

Published in final edited form as:

FEBS Lett. 2002 October 23; 530(1-3): 17–23.

Vitamin E sensitive genes in the developing rat fetal brain: a high-density oligonucleotide microarray analysis

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Abstract

Vitamin E (tocopherols & tocotrienols) is essential for normal neurological function. Recently we have reported that the neuroprotective properties of tocotrienols are much more potent than that of the widely studied tocopherols (*J Biol Chem.* 275, 13049–13055). The objective of this study was to evaluate whether i) oral supplementation of tocotrienols during pregnancy is bioavailable to fetal and mother brains; ii) short-term change in dietary vitamin E levels of pregnant rats influences gene expression profile of developing fetal brains. We report that dietary tocotrienol is bioavailable to both mother and fetal brains. The enrichment is more in fetal brain tissue. Using a GeneChip microarray expression profiling approach we have identified a specific set of vitamin E sensitive genes in the developing rat fetal brain.

Keywords

fetus; pregnancy; diet; microarray; tocotrienol; tocopherol

1. INTRODUCTION

Vitamin E is essential for normal neurological function [1,2]. Based on symptoms of primary vitamin E deficiency, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function [2]. Most of the vitamin E-sensitive neurological disorders are associated with elevated levels of oxidative damage markers. This has led to the popular hypothesis stating that the neuroprotective effects of vitamin E are mediated by its antioxidant property [3]. Vitamin E is a generic term for all tocopherols and their derivatives having the biological activity of RRR- α -tocopherol [4,5]. In nature, eight substances have been found to have vitamin E activity: α -, β -, γ - and δ -tocopherol; and α -, β -, γ - and δ -tocotrienol. Often, the term vitamin E is synonymously used with α -tocopherol. While d- α -tocopherol (RRR- α -tocopherol) has the highest bioavailability and represents the standard against which all the others must be compared, it is only one out of eight natural forms of vitamin E.

Tocotrienols, formerly known as ζ , ϵ or η -tocopherols, are chemically similar to tocopherols except that they have an isoprenoid tail with 3 unsaturation points instead of a saturated phytol tail. While tocopherols are predominantly found in corn, soybean and olive oils, tocotrienols are particularly rich in palm, rice bran and barley oils [4,5]. Tocotrienols have been long known to possess powerful antioxidant, anti-cancer and cholesterol-lowering properties. Tocotrienols are thought to have more potent antioxidant properties than α -tocopherol [6,7]. The unsaturated

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side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver [8]. Superior antioxidant, free radical scavenging effects of tocotrienol compared to that of tocopherol appear to be due to their better distribution in the fatty layers of the cell membrane [8]. While tocotrienols have shown better beneficial effects than α -tocopherol in a limited number of situations indicated above, little is known about the exact mechanism of action. Micromolar amounts of tocotrienols, but not tocopherols, have been shown to suppress the activity of hydroxy-3-methylglutaryl coenzyme A reductase, the hepatic enzyme responsible for cholesterol synthesis [9,10]. We have observed that α -tocotrienol is much more potent than α -tocopherol in inhibiting glutamate-induced signal transduction pathways leading to neurodegeneration [11]. Previously it has been suggested that dietary α -tocotrienol does not reach the brain [12]. The objective of this study was two-fold. First, to confirm whether tocopherol and tocotrienol fed to pregnant rats reach the mother and fetal brains using a HPLC-CoulArray technique recently developed in our laboratory [13]. Second, we utilized a high-density oligonucleotide microarray approach to screen the developing (day 17th of gestation) fetal brain transcriptome for vitamin E sensitive genes.

2. MATERIAL AND METHODS

Animals and supplementation protocol

Pregnant (three-day) rats (10 wks old; Sprague Dawley; Harlan, Indianapolis, IN) were randomly divided into following two groups: *i) E⁺ group* – fed a standard rat chow that is enriched in α -tocopherol (~200 nmol/g diet). Additionally, this group was supplemented for 2 weeks with a daily gavage of tocotrienol rich fraction (TRF2) suspended in vitamin E-stripped corn oil (Harlan). A mixture of 110 mg α -tocopherol and 119 mg of α -tocotrienol contained in 1g TRF was fed to pregnant rats on a per kg bodyweight basis. TRF was provided in the form of Tocomin® 50% provided by Carotech Sdn Bhd (Perak, Malaysia); *ii) E⁻ group* – fed a vitamin E deficient diet (TD88163, Harlan; α -tocopherol/tocotrienol levels below detection limits) and supplemented with a matched volume of vitamin E-stripped corn oil. All rats were maintained under standard conditions at $22 \pm 2^\circ\text{C}$ with 12:12 h dark : light cycles. All animal protocols were approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) of the Ohio State University, Columbus, OH. *Sample collection.* On 17th day of gestation, body weights of each rat were recorded. Rats were killed. Mother and fetal brains were removed, rinsed in ice-cold phosphate buffered saline, pH 7.4 (PBS) and snap frozen in liquid nitrogen. Samples were briefly stored in -80°C .

