), **DCBLD2, EDN1**, ERK, ERK1/2, Fgf, **FGFR4, FN1**, Focal adhesion kinase, **HEY2**, Ifn gamma, IL1, Laminin, **LYPD3**, Mapk, **MUC5B, NTRK2, PCDH7, PCOLCE**, Pdgf, PDGF BB, PI3K, Pkc(s), PLC gamma, **PLEKHG2**, Rac, Rap1, **RASGRP3**, TCR, Tgf beta	22	14	Cellular Development, Visual System Development and Function, Cellular Assembly and Organization
4	Actin, Actin-Nrf2, BCL2, **BNIPL, CD248, DSTN, EMCN**, ENDOG, FMO1, FOXF2, GFI1B, **GIMAP5**, GSTT1, **JAG2**, JARID2, LGALS3BP, **MCOLN3**, MEGF6, MEGF8, **MFAP5**, MYH14, **NCALD**, NFE2L2, NQO2, **PDZD4**, SERPINB8, SLC1A4, **SNAPC4**, STARD3, TBP, TNF, TROPONIN, **UACA**, Vacuolar H+ ATPase, **ZNF496**	20	13	Cell Morphology, Cell-To-Cell Signaling and Interaction, Cell Death
5	**CASKIN2**, CDH6, CDH7, CDH8, CDH9, **CDH10**, CDH15, CDH17, CDH18, CDH22, **CLYBL**, CTNNAL1, CTNNB1, **EDIL3**, FBXO8, **FBXO15**, GNB2L1, **GOLM1**, GPX2, Groucho, **HLCS**, HNF1A, **KRT33A**, MIRLET7D (includes EG:406886), **MKL2, NRCAM**, PCCA, **PTCH1**, Scf Trcp beta, SKP1, SRF, **TRIM46**, TSG101, **TSPAN17**, ZNF365	19	13	Cell-To-Cell Signaling and Interaction, Tissue Development, Embryonic Development
6	**ARL3, C10ORF58**, CD70, **CEL, CST4**, DGKA, GIP2, **GPA33**, Hla-abc, IFNA2, **IGSF3**, IkB-Tp53, KLF4, **LAPTM4B**, MT1L, NFKBIA, **PQLC3**, PROM1, PYHIN1 (includes EG:149628), **SCN3B**, SLC19A2, SOD2, **SPHK2, SPON1**, TACC3, TBX3, TEP1, TERT, **TMC1**, TP53, TRIM14, TRIM22, TRIM28, Ube3, **ZNF10**	19	13	Cellular Growth and Proliferation, Cell Cycle, Cell Death
7	**ABCC4**, ACCN1, **ACCN2**, ATXN1, **BCAS1**, beta-estradiol, BICD1, **CACNB1, DDX54**, GMEB2, GRM2, GRM3, HSD17B12, **HUS1B**, IFT122, MAL, MATN2, MIR133A-1, NAPB, NARS, NR1I3, NR3C1, **NSF, OBSCN (includes EG:84033)**, PAPSS2, **PICK1**, PTP4A2, **RAB6A**, SAPS2, SEPT3, SLC1A6, SULT1A1, TGTP1, **ZCCHC2, ZNF804A**	18	12	Cancer, Psychological Disorders, Cell-To-Cell Signaling and Interaction
8	4930444G20RIK, ADAMTS14, alcohol group acceptor phosphotransferase, amino acids, ASTL, CPA5, **DMPK**, DPEP3, **FAM70A, GRAMD1B, HIPK1**, IMMP2L, **KIAA2022, LTK**, MIR129-2 (includes EG:406918), MIR195 (includes EG:406971), MIR362 (includes EG:574030), MMP1B, NAALADL1, **PAK7**, PEPC (includes EG:109616), peptidase, PRKX, **PRPF4B**, PRT5, PRT6, **PTPRD, PTPRM, RNF150**, SENP5 (includes EG:303874),	18	12	Genetic Disorder, Skeletal and Muscular Disorders, Protein Degradation

