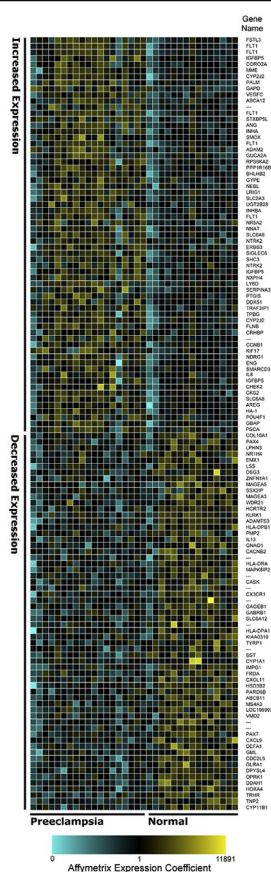


Discovery of antiangiogenic factors in the pathogenesis of preeclampsia



TO THE EDITORS: We report here the historic transcriptomics data that led to the discovery and characterization of antiangiogenic factors in the pathogenesis of preeclampsia. In 2001 and 2002, to identify novel secreted factors playing a pathologic role in preeclampsia, we performed gene expression profiling of placental tissue from 19 women with

FIGURE
Heatmap of up-regulated and down-regulated genes in preeclampsia



Colormap of the predictive gene set for placental mRNA expression in normal vs preeclampsia patients. The rows represent the predictive genes for preeclampsia, whereas the columns represent the expression levels for a given patient relative to the average gene expression. Significantly up-regulated genes include those coding for the fms-like tyrosine kinase 1 family of mRNAs and follistatin-related protein, whereas the significantly down-regulated genes include those coding for cytochrome P450 (CYP11B1). Genes with no names are left blank. However, their gene accession IDs are included in the [Supplemental Table](#).

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preeclampsia and 15 normotensive pregnant women using Affymetrix U95A microarray chips (Affymetrix, Santa Clara, California). Among the preeclamptic women, 89% were nulliparous compared with 19% among the normotensive women. The data were analyzed using Bayesian Analysis of Differential Gene Expression (BADGE method, version 1.0), a computer program implementing a Bayesian approach to identify differentially expressed genes across experimental conditions.¹ We identified a gene set of 127 genes; 65 of them were up-regulated and 62 were down-regulated, at a false positive rate of 1% ([Supplemental Table](#)). The [Figure](#) depicts the heatmap of the up- and down-regulated genes, many of which code for proteins regulating angiogenesis and metabolism. Among the most up-regulated transcripts were the endogenous inhibitor of vascular endothelial growth factor (VEGF) signaling, referred to as fms-like tyrosine kinase 1 (FLT1 or VEGFR1) and its soluble isoforms (sFLT1 or sVEGFR1). We characterized the sFLT1 pathway and noted that in animals, sFLT1 was sufficient to induce hypertension and proteinuria, and phenocopied several features of preeclampsia.² Taken together with the findings that anti-VEGF drugs were inducing preeclampsia-like phenotypes in cancer patients,³ we hypothesized that excess sFLT1 was responsible for the maternal syndrome of preeclampsia.^{2,4} Soluble endoglin, a second antiangiogenic protein in this list, was also further characterized by our group to play a synergistic role in the pathogenesis of preeclampsia.⁵ Nearly 20 years after the initial transcriptomic studies, the characterization of these antiangiogenic protein pathways have led to an improved understanding of the pathophysiology of preeclampsia, new biomarkers for early detection of the disease, and new therapeutic targets for this condition.⁶ Further characterization of other gene products described in the [Supplemental Table](#) may lead to a better understanding of the processes leading to preeclampsia. ■

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S.A.K. is listed as a coinvestigator on patents held by Beth Israel Deaconess Medical Center for the use of biomarkers in the prediction and treatment of preeclampsia. He has financial interest in Aggamin Pharmaceuticals, has served as consultant to Roche Diagnostics and Thermo Fisher Scientific, and reports receiving research funding from Thermo Fisher Scientific and Siemens. T.L. reports no conflict of interests.

This paper is part of a supplement.



