The human fatty acid-binding protein family: Evolutionary divergences and functions

*Rebecca L. Smathers and Dennis R. Petersen**

Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045, USA
**Correspondence to:* Tel: +1 303 724 3397; Fax: +1 303 724 7266; E-mail: dennis.petersen@ucdenver.edu

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Abstract

Fatty acid-binding proteins (FABPs) are members of the intracellular lipid-binding protein (iLBP) family and are involved in reversibly binding intracellular hydrophobic ligands and trafficking them throughout cellular compartments, including the peroxisomes, mitochondria, endoplasmic reticulum and nucleus. FABPs are small, structurally conserved cytosolic proteins consisting of a water-filled, interior-binding pocket surrounded by ten anti-parallel beta sheets, forming a beta barrel. At the superior surface, two alpha-helices cap the pocket and are thought to regulate binding. FABPs have broad specificity, including the ability to bind long-chain (C16–C20) fatty acids, eicosanoids, bile salts and peroxisome proliferators. FABPs demonstrate strong evolutionary conservation and are present in a spectrum of species including *Drosophila melanogaster*, *Caenorhabditis elegans*, mouse and human. The human genome consists of nine putatively functional protein-coding *FABP* genes. The most recently identified family member, *FABP12*, has been less studied.

Keywords: *FABP, intracellular lipid-binding proteins, uptake and trafficking of lipids, metabolic diseases, human genome*

Introduction

Hydrophobic ligands, such as fatty acids (FAs) and their acyl-CoA derivatives (FA-CoA), serve many biological functions within the cell. They serve as metabolic energy sources, substrates for membranes and signalling molecules for metabolic regulation.[1,2] The insoluble properties of FAs make for the requirement for chaperones to bind and transfer them throughout various cellular compartments, including the peroxisomes, mitochondria, endoplasmic reticulum, lipid droplets and nucleus. A family of highly expressed intracellular lipid-binding proteins (iLBPs)—the fatty acid-binding proteins (FABPs)—serves to bind these free ligands with high affinity. FABPs are ubiquitously expressed throughout tissues that are highly active in FA metabolism and comprise several isoforms. To date, nine FABP protein-coding genes have been identified in the human genome. These include liver- (*L-FABP*), intestine- (*I-FABP*), heart- (*H-FABP*), adipocyte- (*A-FABP*), epidermal- (*E-FABP*), ileal- (*Il-FABP*), brain- (*B-FABP*), myelin- (*M-FABP*) and testis-FABP (*T-FABP*). These different isoforms were first named for the organ in which they were first identified or mostly predominate, but their expression profiles are not exclusive to that specific organ. For example, L-FABP is not only expressed in the liver, but also in the intestine, pancreas, kidney, lung and stomach. In addition to the nine known FABPs that have been widely studied, a newer member of the family, FABP12, has recently been discovered. The gene has been identified, but

published reports on the protein encoded by this gene are not yet available. Therefore, in this reviews only the identification of the gene will be addressed.

Domain structure of FABP proteins

Although members of the FABP family share moderate sequence homologies of 20–70 per cent, their tertiary structures are virtually superimposable.[3–5] Sequences of human FABPs are aligned in Figure 1. These ~15-kilodalton proteins comprise ten antiparallel β-barrel (βA-βJ) structures containing a solvent-accessible ligand-binding pocket, capped by an N-terminal helix-turn-helix motif (αI-αII) which is thought to act as the regulatory portal for binding. The helical N-terminus is involved in the regulation of FA transfer from membranes via electrostatic interactions.[6–8] Detailed superimposed images of the FABP family have been demonstrated previously.[5] All FABPs are capable of binding long-chain FAs (LCFAs; C12–20), which differ in their selectivity, affinity and binding mechanism.[1,6] Generally, these lipid chaperones bind only one ligand per molecule of protein, with the exception of L-FABP, which is capable of binding two ligands simultaneously. The ligand-binding process has been hypothesised, suggesting that FA enters a solvent-accessible area of the protein through a dynamic region made of α-helix II and the turns between βC–βD and βE–βF loops before binding in the cavity of the protein.[9] This has been termed the 'portal hypothesis', and is supported by a number of groups. Affinities toward ligands are generally increased with more hydrophobic molecules, and decreased with molecules of decreasing chain length and increasing sites of unsaturation. Dissociation constants have been calculated to be in the nanomolar to micromolar range.[1,10,11] Published reports have also demonstrated that proteins from different tissues reveal large differences in binding thermodynamics. ΔH° values differ by about 15 kcal/mol for different FABPs and by about 21 kcal/mol when compared with cellular retinoic acid-binding protein (CRABP) 1. Heat capacity changes generated by FA binding (ΔC_p) range from 0 to −1.3 kcal/mol/K.[11] X-ray crystallography and nuclear magnetic resonance (NMR) studies have revealed FA-binding characteristics within the water-filled cavity, where bound FAs occupy approximately one-third the total pocket volume and share the cavity with many ordered and disordered water molecules.[12] Generally, the FA is orientated with the carboxylate group facing inwards. In this binding coordination, the carboxylate group of the FA typically involves tyrosine and arginine residues.[12] The binding conformations for an individual FABP vary slightly for different FAs, further distinguishing the differences between FABP family members.