	SOLH, **TMEM132E**, TMPRSS11D, UPG2, YME1L1			
9	ABLIM, Akt, ATP9A, CREBL2, **DHCR7**, DHRS3, DYRK3, **FASN**, FSH, **GATA3**, GK7P, IFN Beta, IgG, IGKV1-117, **IKBKB**, IL12 (complex), Insulin, Interferon alpha, Jnk, Jun-ATF2, Lh, LOC81691, MAS1, P38 MAPK, PI4K2A, Pka, QRFP, **RASAL2, RPA1, TAPBP, TFRC**, TP53I11, **TRIL, USP18, ZNF668**	16	11	Cellular Response to Therapeutics, Lipid Metabolism, Reproductive System Development and Function
10	ACOT2, **ACOT4**, ACOT5, ACOT7, ACOT8, ACOT9, ACOT1 (includes EG:26897), ACOT1 (includes EG:641371), BAAT, C22ORF28, C2ORF47, C3ORF26, **CLEC12B**, FASTKD2, **GOLIM4**, GPX7, GSTK1, HNF4A, LAS1L, MTR, OGFR, palmitoyl-CoA hydrolase, PPT1, PTPN11, **SEL1L3, TCF19, TDRKH**, TLN1, **TMEM63A, TOR1AIP2**, TSC22D1, USMG5, **UTP11L (includes EG:51118), VEPH1, VN1R1**	16	11	Lipid Metabolism, Nucleic Acid Metabolism, Small Molecule Biochemistry
11	ADAMTS3, ADAMTS13, ADK, AKT1, Akt-Calmodulin-Hsp90-Nos3, **ATG9B, ATP13A3 (includes EG:79572)**, C1QC, **CACHD1**, CASP3, **COL6A1**, CORO1C, cyclic AMP, F2, FGL2, FXYD5, Lamin, LOXL2, **LPHN1, MAGI2**, NOS3, PDE11A, **PDE1A**, PDE4C, PDE7A, **PLXNA1**, PPT1, **PTGDS**, SLC12A7, SOLH, **SOX15**, TGFB1, USP25, **WNK3**, ZMIZ1	16	11	Reproductive System Disease, Cell Morphology, Inflammatory Disease
12	**AGRN**, ARPP19, **B9D1,** CEBPB, **CENPV**, CLPX, **COPG2**, Creb, **CXCR6 (includes EG:10663)**, DDX42, DRD1, ENO3, ENTPD2, GPR183, HSPD1, HTT, KLF16, **KPTN, LDB2**, LMO4, MYL4, NDUFA3, PCTP, **PSG4, RLIM**, SCHIP1, SEPP1, **SF3A2**, SMARCA4, SRGAP3, SRRT, TNNI2, TRAP1, VIPR2, ZNF675	14	10	Carbohydrate Metabolism, Cell Signaling, Nucleic Acid Metabolism

*The networks were generated using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These genes were overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment is then assessed using a network score (negative log of p-values of Fisher tests). Focus genes (in bold) are genes identified in our list of differentially expressed genes. Networks shown here are those with network scores > 3.0.

Table 5. Microarray and qRT-PCR expression measurement comparisons

Gene symbol	**Gene description**	**qRT-PCR**		**Microarray**	
		Fold change	P-value	Fold change	P-value
CLEC11A	C-type lectin domain family 11, member A	1.06	0.763	-1.56	0.006
CLEC12A	C-type lectin domain family 12, member A	-1.52	0.22	-1.54	0.018
CLEC12B	C-type lectin domain family 12, member B	-2.08	0.024	-1.59	0.001
MMP25	Matrix metallopeptidase 25	1.09	0.705	1.95	0.019
FGFR1	Fibroblast growth factor receptor 1	-1.24	0.247	-1.52	0.013
LTK	Leukocyte receptor tyrosine kinase	-1.25	0.428	-1.96	0.027
PML	Promyelocytic leukemia protein	-1.08	0.685	-1.86	0.021
PPP1R13L	Protein phosphatase 1, regulatory subunit 13 like	1.05	0.813	-1.51	0.013