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SUPPLEMENTAL TABLE

Differential expression of genes by Bayesian analysis (upregulated in preeclampsia in yellow and downregulated in preeclampsia in blue)

Affy Probe	Probability	Fold	Gene Symbol	Gene Name
33900_at	0.99992	3.849	FSTL3	follistatin-like 3 (secreted glycoprotein)
990_at	0.99990	3.233	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
991_g_at	0.99989	2.727	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
1601_s_at	0.99986	3.254	IGFBP5	insulin-like growth factor binding protein 5
36317_at	0.99982	3.767	CORO2A	coronin, actin binding protein, 2A
1389_at	0.99982	2.299	MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)
501_g_at	0.99980	2.293	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2
37657_at	0.99979	3.089	PALM	paralemmin
HUMGAPDH	0.99978	3.647	GAPD	glyceraldehyde-3-phosphate dehydrogenase
159_at	0.99969	3.343	VEGFC	vascular endothelial growth factor C
31754_at	0.99966	3.737	ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12
1149_at	0.99960	3.241	D16154	Transcription Factor Eb
1545_g_at	0.99959	2.692	FLT1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) ^a
34129_at	0.99953	2.211	STXBP5L	syntaxin binding protein 5-like
1103_at	0.99952	3.141	ANG	angiogenin, ribonuclease, RNase A family, 5
255_s_at	0.99950	2.761	INHBA	inhibin, alpha
1650_g_at	0.99948	2.745	SMOX	spermine oxidase
1964_g_at	0.99946	2.331	FLT1	Soluble fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) ^a
32298_at	0.99940	2.894	ADAM2	a disintegrin and metalloproteinase domain 2 (fertilin beta)
33995_at	0.99939	5.997	GUCA2A	guanylate cyclase activator 2A (guanylin)
32892_at	0.99937	2.014	RPS6KA2	ribosomal protein S6 kinase, 90kDa, polypeptide 2
41577_at	0.99910	2.361	PPP1R16B	protein phosphatase 1, regulatory (inhibitor) subunit 16B
40790_at	0.99903	2.169	BHLHB2	basic helix-loop-helix domain containing, class B, 2
41024_f_at	0.99891	2.617	GYPE	glycophorin E
36426_g_at	0.99879	1.981	NEBL	nebulette
34800_at	0.99868	2.943	LRIG1	leucine-rich repeats and immunoglobulin-like domains 1
36979_at	0.99868	2.389	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
31382_f_at	0.99851	2.065	UGT2B28	UDP glycosyltransferase 2 family, polypeptide B28
40357_at	0.99831	3.380	INHBA	inhibin, beta A (activin A, activin AB alpha polypeptide)

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(continued)

SUPPLEMENTAL TABLE

Differential expression of genes by Bayesian analysis (upregulated in preeclampsia in yellow and downregulated in preeclampsia in blue) (continued)

Affy Probe	Probability	Fold	Gene Symbol	Gene Name
1963_at	0.99822	2.714	FLT1	Soluble fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) ^a
35865_at	0.99815	2.632	NR5A2	nuclear receptor subfamily 5, group A, member 2
39051_at	0.99814	1.805	NNAT	neuronatin
33642_s_at	0.99807	3.236	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
33182_at	0.99804	2.698	NTRK2	neurotrophic tyrosine kinase, receptor, type 2
33639_g_at	0.99802	1.694	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
34483_at	0.99793	2.234	SIGLEC6	sialic acid binding Ig-like lectin 6
1511_at	0.99793	1.771	SHC3	src homology 2 domain containing transforming protein C3
38280_s_at	0.99787	3.286	NTRK2	neurotrophic tyrosine kinase, receptor, type 2
41420_at	0.99785	2.479	IGFBP5	insulin-like growth factor binding protein 5
34088_at	0.99783	2.009	NXPH4	neurexophilin 4
36284_at	0.99781	2.978	LY6D	lymphocyte antigen 6 complex, locus D
33825_at	0.99777	2.575	SERPINA3	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
36533_at	0.99742	2.354	PTGIS	prostaglandin I2 (prostacyclin) synthase
37813_at	0.99735	2.073	DDX51	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51
39202_at	0.99731	1.667	TRAF3IP1	TNF receptor-associated factor 3 interacting protein 1
368_at	0.99721	1.904	TPBG	trophoblast glycoprotein
500_at	0.99716	1.751	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2
38078_at	0.99699	1.774	FLNB	filamin B, beta (actin binding protein 278)
41608_at	0.99693	2.906	CRHBP	corticotropin releasing hormone binding protein
1734_at	0.99656	2.200	TGFB3	Human transforming growth factor beta-3 gene
1945_at	0.99644	1.747	CCNB1	cyclin B1
31990_at	0.99636	1.496	KIF17	kinesin family member 17
36933_at	0.99618	2.050	NDRG1	N-myc downstream regulated gene 1
32562_at	0.99610	1.941	ENG	endoglin (Osler-Rendu-Weber syndrome 1) ^a
32565_at	0.99606	2.098	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3
1369_s_at	0.99601	3.111	IL8	interleukin 8
1678_g_at	0.99589	2.334	IGFBP5	insulin-like growth factor binding protein 5
37887_at	0.99572	1.887	CHEK2	CHK2 checkpoint homolog (S. pombe)
40690_at	0.99568	1.913	CKS2	CDC28 protein kinase regulatory subunit 2
40926_at	0.99559	2.068	SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
34898_at	0.99558	2.179	AREG	amphiregulin (schwannoma-derived growth factor)
33748_at	0.99546	2.523	HA-1	minor histocompatibility antigen HA-1