Vitamin E extraction and analysis

Vitamin E extraction and analysis from mother and fetal brains was performed as described previously using a HPLC-coulometric electrode array detector (Coularray Detector - model 5600 with 12 channels; ESA Inc., Chelmsford, MA) [13]. This system uses multiple channels with different redox-potentials. α -, γ - and δ -Tocopherols and tocotrienols were detected on channels set at 200 mV, 300 mV; and 400 mV, respectively.

Affymetrix GeneChip probe array analysis

Total RNA was extracted by pulverizing the fetal brains in liquid N₂ followed by extraction using Trizol (Gibco BRL) [14,15]. A further clean up of RNA was performed using the RNeasy kit (Qiagen). Targets were prepared for microarray hybridization according to previously described protocols [14]. To assess sample quality the samples were hybridized for 16 hours at 45°C to GeneChip Test-2 arrays. Satisfactory samples were hybridized to Rat Genome arrays (U34A). The arrays were washed, stained with streptavidin-phycoerythrin and were then scanned with the GeneArray scanner (Agilent Technologies) in our facilities.

Raw data were collected and analyzed using Affymetrix Microarray Suite 4.0 (MAS) and Data Mining Tool 2.0 (DMT) software. The following two approaches were utilized to identify differentially expressed genes: **i)** using *Comparison Analysis* in MAS, six pair-wise comparisons were generated from replicates of both E⁺ and E⁻ groups. Average fold-changes were calculated for both up- or down-regulated genes. Genes for which the concordance exceeded 50% in pairwise comparisons were selected, especially if the gene was detected with redundant probe sets; **ii)** T-test was performed using DMT, and genes that significantly ($p < 0.05$) changed (increased or decreased) in the E⁺ group compared to the E⁻ group were selected. The average difference values of selected genes were loaded into the Cluster & TreeView software [16]. The data was adjusted according to the median center for a clear graphic display of vitamin E sensitive genes.

Reverse-transcription and polymerase chain reaction (RT-PCR)

Expression levels of heme oxygenase 3 (HO-3), cyclin D1, high mobility group protein 2 (HMG-2), nucleolar phosphoprotein p130 (NOPP140) and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA were independently determined using RT-PCR as described previously [17]. In brief, the total RNA (1 μ g) was transcribed into cDNA using oligo-dT primer and Superscript II. RT-generated cDNA were amplified by PCR using gene specific primers as described in Table 1. PCR reaction products were electrophoresed in a 1% agarose gel containing 0.25 μ g/ml ethidium bromide. The gel was digitally imaged under conditions of ultraviolet transillumination. Quantification of band intensity was performed using the Scion Image (Scion Corporation) that is based on NIH Image software.

3. RESULTS

Vitamin E levels in mother & fetal brains

α -Tocopherol level in the fetal brain was multi-fold lower than that observed in the mother brain (Figure 1A). Compared to E⁺ group, feeding a vitamin E deficient diet for only 2 weeks during pregnancy did not significantly decrease the α -tocopherol levels in the adult mother brain. However, under similar conditions, fetu from the mothers of E⁻ group had significantly lower α -tocopherol levels in brain compared to the fetu from E⁺ group (Figure 1A). α -Tocotrienol was below detection limits in the brains of mothers as well as fetu of the E⁻ group. Oral supplementation of TRF for 2 weeks to mothers during pregnancy resulted in delivery of α -tocotrienol to the mother as well as fetal brains. Importantly, incorporation of tocotrienol in the fetal brain was significantly higher compared to that in the mother brain (Figure 1B). Of interest, short-term vitamin E deficiency in pregnancy diet did not influence vital statistics of pups such as weight or general health (our unpublished observations).

Transcriptome profiling

The transcriptomes of developing fetal brains from E⁺ and E⁻ groups ($n=3$ in each) were compared using the U34A rat genome high-density oligonucleotide GeneChip array. This array analyzes approximately 7,000 full-length sequences and approximately 1,000 EST clusters. Using raw data from all replicates available from both groups, a total of six pair-wise comparisons were generated. The average (six pair-wise comparisons) fold-changes of all the genes that were differentially expressed were calculated. Data indicated that a majority of genes remained unchanged (Figure 2). A total of 645 (7.3%) genes were up-regulated in vitamin E⁺ group compared to the E⁻ group. Out of which 416 genes increased by a magnitude of 2-fold or more. On the other hand 152 (1.7%) of the genes were down-regulated with 74 of them lowered by 2-fold or more (Figure 2). Using the *t*-test analysis (see *Material and methods* for details), a total 144 genes were observed to have changed significantly ($p < 0.05$) in vitamin E deficiency group compared to the supplemented group. The data was adjusted according to the median center for a clear graphic display of vitamin E sensitive genes (Figures 3A & 3B). Next,

genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. Using this approach of data analysis, a total of 19 probe-sets were found to be up-regulated and 34 repressed in E⁺ group compared to E⁻ group (Tables 2 & 3). Among the up-regulated genes, two probe sets targeting heme oxygenase-3 (HO-3) were increased by 3.9 and 3.1 folds, respectively (Figure 4). In contrast, the expression of maspin, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), apolipoprotein B (apoB) and G protein beta1 subunit (rGb1) genes was highly (3–5 folds) repressed in response to dietary vitamin E.

Validation of GeneChip data using RT-PCR

Select vitamin E sensitive genes identified by the GeneChip approach were verified using conventional semi-quantitative RT-PCR (Figure 4). The band intensity of the PCR products was quantified and fold change for each gene in E⁺ group compared to E⁻ group was calculated. Data showed that fold change detected using both GeneChip or RT-PCR approaches for genes up-regulated E⁺ vs E⁻ groups were comparable (Figure 4). For GAPDH, both microarray as well as RT-PCR data indicated a decrease in expression in E⁺ group compared to E⁻ group. However, the fold-change in GAPDH expression was much higher in the microarray data compared to the RT-PCR data (Figure 4).

4. DISCUSSION

We present first evidence showing that consumption of a vitamin E deficient diet for only 2 weeks during pregnancy can substantially lower the vitamin E levels of fetal brain while not affecting the vitamin E levels of adult brain underscoring the importance of proper levels of this vitamin in the diet during pregnancy. Compared to an adult brain, a higher uptake of the α -tocotrienol form of vitamin E by fetal brain was also observed. Results of this study provides the first global assessment of vitamin E sensitive genes in a developing fetal brain. Of the 8000 genes surveyed, only 17 genes displayed an increase in gene expression levels in fetal brain as a result of vitamin E feeding to mothers, whereas 34 displayed a decrease in expression indicating that a highly specific set of genes are sensitive to the vitamin E levels in a developing fetal brain.

Based on symptoms of primary vitamin E deficiency in adults, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function [2]. However, efforts to systematically evaluate the molecular basis of vitamin E action on the brain are lacking. Our data show that α -tocopherol level in the fetal brain was multi-folds lower than that observed in the mother brain. This data is in accordance with a previous study where α -tocopherol levels in fetal brain were lower compared to that of the brains of 21-day old rats [18]. Furthermore, in humans, the serum α -tocopherol levels in full-term neonates are known to be several folds lower (0.212 \pm 0.127 vs 1.160 \pm 0.513 mg/dL) compared to that of their mothers [19]. We have previously shown that compared to α -tocopherol, tocotrienols are markedly more potent in protecting neuronal cells against glutamate-induced degeneration [11]. However, *in vivo* data demonstrating the availability of dietary tocotrienols in the brain was lacking. The present study provide first evidence that dietary supplementation of TRF during pregnancy leads to a significant enrichment of α -tocotrienol in both maternal and fetal brains. Dietary vitamin E is absorbed in the intestine and carried by lipoproteins to the liver. In the liver, the α -tocopherol fraction is incorporated into VLDL by a α -tocopherol transfer protein and then secreted into the bloodstream [20]. A recent study shows that scavenger receptor class B type I (SR-BI), which mediates cellular selective cholesteryl ester uptake from lipoproteins, facilitates efficient transfer of α -tocopherol from HDL to cultured cells [21]. Furthermore, in SR-BI-deficient mutant mice, relative to wild-type control animals, there was a significant increase in plasma α -tocopherol levels (1.1- to 1.4-fold higher) that was mostly

due to the elevated α -tocopherol content of their abnormally large plasma HDL-like particles [21]. Mechanisms of uptake and transport of tocotrienols in organs and tissues are poorly understood in adults and more so in fetal tissues.

