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Alternative Transcripts of Fatty Acid Desaturase (FADS) Genes

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Abstract

Alternative splicing is a major mechanism for increasing the range of products encoded by the genome. We recently reported positive identification of the first alternative transcripts (AT) of fatty acid desaturase 3 (*FADS3*) and *FADS2* in fetal and neonatal baboons. *FADS3*, a putative polyunsaturated fatty acid (PUFA) desaturase gene with no known function, has 7 AT that are expressed in at least twelve organs in an apparently constitutive manner. At least five of seven AT are expressed in several mammals and the chicken. FADS2, catalyzing 6 and 8 desaturation and having multiple PUFA substrates, has one AT that is missing two exons and portions of two others. Semi-quantitative expression estimates reveal at least 20-fold differential expression of FADS2 AT1 among neonatal baboon organs compared to 2-fold in the same organs for the classically spliced (CS) FADS2 transcript. Expression of four of the FADS3 AT, those with missing putatively active domains, is highly correlated among organs, suggesting coordinated coexpression. AT may serve as templates to generate protein isoforms or as signaling molecules, and their widespread detection and expression patterns suggest that they play an important role in PUFA biosynthesis.

Background

The interconversion of polyunsaturated fatty acids (PUFA) in mammals has long been known to be mediated by desaturation, elongation, and β-oxidation activities. Biosynthesis of long chain PUFA (LCPUFA) from the eighteen carbon precursors linoleic acid (LA, 18:2n-6) and linolenic acid (ALA, 18:3n-3) is especially important because of the prevalence of 18:2n-6 and 18:3n-3 in the human food supply.

Figure 1 is an outline of the currently accepted pathways for LCPUFA biosynthesis starting from 18:2n-6 and 18:3n-3, with genes and associated activities shown. In vertebrates, desaturation and elongation takes place in the endoplasmic reticulum (ER), apart from a report of 5-desaturase activity in the nucleus [1]. β-oxidation activity is found in the peroxisomes and mitochondria, including a critical step leading directly to 22:6n-3 biosynthesis from 24:6n-3 [2]. β-oxidation catalyzes chain shortening interconversion, while the fatty acid chain elongation system operating on PUFA via three genes (ELOVL2, 4, and 5) [3] chain lengthens.

PUFA desaturation activity is mediated by the Fatty Acid Desaturases (*FADS*) gene cluster located within a 100 kb region of the long arm of human chromosome 11q12-13.1, with the homologous genes located on mouse chromosome 19 [4]. *FADS*1, 2, and 3 consist of 12

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exons and 11 introns, and include 3 conserved his box motifs and a cytochrome *b*5 motif. *FADS1* [5] gene products were first established as having Δ5-desaturase activity catalyzing 20:3n-6 → 20:4n-6 (arachidonic acid, ARA) and 20:4n-3 → 20:5n-3 (eicosapentaenoic acid, EPA). *FADS2* [6] gene products have Δ6-desaturase activity catalyzing 18:2n-6 → 18:3n-6 and 18:3n-3 → 18:4n-3; based on *in vitro* assays [7], Δ6-desaturation is generally considered the rate limiting step for LCPUFA biosynthesis. *FADS3* [8] shares sequence homology with *FADS1* and *FADS2*. Despite the congruence of sequence similarity, no function has emerged for *FADS3*.

Recent studies have detected associations between specific single nucleotide polymorphisms (SNP) in *FADS* genes and blood fatty acid levels in normal adults [9-11], adults with cardiovascular disease [12], pregnant and lactating women [13], and in attention-deficit/ hyperactivity disorder (ADHD) [14]. More than 90% of SNPs that are associated with fatty acid concentrations are located in the untranslated regions, in introns, and in the intergene regions in CpG islands. Mechanisms that may be influenced by these non-coding regions are differential promoter binding and changes in methylation patterns that alter transcription.

Another possibility are genome-wide alternative splicing (AS) events that yield alternative transcripts (AT) [15]. The majority of AT result from full or partial exon deletion or intron retention. AT may code for modified proteins with different specificities than the protein coded by the transcript resulting from classical splicing (CS), or serve some regulatory role. AT serve to expand the range of products for which a genome can code. It has been estimated that 90% of multi-exon genes have alternative transcripts. In a remarkable example of AS, the Drosophila gene *Down syndrome cell adhesion molecule* (Dscam) potentially generates over 38,000 transmembrane proteins of the immunoglobulin superfamily [16] that function in the development of neural circuitry, and specifically in dendritic self-avoidance [17]. Metabolic regulation of AT expression has also been reported. For instance, ABCA1, a gene required for lipid transport across the plasma membrane has at least three AT that are regulated in a tissue and diet specific manner [18].