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SUPPLEMENTAL TABLE

Differential expression of genes by Bayesian analysis (upregulated in preeclampsia in yellow and downregulated in preeclampsia in blue) (continued)

Affy Probe	Probability	Fold	Gene Symbol	Gene Name
35940_at	0.99536	2.086	POU4F1	POU domain, class 4, transcription factor 1
32632_g_at	0.99526	2.108	GBAP	glucosidase, beta; acid, pseudogene
33792_at	0.99518	2.318	PSCA	prostate stem cell antigen
38566_at	0.00495	0.730	COL10A1	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)
31740_s_at	0.00488	0.637	PAX4	paired box gene 4
33359_at	0.00485	0.547	LPHN3	latrophilin 3
38519_at	0.00476	0.483	NR1H4	nuclear receptor subfamily 1, group H, member 4
33046_f_at	0.00473	0.492	EMX1	empty spiracles homolog 1 (Drosophila)
39108_at	0.00472	0.616	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
33693_at	0.00451	0.499	DSG3	desmoglein 3 (pemphigus vulgaris antigen)
834_at	0.00436	0.615	ZNFN1A1	zinc finger protein, subfamily 1A, 1 (Ikaros)
34575_f_at	0.00416	0.480	MAGEA5	melanoma antigen, family A, 5
33379_at	0.00407	0.432	SSX2IP	synovial sarcoma, X breakpoint 2 interacting protein
31599_f_at	0.00390	0.420	MAGEA3	melanoma antigen, family A, 3
32935_at	0.00389	0.512	WDR21	WD repeat domain 21
33072_at	0.00361	0.809	HCRTR2	hypocretin (orexin) receptor 2
36777_at	0.00357	0.525	KLRK1	killer cell lectin-like receptor subfamily K, member 1
36269_at	0.00356	0.538	ADAMTS3	a disintegrin-like and metalloprotease (repolysin type) with thrombospondin type 1 motif, 3
38095_i_at	0.00351	0.596	HLA-DPB1	major histocompatibility complex, class II, DP beta 1
36272_r_at	0.00319	0.335	PMP2	peripheral myelin protein 2
494_at	0.00307	0.610	IL13	interleukin 13
34698_at	0.00300	0.522	GNAO1	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide 0
39646_at	0.00291	0.414	CACNB2	calcium channel, voltage-dependent, beta 2 subunit
36049_at	0.00278	0.497	W27899	CDNA clone IMAGE:4940887, partial cds
37039_at	0.00277	0.602	HLA-DRA	major histocompatibility complex, class II, DR alpha
37588_s_at	0.00262	0.621	MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2
33846_at	0.00260	0.522	AA620377	Cluster Incl. AA620377:ae57a07.s1 Homo sapiens cDNA, 3 end /clone=IMAGE-950964
36416_g_at	0.00259	0.512	CASK	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
1298_at	0.00256	0.447	X86816	Human estrogen receptor mRNA, alternatively spliced transcript H, partial cds.
40646_at	0.00235	0.562	CX3CR1	chemokine (C-X3-C motif) receptor 1
37108_at	0.00229	0.529	X72755	MRNA; cDNA DKFZp779B1535 (from clone DKFZp779B1535)
32997_at	0.00228	0.363	GAGEB1	G antigen, family B, 1 (prostate associated)
35028_at	0.00227	0.438	GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1