HO-3 was one of the few vitamin E sensitive genes up-regulated in fetal brains. Heme oxygenase isozymes, HO-1, HO-2 and HO-3, are HSP32 protein cognates with a known function of catalyzing the isomer-specific oxidation of the heme molecule, including that of NO synthase [22]. HO-1 is highly inducible, whereas HO-2 and HO-3 are constitutively expressed. These proteins play a central role in the cellular defense mechanisms. HO activity is responsible for the production of equimolar amounts of CO, biliverdin and free Fe [22,23]. Recent findings with the hemeoxygenases suggest that these proteins may serve as an intracellular "sink" for NO [24]. LINE1 was identified to be another vitamin E sensitive transcript. The LINE-1, or L1 family of interspersed repeats accounts for at least 10% of the mammalian genome. Like other interspersed repeat DNA families in genomes of other organisms, L1 is dispersed and amplified throughout the genome by a series of duplicative transposition events. Due to the high copy number of L1 sequences in the genome, L1 is abundantly represented in the RNA population of most cells. However, most of the transcripts that contain L1 are the result of fortuitous transcription and are not intermediates in L1 retrotransposition. This high background of L1-containing transcripts, many of which are truncated and rearranged, makes it difficult to distinguish the transcript encoded by an active L1 element(s) [25]. ApoB mRNA was one of top candidates that were lower in E⁺ group compared to the E⁻ fetal group. ApoB plays a central role in lipoprotein metabolism and exists in 2 isoforms in plasma, apoB-100 and apoB-48 [26]. High levels of apoB and LDL cholesterol have been associated with an increased risk for coronary heart disease [27]. An earlier study has shown that administration of TRF (100 mg/day) decreases serum apoB [28]. Tocopherol has been shown to inhibit PKC activity in cells [29]. Protein kinase C-regulated chloride channel was one of the genes that was observed to be suppressed in the E⁺ group.

Taken together, these results provide evidence that compared to the adult brain, fetal brain is deficient in vitamin E and that fetal brain vitamin E status is tightly linked to the dietary vitamin E intake of the mother. Dietary tocotrienol is bioavailable to the brain. Gene expression patterns in response to dietary vitamin E suggest that vitamin E in pregnancy diet favorably influences the gene expression profile of the developing fetal brain.

Acknowledgements

Supported by NIH RO1NS42617 to CKS. The Laboratory of Molecular Medicine is the research wing of the Center for Minimally Invasive Surgery. SR and BL equally contributed to this work and should therefore be credited as joint first authors.

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Abbreviations

apoB	apolipoprotein B
CO	carbon monoxide
DMT	Data Mining Tool 2.0
GAPDH	glyceraldehyde-3-phosphate-dehydrogenase
HO	hemeoxygenase
HMG2	high mobility group protein 2
HSP	heat shock protein
MAS	Affymetrix Microarray Suite 4.0
NO	nitric oxide
NOPP140	

nuclear phosphoprotein p130

PKC

protein kinase C

RT-PCR

reverse-transcription-polymerase chain reaction

TRF

tocotrienol rich fraction

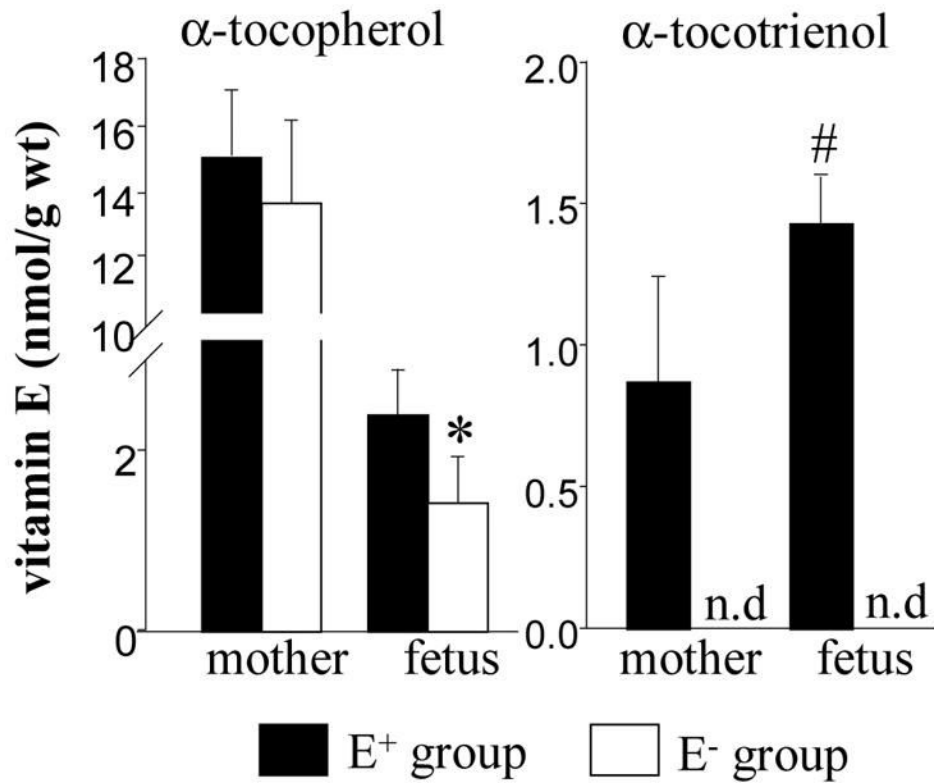


Figure 1. Vitamin E levels in fetal and mother rat brains

Pregnant (three-day) rats were randomly divided into i) *E⁺ group* – fed a standard rat chow that is enriched in α -tocopherol. Additionally, this group was supplemented for 2 weeks with a daily gavage of TRF suspended in vitamin E-stripped corn oil; and ii) *E⁻ group* – fed a vitamin E deficient diet and supplemented with a matched volume of vitamin E-stripped corn oil. On 17th day of gestation, brains were collected and vitamin E analysis was performed using HPLC. *, $p < 0.05$ significantly different compared to the *E⁺ group*. #, $p < 0.05$ significantly different compared to mother brain. n.d., not detected