Recently, we have reexamined various aspects of the pathways of LCPUFA biosynthesis, showing, for instance, that the *FADS2* gene product catalyzes the Δ8-desaturation of 20:2n-6 → 20:3n-6 and 20:3n-3 → 20:4n-3 [19]. In the course of these molecular studies we discovered AT associated with *FADS3* [20] and, most recently, *FADS2* [21]. We discuss here salient aspects of these first reports of FADS AT and their possible implications for LCPUFA biosynthesis.

Table 1 outlines the number of transcripts, proteins, and activities reported for the mammalian *FADS* genes. *FADS1* function is limited to Δ5-desaturation of the 20 carbon precursors of ARA and EPA. *FADS2* has a much wider substrate spectrum. Its microsomal activity is about one quarter that of Δ5-desaturation [7], and it prefers n-3 to n-6 PUFA. It also shows small Δ8-desaturation activity toward 20 carbon PUFA in the presence of 18 carbon substrates [19]. Finally, *FADS2* apparently mediates the biosynthesis of sapienic acid (16:1n-10), the most abundant unsaturated fatty acid on human skin, though the activity on 16:0 as a substrate is low [22]. *FADS3* has no known substrates. Analogous PUFA desaturase genes reported in other species, including fish [23], *C. elegans* [24], and many plant species (e.g. [25]) have a much wider range of substrates and specificities than those of mammals.

FADS3 AT

We recently described the detection of 7 AT of *FADS3* in 12 tissues of three month old baboons [20]. AT are numbered sequentially according to the order in which they were discovered. Figure 2 is an alignment of predicted amino acid sequences for the *FADS3* CS

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and AT. AT1 and AT2 are missing exons 3 or 6, respectively. FADS3 AT3 has a truncated version of exon three; AT4 has truncated exons 1 and 3, and is missing exons 2 and 8; AT5 has truncated exons 1 and 4 and is missing exons 2 and 3, as well as retaining intron 8-9; AT6 skips the span from part of exon 4 through part of exon 9; AT7 skips part of exon 8 and all of exons 9 and 10. Predicted protein sequences indicate that AT1, AT3, and AT7 would code for shorter proteins; AT1 and AT3 retain all conserved motifs characteristic of PUFA desaturases (HPGG cytochrome *b*5 and three histidine repeats "HDLGH, HFQHH, QIEHH"). The deletion of part of exon 8 and all of exons 9-10 results in the loss of the last histidine repeat QIEHH in AT7. AT2, AT4, AT5, and AT6 would produce truncated proteins missing one or more motifs.

The FADS3 AT were cloned into yeast (*Saccharomyces cerevisiae*) and mammalian cells, but showed no activity toward a variety of PUFA substrates (our unpublished data, 2009). Further studies were carried out to detect FADS3 AT expression in baboon tissue and cultured human neuroblastoma cells using AT-specific PCR primers bridging deleted parts of exons. Remarkably, all seven AT, were detected in all baboon tissues and in human cells [20]. Figure 3 shows expression relative to β-actin for 12 tissues. Summed expression of all AT was highest in hippocampus and more than double that in pancreas; summed expression in all tissues was highest for AT6, and more than triple that of AT2. Correlation analysis of these data was performed using JMP 7.0 (SAS Institute, Cary, NC, USA) for the 21 possible pairs of 7 different AT. Results are shown in Table 2 for the significant correlations ($p<0.05$). Six of the seven correlations are among FADS3 AT4, 5, 6, and 7. These findings imply coordinated expression of these AT in tissues, and also imply that expression of the other AT are independent. FADS4,5,6,and 7 putatively code for truncated proteins that, individually, are all missing one or two of the four conserved motifs, and all are missing the QIEHH at the 3′ end. It is tempting to speculate that simultaneous translation of all these ATs would code for proteins that could bind to form one or more functional desaturases, possibly to enable activity toward multiple substrates or for purposes of regulation.