The overall gene structure of *FABP*s is highly conserved, consisting of four exons separated by three introns.[13–16] The position of exons and introns are similar for all *FABP*s, but the intron length is variable between isoforms. Typically, the lengths of exons are relatively short, with 23–24 amino acids encoded by exon 1, 57–58 amino acids by exon 2, 34–36 amino acids by exon 3 and 16–17 amino acids by exon 4.[17,18] The length of intron 1 in human *FABP3*, *FABP8* and *FABP2* differs considerably, at 3.4, 2.3 and 1.2 kilobases (kb), respectively.[13,15,19] The conservation of gene structure is also maintained in other members of the iLBP family, where the genes encoding CRABPs and cellular retinol-binding proteins (CRBPs) all contain four exons and three introns, although the average lengths of introns are significantly longer than those of *FABP* genes.[20,21] Additionally, *FABP* genes contain the canonical TATA box upstream of the transcription start site.[6] For example, the TATA box sequence of *FABP4* (5′-TTTAAAA-3′) is located 32 nucleotides upstream of the TCA transcription start site.[22]

All FABPs share a conserved three-element fingerprint domain, separated by motifs termed FATTYACIDBP1–3 (Kyoto Encyclopedia of Genes and Genomes, PRINTS: PR00178). Motif 1 contains a G-x-W triplet, which is involved in the initial formation of β-strand A (βA) and is homologous with a similar motif in another small hydrophobic ligand-binding protein family, the lipocalins.[1,23] Motif 2 contains strands 4 and 5 (βD-βE) and motif 3 spans strands 9 and 10 (βI-βJ). Each motif is outlined in Figure 1. Potential