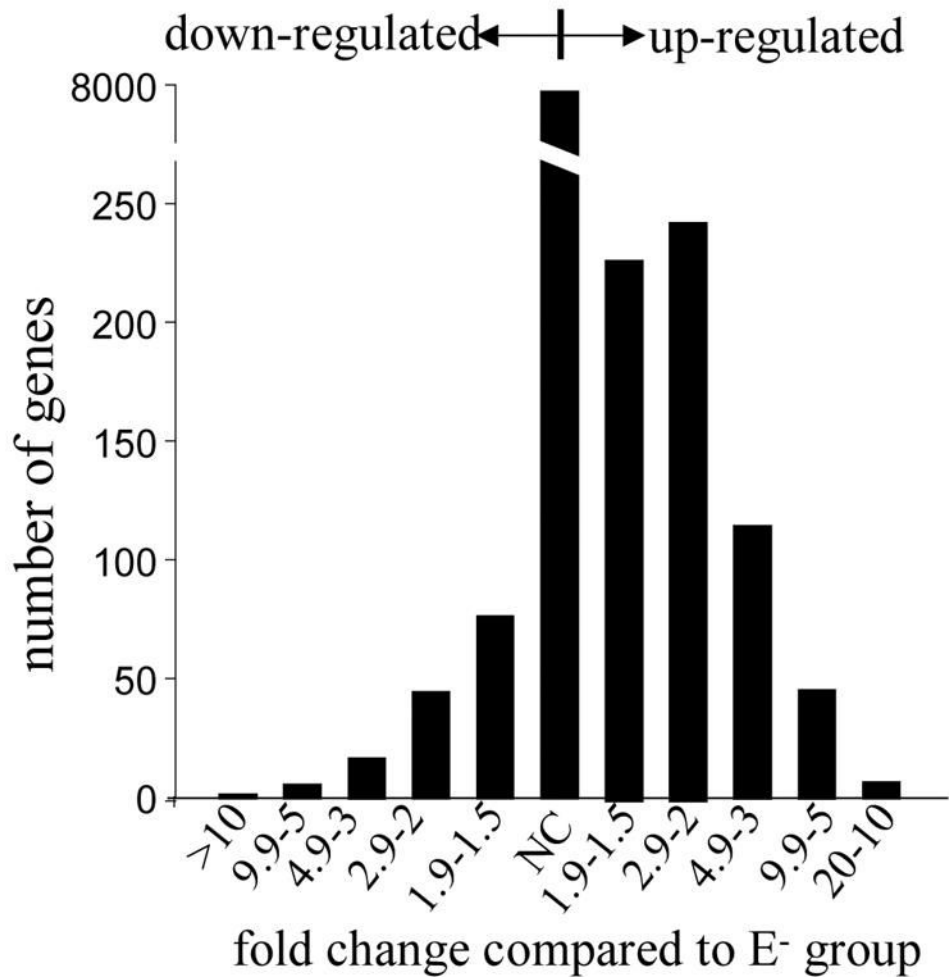


Figure 2. Range of the average fold changes of differentially expressed genes in E⁺ and E⁻ groups
 Six pair-wise comparisons were generated from replicates of both E⁺ and E⁻ groups using *Comparison Analysis* in MAS. Average fold changes were calculated for both up- or down regulated genes.