FADS2 AT

Figure 4 presents quantitative data on the expression of FADS2 CS and AT1 in the same twelve organs of baboon neonate as in Figure 3. Liver expression of FADS2 CS is 4-fold that of the average expression of the other organs. The range of expression in the other organs is 2-fold, from the highest in occipital lobe (cerebral cortex) to the lowest in skeletal muscle. In contrast, the range for FADS2 AT1 in these same tissues varies over a factor of 20, from highest again in occipital lobe to the lowest in thymus. The dramatic difference in expression suggests a difference in function and independent regulation.

Preliminary observations on species conservation of FADS AT

The conservation of alternatively spliced transcript variants of *FADS AT* was examined in several animal species, as shown in Figure 5. Using the *baboon-specific primers* [21] for each of the *FADS AT*, we performed RT-PCR analysis using liver cDNA from mouse, pig, dog, fox, chicken and horse. At least five of the seven *FADS3 AT* and *FADS2 AT1* are reproducible in many species. These PCR products have not yet been confirmed by sequencing, but are consistent with the hypothesis that FADS AT perform some functionally important role, encoding proteins or performing a regulatory role.

Some investigators of desaturase function have suggested the existence of at least two different 6-desaturases as a possible explanation of the findings of functional studies [26,27]. To our knowledge, isozymes of a Δ6-desaturase have been described only in the arachidonic acid producing fungus Mortierella sp. and not for other organisms [4,28]. In previous work, we and others have also suggested that specific LCPUFA synthases would

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explain results that are not easily accounted for by the accepted LCPUFA synthesis pathways [29,30]. Although a function has yet to emerge for FADS3 and for the various AT that we have described, they represent a putative molecular basis on which alternative regulation and LCPUFA biosynthetic pathways could be based.

Hypothetically, FADS AT as mediators of LCPUFA biosynthesis or regulation could have implications for establishing variability in individual and population LCPUFA dietary requirements. Among the few studies that have reported on stable isotope tracers in humans, at least one in preterm infants showed wide variability to form 22:6n-3 and 20:4n-6 [31]. Differential expression of AT among individuals in response to diets could imply differential LCPUFA requirements. For instance, some populations subsisting on diets that provide only 18:2n-6 and 18:3n-3 as PUFA may require supplementary LCPUFA because of low expression of putative AT-related LCPUFA synthetic proteins, while others might synthesize sufficient LCPUFA from precursors. Group differences of this type have been suggested [32] but no compelling data has been presented. The novel findings of FADS AT suggest that investigations to elucidate molecular mechanisms and nutritional factors regulating their expression are required to elucidate factors mediating human PUFA desaturation.

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Abbreviations

LCPUFA	long chain polyunsaturated fatty acids
EPA	eicosapentaenoic acid
ARA	arachidonic acid
DHA	docosahexaenoic acid
FAME	fatty acid methyl ester

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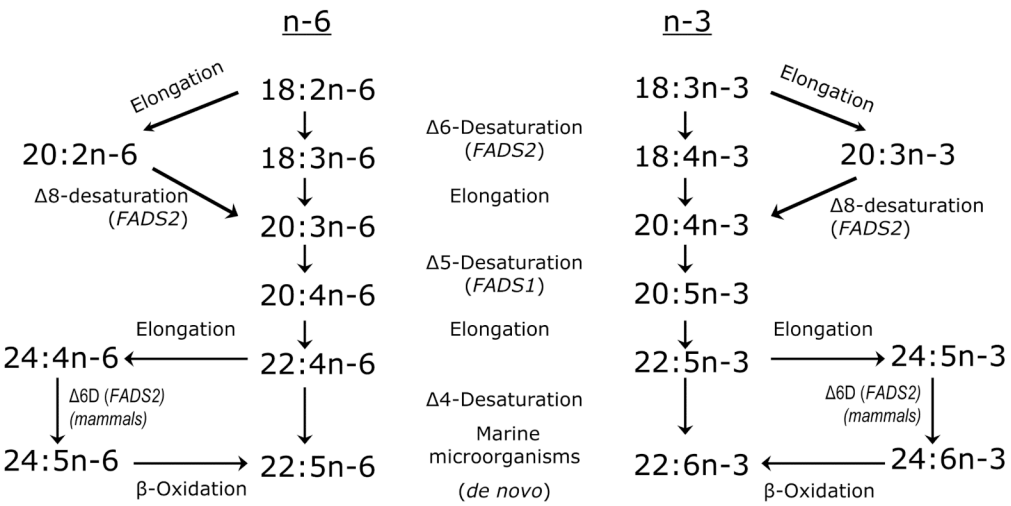

Figure 1.