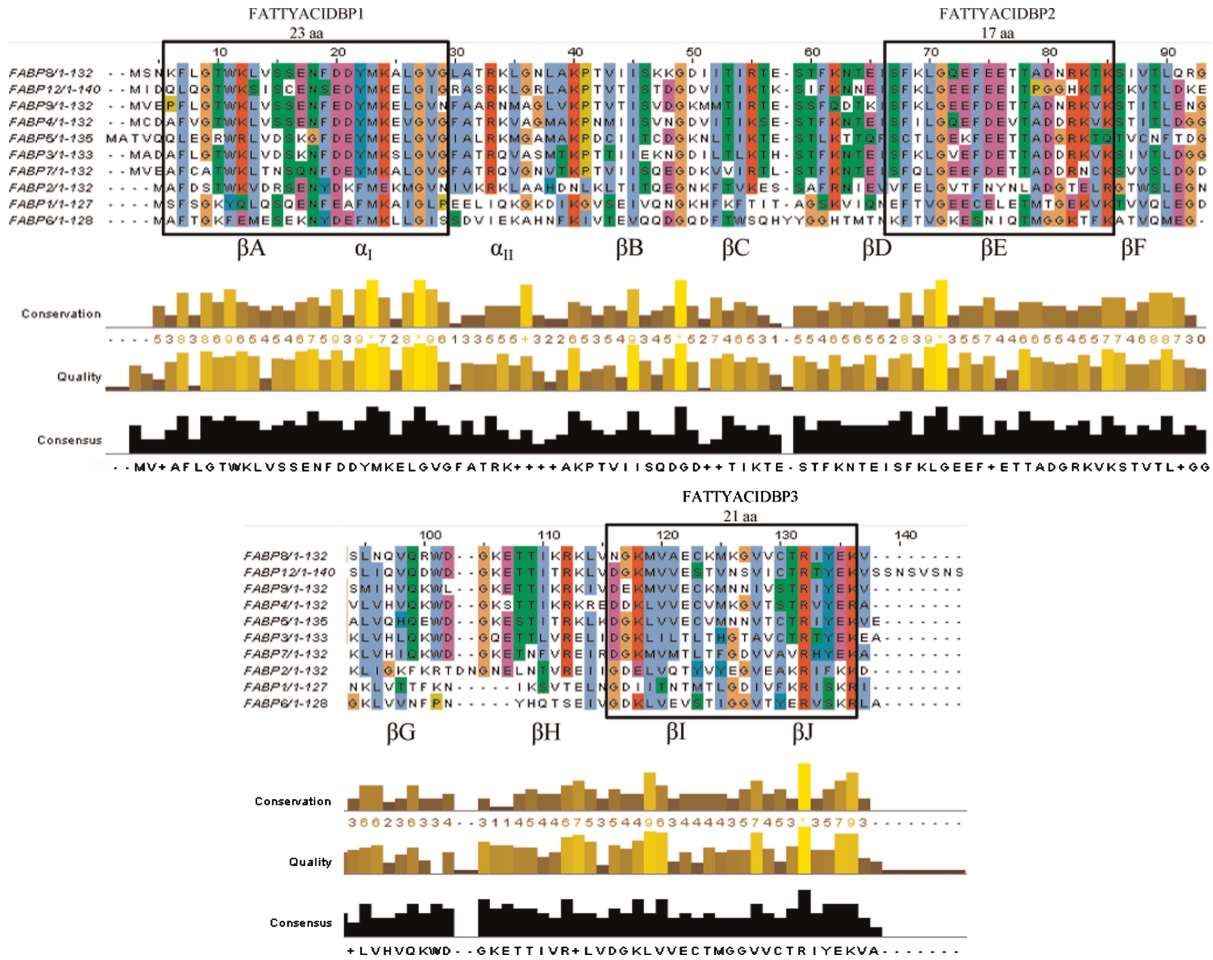

Figure 1. Amino acid sequences of the human FABP family members 1–9 and 12 are demonstrated using the ClustalW2 multiple sequences alignment software program,[176] demonstrated in Jalview.[177] The colour of amino acids is determined by the default annotation created by the analysis of multiply aligned sequences (AMAS), where it allows the identification of functional residues by comparing subgroups of sequences arranged on a tree (http://www.compbio.dundee.ac.uk/Software/Amas/amas.html). Conservation between amino acids is demonstrated below the alignment, where yellow (value of 10) is 100 per cent conserved, and absence of bars (value of 0) represents zero conservation among the sequences at that point. The consensus sequence for the family is shown below the conservation annotation. Common among FAPBs is a three-element fingerprint domain, separated by motifs named FATTYACIDBP1-3 (Kyoto Encyclopedia of Genes and Genomes, PRINTS: PR00178). Each motif is identified by a black box outline. Characteristic structural elements of all iLBP family members, alpha helices and beta-sheets, are all shown in grey boxes. Amino acid sequences were obtained from the National Center for Biology Information (NCBI) website (www.ncbi.nlm.nih.gov/): *FABP1* (GenBank: CAG46887.1), *FABP2* (GenBank: AAH69617.1), *FABP3* (GenBank: CAG33148.1), *FABP4* (GenBank: CAG33184.1), *FABP5* (NCBI Reference Sequence: NP_001435.1), *FABP6* (GenBank: AAH22489.1), *FABP7* (GenBank: CAG33338.1), *FABP8* (NCBI Reference Sequence: NP_002668.1), *FABP9* (NCBI Reference Sequence: NP_001073995.1) and *FABP12* (NCBI Reference Sequence: NP_001098751.1).

functional domains of *FABP*s include those found in the *FABP4* gene, which has a nuclear localisation signal, nuclear export site and hormone-sensitive lipase (HSL)-binding site.[24–26]

Chromosomal mapping of *FABP* family members has shown both dispersion and synteny, as demonstrated in Table 1. *FABP1–3*, *6* and *7* all exist on separate chromosomes in the human, whereas *FABP4*, *5*, *8* and *9* all co-localise at chromosome (Chr) 8q21. A more detailed analysis within the chromosome containing these genes reveals clustering within a 300,000-base pair (bp)

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Table 1. Human *FABP* genes, as listed in the Human Gene Nomenclature Committee (HGNC) and Online Mendelian Inheritance in Man (OMIM) databases