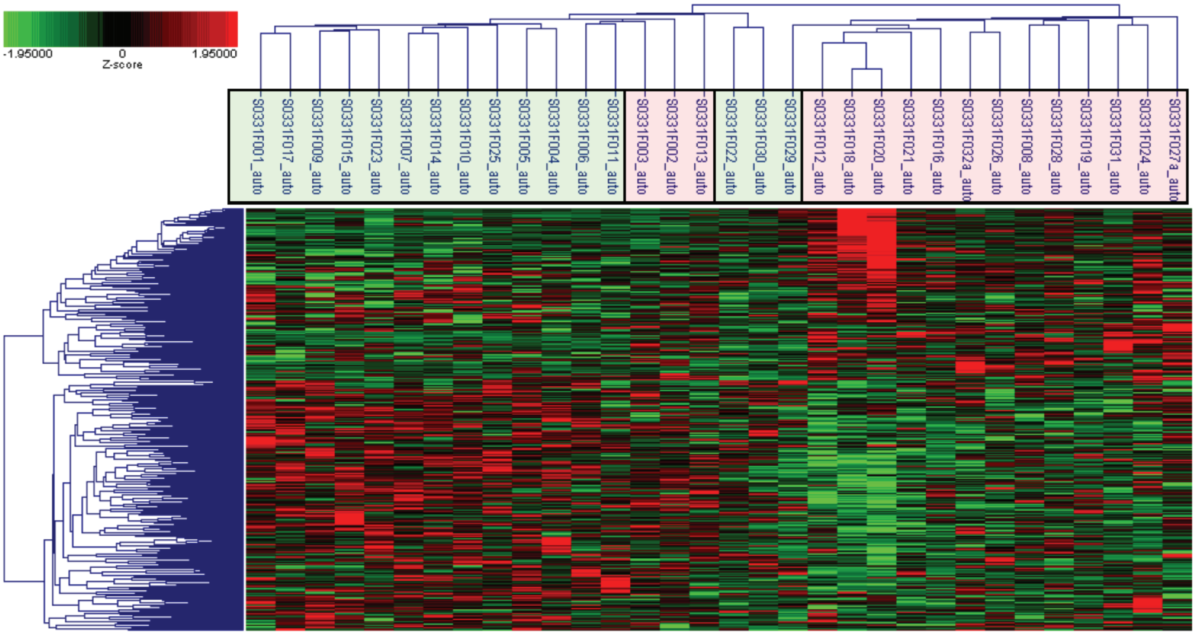


Figure 1 Hierarchical clustering of participants and differentially expressed genes. Probes (N=356) representing differentially expressed genes (N=247) (upregulated: shades of red and downregulated: shades of green) (rows) and participants (columns, cases=pink and controls=green) grouped according to level and nature of expression and similarity of expression profiles (participants) and subjected to hierarchical tree clustering.

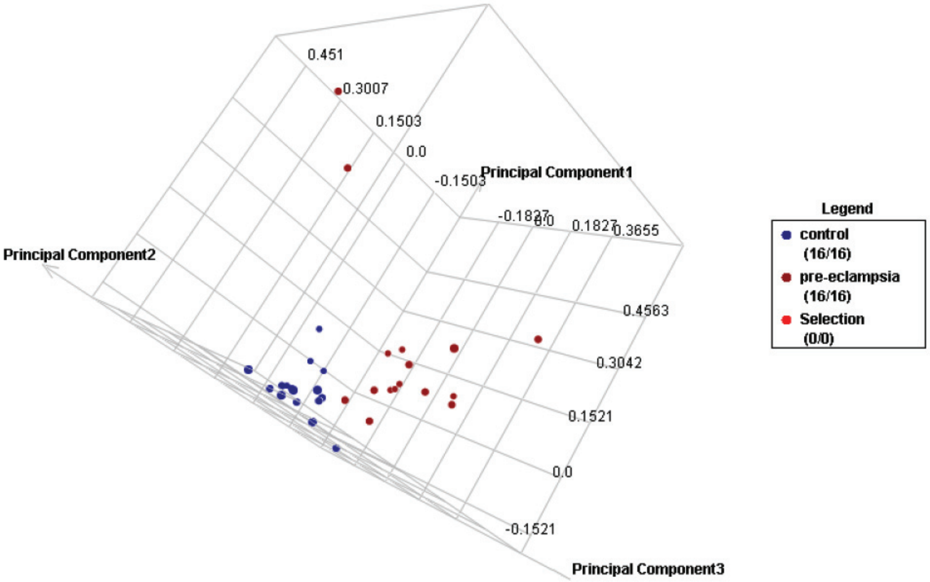


Figure 2 Principal components analysis. Principal component analysis results of all samples (16 preeclampsia cases and 16 controls) using expressions measured by probes (N=356) representing differentially expressed genes (N=247). (Red/right: cases, Blue/left: controls).