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SUPPLEMENTAL TABLE

Differential expression of genes by Bayesian analysis (upregulated in preeclampsia in yellow and downregulated in preeclampsia in blue) (continued)

Affy Probe	Probability	Fold	Gene Symbol	Gene Name
40679_at	0.00213	0.458	SLC6A12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12
39498_at	0.00213	0.497	AA044810	Cluster Incl. X86400:H.sapiens mRNA for gamma subunit of sodium potassium ATPase
38833_at	0.00199	0.670	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1
35031_r_at	0.00183	0.281	KIAA0316	KIAA0316 gene product
36911_at	0.00180	0.433	TYRP1	tyrosinase-related protein 1
31494_at	0.00175	0.434	L12691	Cluster Incl. D25272:Homo sapiens mRNA, clone-RES4-16
37782_at	0.00170	0.654	SST	somatostatin
36767_at	0.00164	0.302	CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1
35539_at	0.00159	0.386	IMPG1	interphotoreceptor matrix proteoglycan 1
38330_at	0.00159	0.371	FRDA	Friedreich ataxia
35061_at	0.00152	0.272	CXCL11	chemokine (C-X-C motif) ligand 11
34002_at	0.00139	0.627	HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2
32017_at	0.00139	0.531	PAR6B	par-6 partitioning defective 6 homolog beta (C. elegans)
31398_at	0.00132	0.440	ABCB11	ATP-binding cassette, sub-family B (MDR/TAP), member 11
32451_at	0.00131	0.556	MS4A3	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific)
34045_at	0.00131	0.503	LOC196993	hypothetical protein LOC196993
36428_at	0.00130	0.569	VMD2	vitelliform macular dystrophy (Best disease, bestrophin)
AFFX-DapX-3_a	0.00122	0.469	M33197	L38424 B subtilis dapB, jojF, jojG genes corresponding to nucleotides 1358-3197 of L38424
31324_at	0.00116	0.484	AF016492	U82303:Homo sapiens unknown protein mRNA
32474_at	0.00111	0.644	PAX7	paired box gene 7
37219_at	0.00098	0.395	CXCL9	chemokine (C-X-C motif) ligand 9
31506_s_at	0.00097	0.288	DEFA1	defensin, alpha 1, myeloid-related sequence
378_s_at	0.00075	0.529	GML	GPI anchored molecule like protein
41820_s_at	0.00073	0.570	CDC2L5	cell division cycle 2-like 5 (cholinesterase-related cell division controller)
31310_at	0.00061	0.523	GLRA1	glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)
39502_at	0.00046	0.553	DPYSL4	dihydropyrimidinase-like 4
35024_at	0.00031	0.272	OPRK1	opioid receptor, kappa 1
36220_at	0.00030	0.346	DDAH1	dimethylarginine dimethylaminohydrolase 1
204_at	0.00022	0.601	HOXA4	homeo box A4
750_at	0.00021	0.389	TRHR	thyrotropin-releasing hormone receptor

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(continued)

SUPPLEMENTAL TABLE**Differential expression of genes by Bayesian analysis (upregulated in preeclampsia in yellow and downregulated in preeclampsia in blue) (continued)**

Affy Probe	Probability	Fold	Gene Symbol	Gene Name
33478_at	0.00009	0.296	TNP2	transition protein 2 (during histone to protamine replacement)
1412_g_at	0.00008	0.560	CYP11B1	cytochrome P450, family 11, subfamily B, polypeptide 1

Genes selected with a 1.0% false positive error rate for a total of 127 genes, 65 of these are upregulated. Genes with no names are labeled with Genbank accession numbers.

^a FLT1, sFLT1 and Endoglin genes.

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