Gene	Common name	Aliases for proteins	Previous symbols	Localisation	Chromosomal location	OMIM ID/ HGNC ID	Number of amino acids
FABP1	Liver FABP	L-FABP, hepatic FABP, Z protein, heme-binding protein		Liver, intestine, pancreas, kidney, lung, stomach	2p11	134650/ 3555	127
FABP2	Intestinal FABP	I-FABP, gut FABP (gFABP)		Intestine, liver	4q28–q31	134640/ 3556	132
FABP3	Heart FABP	H-FABP, O-FABP, mammary-derived growth inhibitor (MDGI)	*FABP11*	Cardiac and skeletal muscle, brain, kidney, lung, stomach, testis, adrenal gland, mammary gland, placenta, ovary, brown adipose tissue	1p33–p31	134650/ 3557	133
FABP4	Adipocyte FABP	A-FABP, aP2		Adipocytes, macrophages, dendritic cells, skeletal muscle fibres	8q21	600434/ 3559	132
FABP5	Epidermal FABP	E-FABP, keratinocyte-type FABP (KFABP), psoriasis-associated-FABP (PA-FABP)		Skin, tongue, adipocyte, macrophage, dendritic cells, mammary gland, brain, stomach, intestine, kidney, liver, lung, heart, skeletal muscle, testis, retina, lens, spleen, placenta	8q21.13	605168/ 3560	135
FABP6	Ileal FABP	II-FABP, Ileal lipid-binding protein (ILLBP), intestinal bile acid-binding protein (I-BABP), gastrophin		Ileum, ovary, adrenal gland, stomach	5q23–q35	600422/ 3561	128
FABP7	Brain FABP	B-FABP, brain lipid-binding protein (BLBP), MRG		Brain, central nervous system (CNS), glial cell, retina, mammary gland	6q22–q23	602965/ 3562	132
FABP8	Myelin FABP	M-FABP, peripheral myelin protein 2 (PMP2)		Peripheral nervous system, Schwann cells	8q21.3–q22.1	170715/ 9117	132
FABP9	Testis FABP	T-FABP, testis lipid-binding protein (TLBP), PERF, PERF 15		Testis, salivary gland, mammary gland	8q21.13	—/3563	132
FABP12	——	——		Retinoblastoma cell,[a] retina (ganglion and inner nuclear layer cells),[b] testicular germ cells,[b] cerebral cortex,[b] kidney,[b] epididymis[b]	8q21.13	—/34524	140

[a]Expression found in humans, versus [b]rodents

region.[18] In this same region, prediction programs identified an additional gene homologous to *FABP8*. It has since been cloned and named as the tenth member of the human *FABP* family, *FABP12*.[18] In addition to protein-coding *FABP*s, pseudogenes for *FABP3* and *FABP5* have been identified and do not code for functional proteins in humans.[27,28] A list of known *FABP* pseudogenes to date is provided in the Human Gene Nomenclature Committee (HGNC) database (http://www.genenames.org).

Evolution of FABPs

It has been well established that significant structural homology between the members of family FABP exist, not only between human isoforms but between other species as well. This is highly suggestive of duplication and divergence from a single ancestral lipid-binding gene. FABPs belong to a larger family of iLBPs that also contain the CRABPs and CRBPs.[29] The iLPB family is divided into four subfamilies based on the types of ligands to which they bind.[5] The first subfamily binds vitamin A derivatives and contains the CRABPs and CRBPs. Subfamily II includes FABP1 and FABP6, binding bulkier hydrophobic groups such as bile acids (BAs), heme and acyl-CoA. The third subfamily includes FABP2, which exclusively binds LCFA. The fourth, and largest, subfamily includes FABP3, FABP4, FABP5, FABP7, FABP8 and FABP9, which bind a variety of ligands including LCFA, eiscosanoids and retinoids. The iLBP family is also part of a superfamily of lipophilic ligand-binding proteins which are widely expressed throughout the animal kingdom, the calycins.[30] Other members of the calycin superfamily include the avidins and the lipocalins.[23,30,31] Interestingly, the homology between these family members is relatively low ($\leq$10 per cent amino acid identity), yet they still share the characteristic β-barrel tertiary structure.[30,32] iLBPs are found in both vertebrates and invertebrates throughout the animal kingdom.[32]

The phylogenesis of human FABPs is summarised as a dendrogram in Figure 2, demonstrating the divergence of the primary sequences over time. It has been estimated that the iLBP family evolved approximately 1,000 million years ago (MYA) by subsequent duplications of a single ancestral gene, further propagating the increasing number of tissue-specific homologues that we know today.[33] Homology searches between either ancestral *iLBP* genes or present-day *iLBP* sequences have demonstrated the absence of this family in yeast (*Saccharomyces cerevisiae*) and plant (*Arabidopsis thaliana*) genomes, thus confining this family to the animal kingdom. This further suggests evidence for emergence of the ancestral *iLBP* gene post-divergence of animals from plants and fungi (1,200–1,000 MYA).[32] The evolutionary rates of iLBP members have been estimated using the average distance between mammalian orthologues (rodents, man, rabbit, cow and pig), indicating that the amino acid substitution rate