39 weeks of gestation [18]. Purwosunu et al, in a qRT-PCR based study of peripheral blood samples collected around 39 weeks of gestation, reported that expression of CRH, PLAC1 and P-Selectin were up-regulated in women with preeclampsia [19]. In another qRT-PCR based study, Purwosunu and colleagues have reported differential regulation of angiogenesis-related genes including Flt-1 and VEGF in peripheral blood of women with preeclampsia at 38-39 weeks of

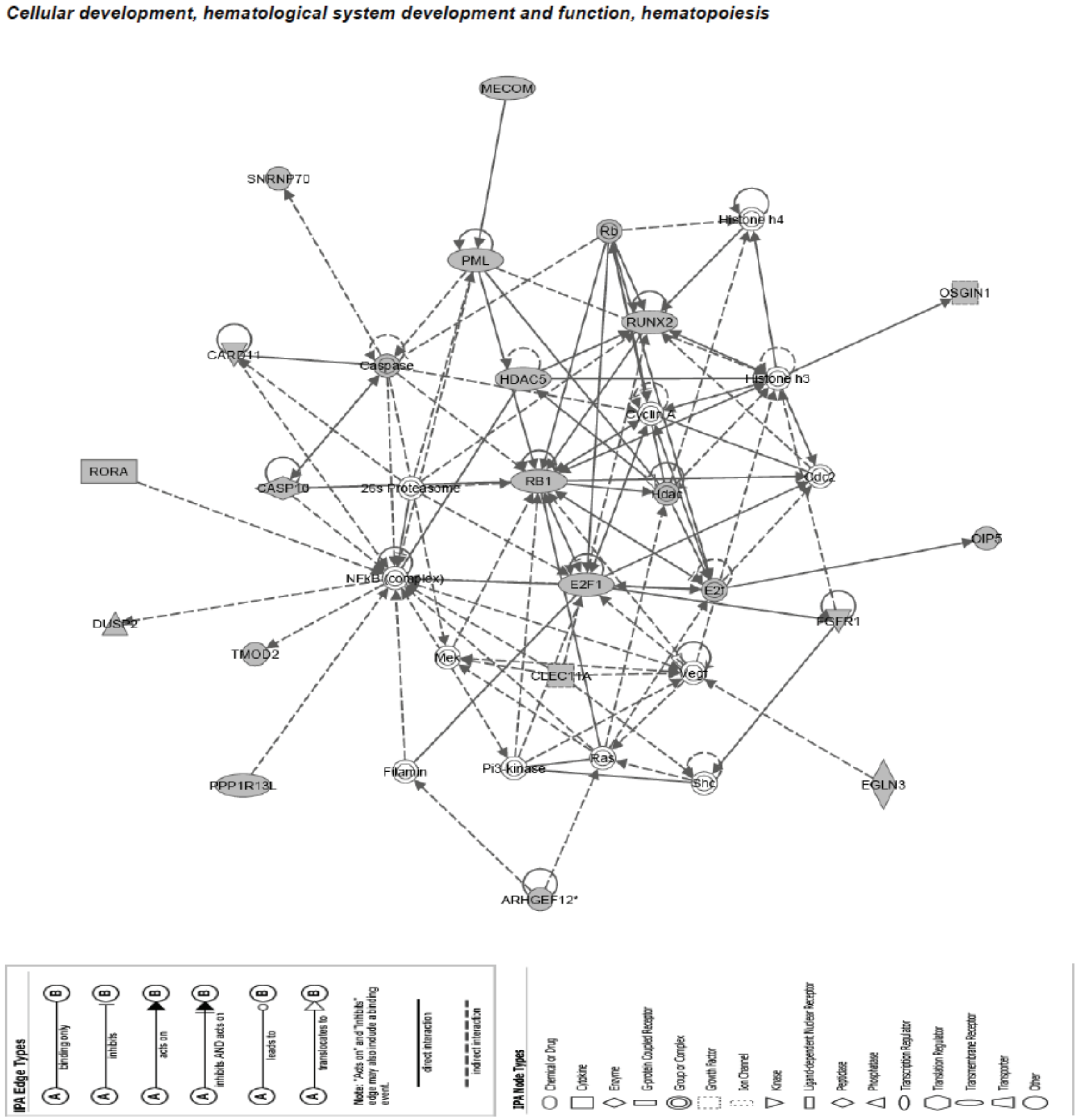


Figure 3. Top network overrepresented by differentially expressed genes. The networks were generated using Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These genes were overlaid onto a global molecular network developed from information contained in the IPKB. Network enrichment is then assessed using a network score (negative log of p-values of Fisher tests). Focus genes (shaded) are genes identified in our list of differentially expressed genes.