Fructose-6-phosphate-2-kinase / fructose 2-6 biphosphatase3	Metabolism / Biosynthesis
EST, similar to hypothetical protein MGC 14833 [H. sapiens]	Unknown
F-spondin	Neuron
EST, weakly similar to 810024JJ URF 4 [H. sapiens]	Unknown
Protein tyrosine phosphatase, no-receptor type substrate 1	Immune response signaling
PAM COOH-terminal interactor protein 1	Sensing / signaling / Cell communication
Rat mixed-tissue library Rx03955	Unknown
Cortactin-binding protein 1/ Proline rich synapse associated protein 1	Neuron
Tyrosine kinase receptor ligand 2 (RETL 2)	Neuron
Rat mixed-tissue library Rx04977	Unknown
14-3-3 protein beta-subtype	Neuron
Aldolase A, fructose biphosphate	Metabolism / Biosynthesis
Neural visinin-like protein	Neuron
Heart myosin light chain	Metabolism / Biosynthesis
Potassium voltage-gated channel, subfamily H (age related), member 3	Metabolism / Biosynthesis
Quinoid dihydropteridine reductase	Neuron
Signal transducer and activator of transcription 6	Sensing / signaling / Cell communication
Pro-protein convertase 5 isoform B	Neuron & Development
EST, moderately similar to Y053 human hypothetical protein KIAA0053 [H. sapiens]	Unknown
EST, weakly similar to JC 5238 galactosylceramide-like protein, GCP [H.sapiens]	Neuron
ADP-ribosylation factor related protein 1	Sensing / signaling / Cell communication
G protein coupled receptor (RTA)	Metabolism / Biosynthesis
Putative G-protein coupled receptor RA1c	Olfaction (Brain)
Developmentally-regulated cardiac factor	Vascular
Sulfonylurea receptor	Metabolism / Biosynthesis
Non-selective type endothelin receptor	Vascular
Adenylyl cyclase type VIII	Neuron
EST, moderately similar to protein kinase inhibitor alpha	Sensing / signaling / Cell communication
Nucleolar phosphoprotein of 140 kD	Cell cycle
Mu opioid receptor	Neuron
EST, highly similar to rat pyruvate dehydrogenase E1 component, beta subunit	Metabolism / Biosynthesis
Rat mixed-tissue library Rx03063	Unknown
Uncoupling protein-3 (nuclear gene encoding mitochondrial protein)	Metabolism / Biosynthesis
Chondroadherin	Development
EST, highly similar to cyclin M4 [H. sapiens]	Cell cycle
Malic enzyme 1	Metabolism / Biosynthesis
Arylalkylamine N-acetyltransferase	Neuron
Pancreatic islet	Immune response