Biosynthesis of LCPUFA with emphasis on the role of FADS genes, as generally accepted. β-oxidation may chain shorten any LCPUFA. Not shown are 16:2n-6 and 16:3n-3 which can be elongated to 18:2n-6, and 18:3n-3, respectively.

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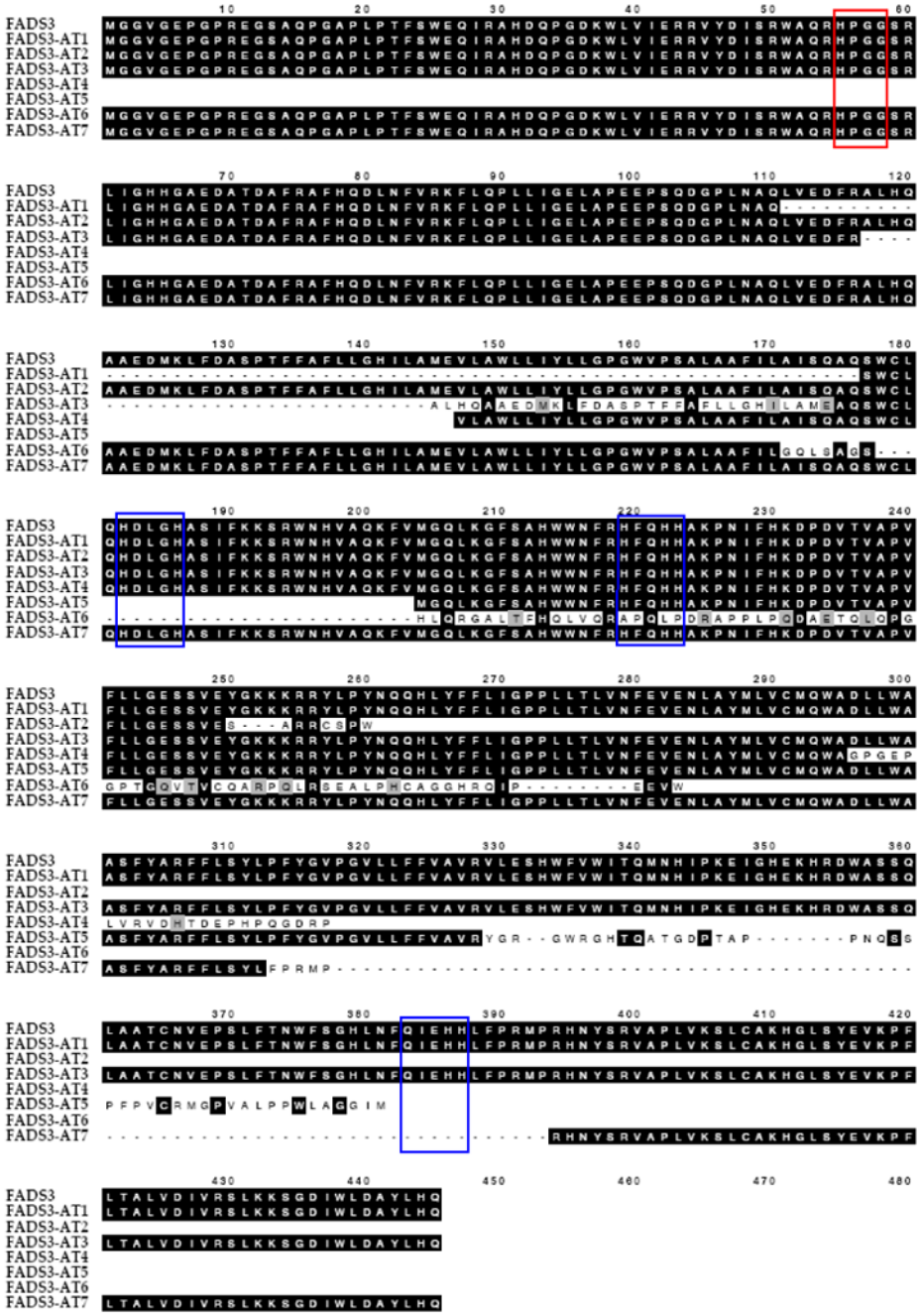

Figure 2.