gestation [20]. Sun et al reported that 72 genes involved in cell proliferation, smooth muscle contraction and immune response were differentially expressed in peripheral blood of women with preeclampsia at 24-32 weeks of gestation [21]. In a follow-up study of chorionic villus gene expression study in early pregnancy (11 weeks), Farina et al investigated expressions of selected genes in third trimester peripheral blood of women who developed preeclampsia [10]. They reported up regulation of ADD1, BTD7, CLDN6, LTF and MAS1 in third trimester peripheral

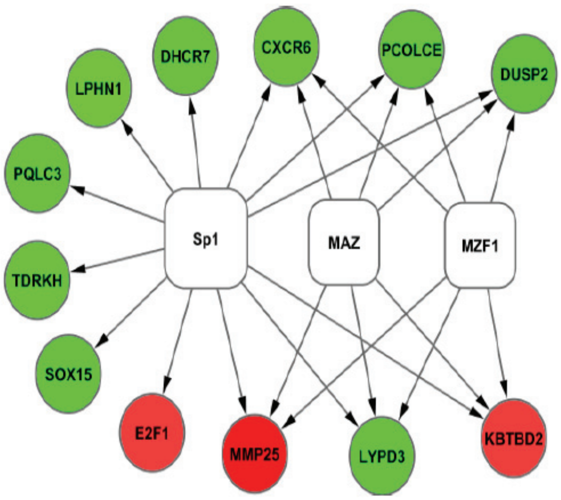
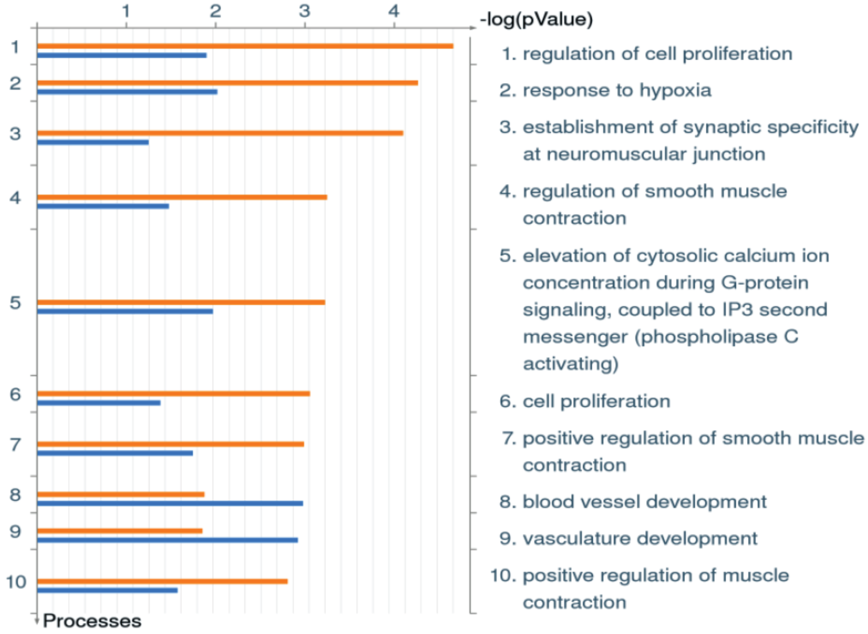


Figure 4. Promoter analysis results of differentially expressed genes. Inferred network of differentially expressed genes (Red=up regulated and Green=down regulated) in preeclampsia and transcription factors (White). Transcription factors were identified by their binding to over expressed promoter sequences in the differentially expressed genes.

blood of women with preeclampsia. Recently, Sekizawa et al investigated expressions of selected candidate genes in peripheral blood at 16-17 weeks to identify early pregnancy markers of preeclampsia [12]. In their study, expressions of FLT-1, ENG, P-Selectin, PLAC1, P1GF and HO-1 were deregulated in the case group while no differences were present between cases and controls in expressions of TGFB1, VEGF and SOD [12].