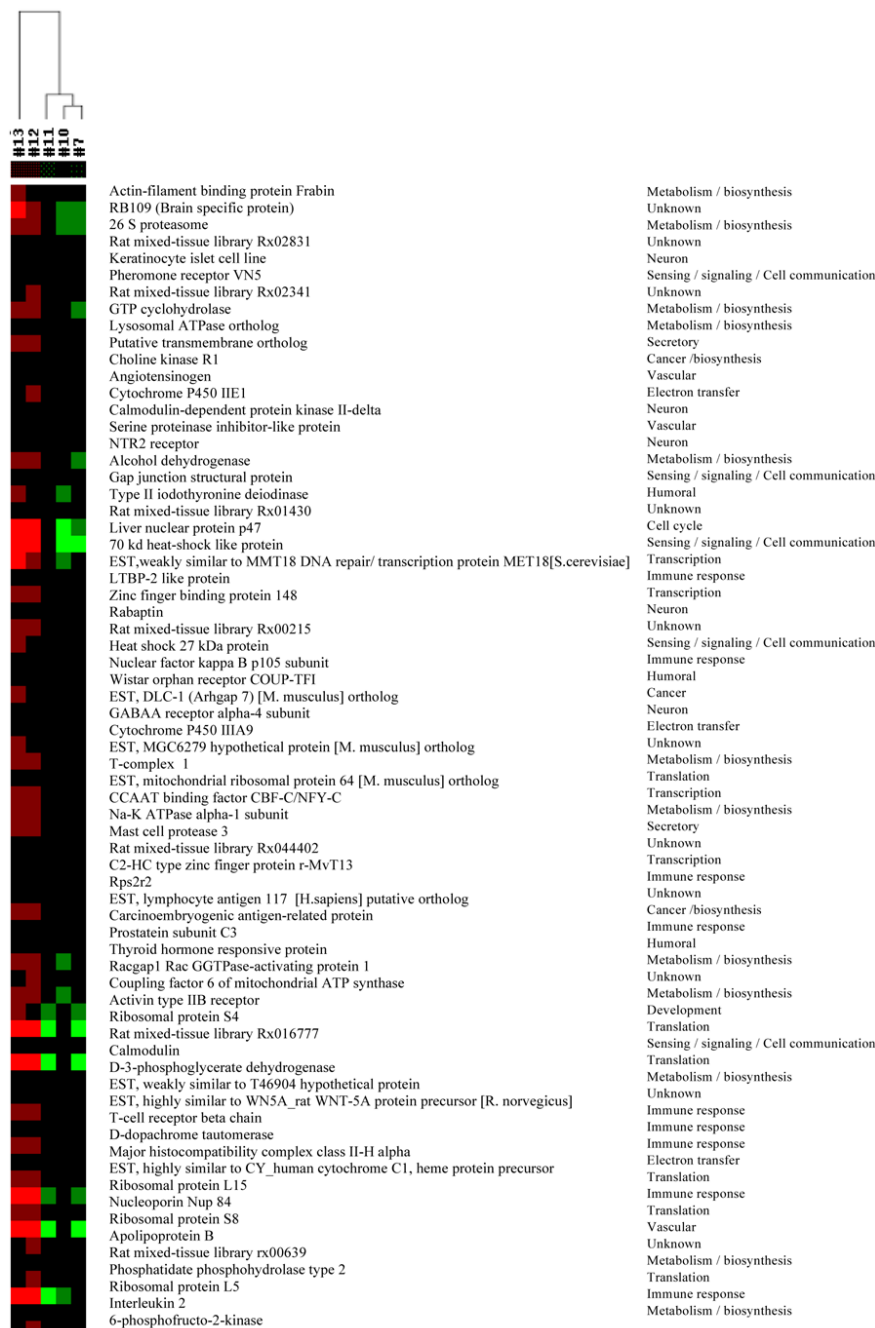


Figure 3. Cluster image illustrating the genes differentially expressed in fetal brains of E⁺ group
 For a clear graphic display of vitamin E sensitive genes, T-test was performed on replicate samples of E⁺ group (animal #7, #10, #11) and E⁻ group (animal # 12 and #13) that significantly (p<0.05) changed (increase or decrease) in the E⁺ group as compared to the E⁻ group were selected. The average difference values of selected genes were loaded into the Cluster & TreeView software where the data was adjusted according to the median center. Red to green gradation in color represent higher to lower expression signal compared to median center. In some cases due to very high expression levels the signal is saturated and shows red in all samples. The scale is adjustable. A medium intensity (not optimal for all data presented) scale

is shown. For details of vitamin E feeding and tissue collection, see legend of Figure 1. **A)** upregulated; or **B)** down-regulated genes in E⁺ group compared to E⁻ group.

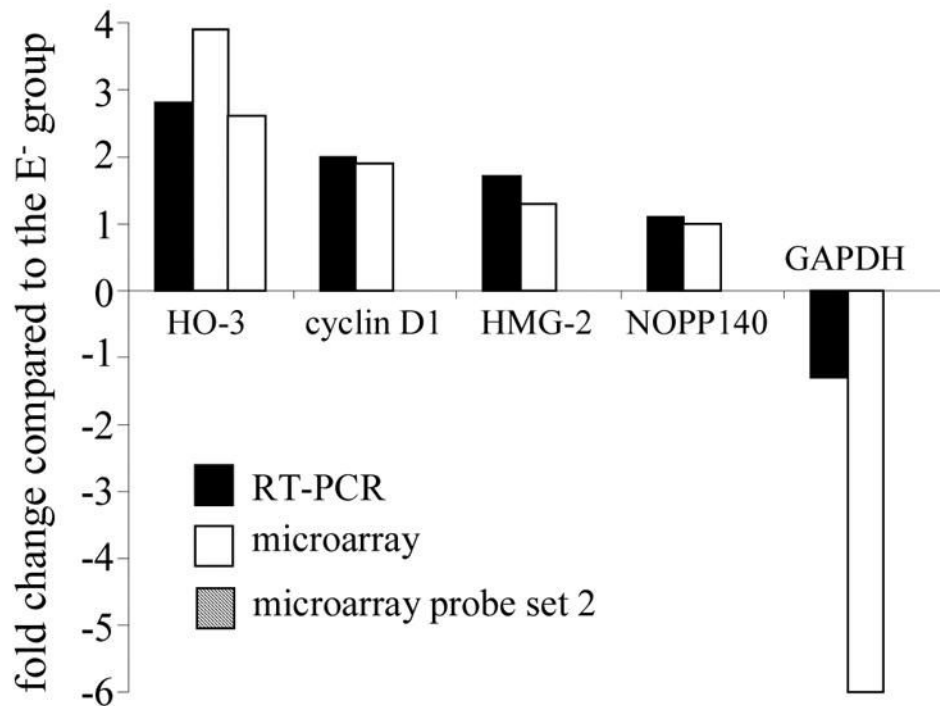


Figure 4. RT-PCR validation of GeneChip microarray expression analysis

Expression levels of were independently determined using RT-PCR. The following genes identified as differentially expressed in E^+ group compared to E^- group using GeneChip microarray analysis were verified using RT-PCR: heme oxygenase 3 (HO-3), cyclin D1, HMG-2, NOPP140 and GAPDH. The band intensity of the PCR products was quantified and fold change for each gene in E^+ group compared to E^- group was calculated (solid bars). For comparison, fold changes observed in the expression of a specific gene using GeneChip microarray analysis (one or more probe sets) was also plotted (empty and hatched bars).