Alignment of Baboon FADS3 alternative transcripts using MacVector software and ClustalW alignment. Well conserved motifs common for desaturases are depicted in boxes. The “HPGG” characteristic of Cytochrome b5 is boxed in red, whereas three conserved histidine motifs “HDLGH, HFQHH and QIEHH” are shown in blue boxes.

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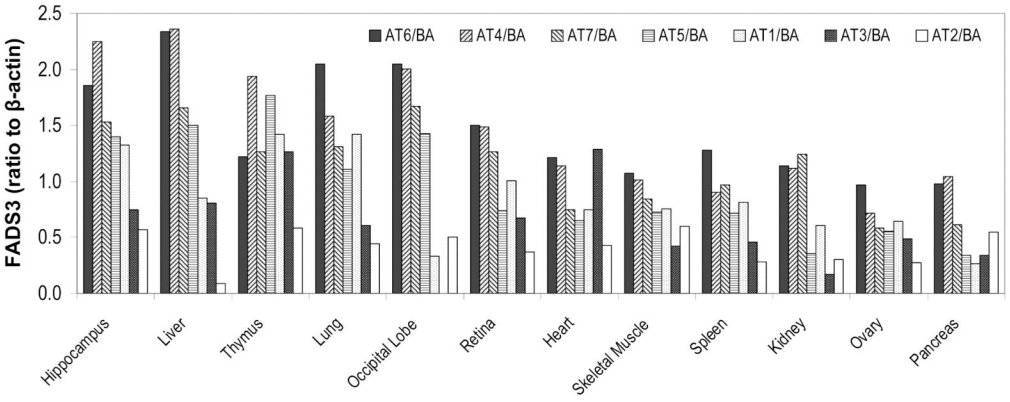

Figure 3.

Expression of the seven FADS3 AT in 12 tissues of a 12 week old baboon. Data are arranged left to right in order of total expression relative to β-actin. Gels were scanned and quantified manually using ImageJ <http://rsbweb.nih.gov/ij/>.

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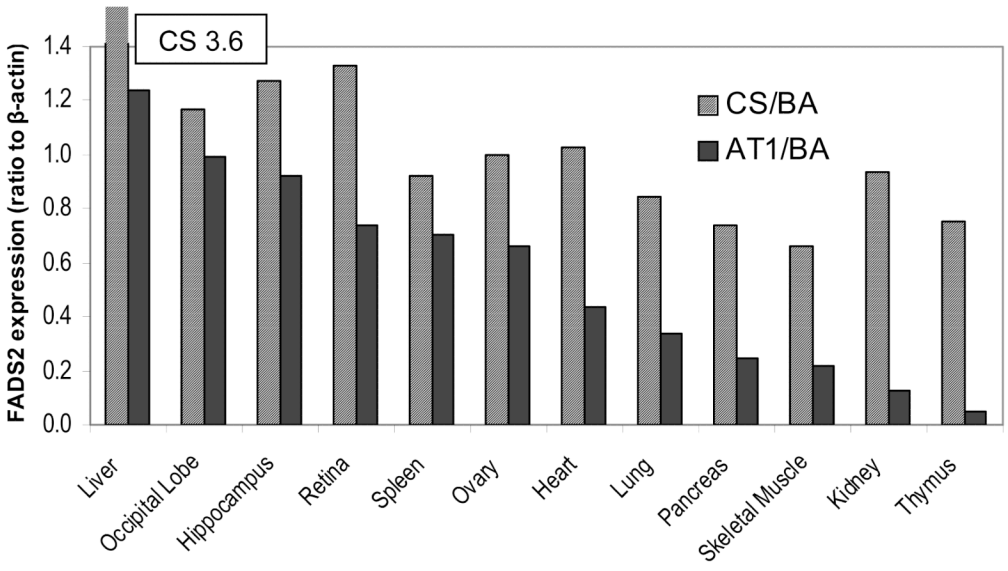

Figure 4.

Expression of the FADS2 classically spliced (CS) transcript and AT1 in various tissues of a 12 week old baboon. The y-axis is plotted with liver FADS2 CS off-scale because it is four-fold the average of the others. The range of expression for FADS2 AT1 is much greater among the 11 tissues (other than liver) than for the CS.