Investigators have also studied gene expression profiles in chorionic villus tissue samples collected in early pregnancy in relation to risk of preeclampsia [10-11]. Farina et al reported preeclampsia related differential expression of genes involved in trophoblast invasion, inflammation, endothelial dysfunction, angiogenesis and blood pressure control in chorionic villus samples in early pregnancy (11 weeks) [10]. Similarly, Founds et al reported deregulation of genes related to inflammation/immune regulation in early pregnancy (10-12 weeks) chorionic villus samples in women who later developed preeclampsia [11]. Genes involved in hypoxia or oxidative stress responses were not differentially expressed in their samples, similar to our findings. In sum, review of current and previous preeclampsia related gene expression profiles from blood and placental tissues suggest early pregnancy changes consistent with alterations in angiogenesis and immune/inflammatory response in contrast to late pregnancy changes which are consistent with alterations in response to hypoxemia or oxidative stress and subsequent endothelial dysfunction.



Note: *Yellow:* Preeclamptic placenta, and
Blue: Early pregnancy peripheral blood

Figure 5. Comparison of gene ontology processes. Gene ontology (GO) processes over represented by differentially expressed genes in early pregnancy maternal peripheral blood (blue) and preeclamptic placenta (yellow).

In our study, several genes that participate in abnormal placentation were differentially expressed in preeclampsia. In their candidate gene study, Goddard et al reported associations of variations in the COL1A1 gene with risk of preeclampsia [22]. COL1A1 is a gene coding for a protein in collagen metabolism (similar to COL5A3, also differentially expressed in our study) which influence maternal extracellular matrix composition and subsequently trophoblast migration [23]. NRTK2 is a brain derived neurotrophin family of proteins known to activate the high-affinity tyrosine kinase [24]. Kawamura et al, using *in vitro* and *in vivo* studies, have previously demonstrated important roles of the tyrosine kinase B signaling system and related neurotrophins in implantation and placental development through regulation of trophoblast cell growth [24].

Several genes in the immune response/ inflammation and cell cycle pathways were also differentially expressed related to preeclampsia in our study. For instance, genes constituting the CLEC family of genes (e.g. CLEC11A, CLEC12A and CLEC12B) were down regulated. These C-type lectin receptors play crucial roles in immunity and homeostasis, particularly in pathogen and self-antigen recognition, pathomechanisms that have been implicated in preeclampsia [25-27]. Regulatory signal pathways of the inflammatory system involving TNFRSF1ATRAFs, IKBKB and NFKB genes have been described [28]. IKBKB was differentially expressed in our study, while NFKB plays a central role in the top network that was over represented by differentially expressed genes. Genes participating in the RB_E2R1 cell cycle pathway were also differentially expressed in our study. While most research in this pathway has been done in cancer research, recently, interest in this pathway related to vascular disorders has increased following identification of E2F1 binding sites in promoters of angiogenesis related genes (e.g. FLT-1) [29].

We identified putative transcription factors (i.e., Sp1, MAZ and MZF1) that may be responsible for co-expression of differentially expressed genes. Sp1 has been associated with transcription of genes involved in syncytiotrphoblast differentiation such as the PSG family of genes (e.g. PSG4 up regulated in our study), endoglin and TGFβ1 and 2 other genes [30]. Further research in this area may enhance understanding of mechanisms of abnormal syncytiotrophoblast differentiation and related pathologies such as preeclampsia.

Our study has several strengths and limitations. It is the first global microarray based study investigating risk of preeclampsia and early pregnancy differential gene expression in peripheral blood, to our knowledge. Evaluation of functions and functional relationships of differentially expressed genes, for example using GO processes, as observed in past reports, enhances comparison of findings across studies [31]. By comparing preeclampsia related differential gene expression in early pregnancy peripheral blood and placenta at-delivery, we were able to present corroborative evidence for recent hypotheses that seek to elucidate gestational age and/or tissue specific gene expression changes associated with preeclampsia [4].

Several limitations of our study deserve mention. Single measurement of peripheral blood gene expression may not provide a full picture of gene expression changes across gestation. Evaluation of whole blood gene expression, a potentially heterogeneous cell population, does not allow comparisons of expression differences across similar cell subtypes. We were able to confirm microarray-based measurement for approximately 75% of genes in our confirmatory qRT-PCR study. This is comparable to other previous reports that range between 60-75% [32-33]. Further, most fold change differences observed were in the same direction in both experiments. For the two genes with different fold change directions (up or down regulation) between the two experiments, the qRT-PCR based gene expression differences were close to 1 (1.05 for PPP1R13L and 1.06 for CLEC11A).