Table 1

Primers used for RT-PCR.

mRNA	Primer Sequence 5' to 3'
CyclinD1	CTG CAT GTT CGT GGC CTC TAA GAT CCA GAA GGG CTT CAA TCT GTT CCT
GAPDH	TAT GAC TCT ACC CAC GGC AAG TTC A CAG TGG ATG CAG GGA TGA TGT TCT
HMG2	TCC TCC CAA AGG TGA TAA GAA AGG A TGG CAC GGT ATG CAG CAA TA
HO-3	ATG GCA TCA GAG AAG GAA AAC CAT T CCC ATC AAG TAT TGA GAG CCC ATT C
NOPP140	TCA GTG CCA CCA AGA GTC CCT TAA CTT CTT CAC TGG AAT CTT CGG AGG A

Table 2Genes up-regulated in fetal brains of E⁺ group¹.

Affymetrix Accession Number	Description
M13100cds#1_at	Heme oxygenase 3 or RATLIN3A
X05472cds#2_at	RNREP24R Rat 2.4 kb repeat DNA right terminal region
X07686cds_s_at	Rat L1Rn B6 repetitive DNA element
M13100cds#1_g_at	Heme oxygenase 3 or LINE3 (L1Rn)
rc_AA800912_g_at	GTF2I repeat domain-containing 1 putative ortholog
rc_AA924591_at	Cyp4a locus, encoding cytochrome P450 (IVA3)
X62951mRNA_s_at	mRNA (pBUS19) with repetitive elements
rc_AI231445_at	unr protein
rc_AA852046_s_at	VL30 element mRNA
AB010154_at	PKN mRNA for serin/threonine protein kinase expressed in hippocampus
rc_AI231257_at	Cyclin D1
X53581cds#3_f_at	long interspersed repetitive DNA containing 7 ORFs
AF055714UTR#1_at	hypertension-regulated vascular factor-1C-4
X05472cds#3_f_at	RNREP24R Rat 2.4 kb repeat DNA right terminal region
S76466_at	type I serine-threonine kinase receptor
S78556_at	grp75=75 kda glucose regulated protein
rc_AA998882_s_at	nucleolar phosphoprotein p130 (NOPP140)
D84418_r_at	chromosomal protein HMG2
U35244_g_at	vacuolar protein sorting homolog r-vps33a

¹ Six pairwise comparisons among brains obtained from individual fetus, the mothers of whom were fed vitamin E⁺ and vitamin E⁻ diet during pregnancy for 2 wks. Genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. ESTs for which no description is available were excluded.

Table 3Genes down-regulated in fetal brains of E⁺ group¹.

Affymetrix Accession Number	Description
U58857_at	maspin
AFFX_Rat_GAPDH_M_st	glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)
M27440_at	apolipoprotein B (apoB)
U88324_g_at	G protein beta1 subunit (rGb1)
AFFX_Rat_beta-actin_3_st	cytoplasmic beta-actin
rc_AI045858_at	Weakly similar to T14794 hypothetical protein DKFZp586P1522
rc_AI008852_at	eukaryotic translation elongation factor
U46118_at	cytochrome P450 3A9
L27112_s_at	stress activated protein kinase alpha II
rc_AA965261_at	H2A histone family, member Y
AF091563_r_at	QIL-LD1 olfactory receptor
AB013454_at	NaPi-2 beta
M27467_at	cytochrome oxidase subunit VIc (COX-VIc)
U50412_at	phosphoinositide 3-kinase regulatory subunit p85alpha
U88324_at	G protein beta1 subunit (rGb1)
M32474_at	carcinoembryonic antigen-related protein (CGM1)
AF037071_at	carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase
AB010436_at	cadherin-8
L03386_i_at	zinc finger protein
rc_AI072341_at	vacuolar protein sorting homolog r-vps33b
rc_AI073204_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activatioprotein
rc_AI103498_at	ribosomal protein L5
D16308_at	cyclin D2
rc_H31847_at	dynein light intermediate chain
D17521_at	protein kinase C-regulated chloride channel
D90038_at	70-kDa peroxisomal membrane protein (PMP70)
rc_AI171630_s_at	p38 mitogen activated protein kinase
AF083330_at	kinesin-like protein KIF3C (KIF3C)
M65251_s_at	Rat angiotensinogen gene-inducible enhancer-binding protein 1
rc_AA892378_at	AF151893 1 CGI-135 protein
rc_AA800194_at	similar to T08812 probable succinate-CoA ligase
AF090867_g_at	guanosine monophosphate reductase mRNA
D14045_s_at	DNA topoisomerase IIA
rc_AI179150_s_at	CDK110

¹ Six pairwise comparisons among brains obtained from individual fetus, the mothers of whom were fed vitamin E⁺ and vitamin E⁻ diet during pregnancy for 2 wks. Genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. ESTs for which no description is available were excluded.