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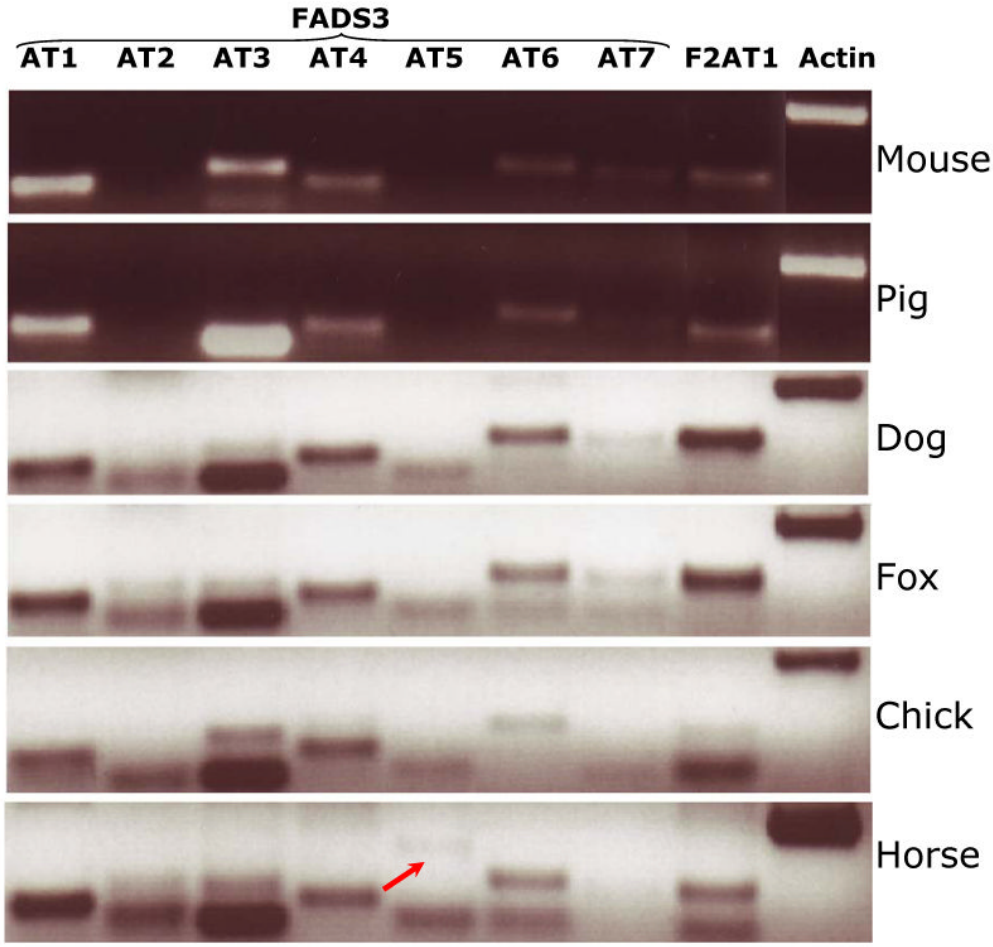

Figure 5.
Expression of FADS3 AT and FADS2 AT1 in five mammalian species and in the chick, analyzed using baboon-specific primers. All but FADS3 AT2 and FADS3 AT5 are expressed in most of the species. FADS3 AT5 is detected in horse.

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Table 1

Current knowledge of primate PUFA desaturases.

Gene Transcript(s)	***FADS1***	***FADS2***	***FADS3***
	1	**2**	**8 (9?)**
Protein		*Not characterized*	
Activities	Δ5-desaturation 20:3n-6→20:4n-6 20:4n-3→20:5n-3	Δ6-desaturation 18:2n-6→18:3n-6 18:3n-3→18:4n-3 24:5n-3→24:6n-3 24:4n-6→24:5n-6 16:0→16:1n-10 Δ8-desaturation 20:2n-6→20:3n-6 20:3n-3→20:4n-3	*Not identified*

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Table 2

Significant correlations between AT in various tissues.[1]

	r^2	P
AT7 – AT4	0.79	0.0001
AT5 – AT4	0.77	0.0002
AT7 – AT6	0.74	0.0003
AT4 – AT6	0.68	0.0010
AT5 – AT7	0.55	0.0055
AT5 – AT6	0.46	0.0150
AT3 – AT1	0.37	0.0352

[1] Calculated with JMP 7.0 <http://www.jmp.com/>, “Multivariate Methods >Multivariate” menu and activation of “pairwise correlations” to obtain p values.