In summary, we demonstrated maternal early pregnancy peripheral blood gene expression in early pregnancy. Differentially expressed genes participate in cellular processes of placentation, immune function/inflammation and cell growth (cell cycle). Besides improving understanding of pathogenesis of preeclampsia, early pregnancy peripheral blood gene expression profiling may provide critical windows of opportunity for disease prevention, early detection and/or treatment.

Acknowledgments

The authors are indebted to the participants of the Omega study for their cooperation. They are also grateful for the technical expertise of staff of the Center for Perinatal Studies, Swedish Medical Center. This work was supported by grants from the National Institute of Child Health and Human Development, National Institutes of Health (HD/HL R01-32562 and R01-055566).

Please address correspondence to: Daniel A. Enquobahrie, MD, PhD, Center for Perinatal Studies, Swedish Medical Center, 1124 Columbia Street, Suite 750, Seattle, WA 98104, Tel: 206 215 2595 Fax: 206 215 6995, E-mail: danenq@u.washington.edu

References

[1] Baumwell S, Karumanchi SA. Pre-Eclampsia: Clinical Manifestations and Molecular Mechanisms. Nephron Clin Pract 2007;106:c72-c81.

[2] Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. Microvasc Res 2008;75:1-8.

[3] Kanasaki K, Kalluri R. The biology of preeclampsia. Kidney Int 2009;76:831-837.

[4] Foidart JM, Schaaps JP, Chantraine F, Munaut C, Lorquet S. Dysregulation of anti-angiogenic agents (sFlt-1, PLGF, and sEndoglin) in preeclampsia--a step forward but not the definitive answer. J Reprod Immunol 2009;82:106-111.

[5] Chappell S, Morgan L. Searching for genetic clues to the causes of pre-eclampsia. Clin Sci (London) 2006;110:443-458.

[6] Sitras V, Paulssen RH, Grønaas H, Leirvik J, Hanssen TA, Vartun A, Acharya G. Differential placental gene expression in severe preeclampsia. Placenta 2009;30:424-433.

[7] Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS, Williams MA. Differential placental gene expression in preeclampsia. Am J Obstet Gynecol 2008;199:566.e1-11.

[8] Nishizawa H, Pryor-Koishi K, Kato T, Kowa H, Kurahashi H, Udagawa Y. Microarray analysis of differentially expressed fetal genes in placental tissue derived from early and late onset severe pre-eclampsia. Placenta 2007;28:487-497.

[9] Reimer T, Koczan D, Gerber B, Richter D, Thiesen HJ, Friese K. Microarray analysis of differentially expressed genes in placental tissue of pre-eclampsia: up-regulation of obesity-related genes. Mol Hum Reprod 2002;8:674-680.

[10] Farina A, Morano D, Arcelli D, De Sanctis P, Sekizawa A, Purwosunu Y, Zucchini C, Simonazzi G, Okai T, Rizzo N. Gene expression in chorionic villous samples at 11 weeks of gestation in women who develop preeclampsia later in pregnancy: implications for screening. Prenat Diagn 2009;29:1038-1044.

[11] Founds SA, Conley YP, Lyons-Weiler JF, Jeyabalan A, Hogge WA, Conrad KP. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. Placenta 2009;30:15-24.

[12] Sekizawa A, Purwosunu Y, Farina A, Shimizu H, Nakamura M, Wibowo N, Rizzo N, Okai T. Prediction of pre-eclampsia by an analysis of placenta-derived cellular mRNA in the blood of pregnant women at 15-20 weeks of gestation. BJOG 2010;117:557-564.

[13] Rainen L, Oelmueller U, Jurgensen S, Wyrich R, Ballas C, Schram J, Herdman C, Bankaitis-Davis D, Nicholls N, Trollinger D, Tryon V. Stabilization of mRNA expression in whole blood samples. Clin Chem 2002;48:1883-1890.

[14] Weng L, Dai H, Zhan Y, He Y, Stepaniants SB, Bassett DE. Rosetta error model for gene expression analysis. Bioinformatics 2006;22:1111-1121.

[15] Yeung KY, Ruzzo WL. Prinicipal component analysis for clustering gene expression data. Bioinformatics 2001;17:763-774.

[16] Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863-14868.

[17] Hooghe B, Hulpiau P, van Roy F, De Bleser P. ConTra: a promoter alignment analysis tool for identification of transcription factor binding sites across species. Nucleic Acids Res 2008;36:W128-W132.

[18] Okazaki S, Sekizawa A, Purwosunu Y, Farina A, Wibowo N, Okai T. Placenta-derived, cellular messenger RNA expression in the maternal blood of preeclamptic women. Obstet Gynecol 2007;110:1130-1136.

[19] Purwosunu Y, Sekizawa A, Farina A, Wibowo N, Okazaki S, Nakamura M, Samura O, Fujito N, Okai T. Cell-free mRNA concentrations of CRH, PLAC1, and selectin-P are increased in the plasma of pregnant women with preeclampsia. Prenat Diagn 2007;27:772-777.

[20] Purwosunu Y, Sekizawa A, Yoshimura S, Farina A, Wibowo N, Nakamura M, Shimizu H, Okai T. Expression of angiogenesis-related genes in the cellular component of the blood of preeclamptic women. Reprod Sci 2009;16:857-864.

[21] Sun CJ, Zhang L, Zhang WY. Gene expression profiling of maternal blood in early onset severe preeclampsia: identification of novel biomarkers. J Perinat Med 2009;37:609-616.

[22] Goddard KA, Tromp G, Romero R, Olson JM, Lu Q, Xu Z, Parimi N, Nien JK, Gomez R, Behnke E, Solari M, Espinoza J, Santolaya J, Chaiworapongsa T, Lenk GM, Volkenant K, Anant MK, Salisbury BA, Carr J, Lee MS, Vovis GF, Kuivaniemi H. Candidate-gene association study of mothers with pre-eclampsia, and their infants, analyzing 775 SNPs in 190 genes. Hum Hered 2007;63:1-16.

[23] Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbecev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J, Fazleabas AT, Fisher SJ, Giudice LC. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. Biol Reprod 2007;76:102-117.

[24] Kawamura K, Kawamura N, Sato W, Fukuda J, Kumagai J, Tanaka T. Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival. Endocrinology 2009;150:3774-3782.

[25] Hoffmann SC, Schellack C, Textor S, Konold S, Schmitz D, Cerwenka A, Pflanza S, Watzl C. Identification of CLEC12B, an inhibitory receptor on myeloid cells. J Biol Chem 2007;282:22370-22375.

[26] Thebault P, Lhermite N, Tilly G, Le Texier L, Quillard T, Heslan M, Anegon I, Soulillou JP, Brouard S, Charreau B, Cuturi MC,, Chiffoleau E. The C-type lectin-like receptor CLEC-1, expressed by myeloid cells and endothelial cells, is up-regulated by immunoregulatory mediators and moderates T cell activation. J Immunol 2009;183:3099-3108.

[27] Bonney EA. Preeclampsia: a view through the danger model. J Reprod Immunol 2007;76:68-74.

[28] Li W, Li H, Bocking AD, Challis JR. Tumor Necrosis Factor Stimulates Matrix Metalloproteinase 9 Secretion from Cultured Human Chorionic Trophoblast Cells Through TNF Receptor 1 Signaling to IKBKB-NFKB and MAPK1/3 Pathway. Biol Reprod. 2010 May 12. [Epub ahead of print]

[29] Pillai S, Kovacs M, Chellappan S. Regulation of vascular endothelial growth factor receptors by Rb and E2F1: role of acetylation. Cancer Res 2010;70:4931-4940.

[30] Nores R, Blanchon L, López-Díaz F, Bocco JL, Patrito LC, Sapin V, Panzetta-Dutari GM. Transcriptional control of the human pregnancy-specific glycoprotein 5 gene is dependent on two GT-boxes recognized by the ubiquitous specificity protein 1 (Sp1) transcription factor. Placenta 2004;25:9-19.

[31] Toxicogenomics Research Consortium. Standardizing global gene expression analysis between laboratories and across platforms. Nature Methods 2005;2:351-356.

[32] Jenson SD, Robetorye RS, Bohling SD, Schumacher JA, Morgan JW, Lim MS, Elenitoba-Johnson KS. Validation of cDNA microarray gene expression data obtained from linearly amplified RNA. Mol Pathol 2003;56:307-312.

[33] Rajeevan MS, Vernon SD, Taysavang N, Unger ER. Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. J Mol Diagn 2001;3:26-31.