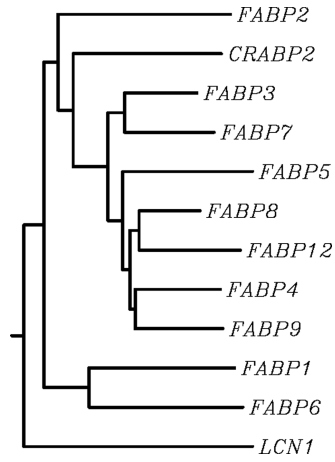

Figure 2. Phylogenetic analysis of human FABP family members. Using ClustalW alignment software of known peptide sequences for FABP1-9, FABP12, CRABP2 and LCN1, a dendogram with branch lengths was constructed. Clustering analysis showed divergence of iLBP family members by their known and established subfamilies: Subfamily I – FABP1, FABP6, Subfamily III – FABP2, Subfamily IV – FABP3, FABP4, FABP5, FABP7, FABP8, FABP9, and the newly established FABP family member FABP12. Subfamily II includes CRABP and CRBP: only CRABP2 was included in this analysis, to demonstrate similarities in the divergences of the iLBP family. Additionally, a member of the calcyin superfamily of hydrophobic ligand-binding proteins, lipocalin 1 (LCN1, GenBank accession no. NP_002288), was included in this analysis as an outgroup, as calcyins share $\leq$10 per cent homology with iLBP members.

within the family differs widely (0.016–1.24 amino acid substitutions per amino acid site per billion years).[32] The amino acid substitution rate for the FABP family ranges from 0.60–1.24. Earlier analyses of the evolutionary relatedness of proteins within the FABP family showed similarity, while identifying the positions of at least 14 gene duplications.[20,34] In addition, an index of evolutionary conservation between the family averages 1.16, with CRABP being the most highly conserved proteins in the iLBP family.[32]

The human *FABP* gene family

As mentioned previously, FABPs are ubiquitously expressed but differ in stoichiometry, affinity and specificity toward ligands. Expression of these proteins is mostly regulated at the transcriptional level, due to resulting synergistic interactions at conserved motifs within gene promoters.[6,35] Multiple *cis*-acting regulatory elements in the 5′-flanking region of these genes and *trans*-acting nuclear factors that either activate or repress transcription have been identified, and will be discussed separately for each *FABP* gene in the following sections of this review. Often, the expression of FABPs in a tissue directly reflects its lipid-metabolising capacity; that is, in hepatocytes, adipocytes and cardiomyocytes, FABPs make up 1–5 per cent of all soluble cytosolic proteins, due to the high concentration of FAs used for lipid biosynthesis, storage and metabolism.[36] In other tissues, less involved in active lipid metabolism, FABPs are not as highly expressed.

Numerous functions have been proposed for FABPs. Their presence in the cell is essential for the binding of hydrophobic molecules, reducing the detergent-like properties of high FA concentrations and making them more soluble. Additionally, FABPs traffic these ligands to various cellular compartments for storage, oxidation, membrane synthesis, signalling and activation of nuclear receptors. It has been demonstrated that FABPs can target FA to transcription factors such as the peroxisome proliferator-activated receptor (PPAR) family in the lumen of the nucleus, where the *FABP1*, *FABP3*, *FABP4* and *FABP5* genes are all regulated by PPARs via activation from FA or other ligands.[37,38] Many FABPs interact physically with phospholipid-rich membranes and other proteins for ligand transfer. For example, L-FABP interacts with membranes, the mitochondrial protein carnitine palmitoyl transferase 1 and transcription factors (PPARα).[7,39,40] Additionally, FABPs also bind eicosanoid intermediates and protect these substrates against peroxidation, suggesting antioxidant-type behaviour.[41,42] This further suggests that, in addition to the general binding and trafficking functions of FABPs, they must have diverse and highly specified roles in regulating the metabolism and actions of the ligands they bind.

FABP1 (L-FABP)

The *FABP1* human gene is located at Chr 2p12-q11.[15,43] The gene has several regulatory elements, differing in specificity and function. For example, one TATA-box (5′-TATAAAA-3′) is located within the promoter region and is recognised by the Ets superfamily of proteins.[15,29] The gene promoter of *FABP1* also contains sterol response elements-1 and -2 (SRE1, SRE2), two hepatocyte nuclear factor (HNF) 1-binding sites, activator protein-1 (AP-1)-binding sites and a CCAAT/enhancer-binding protein (C/EBP) site.[6,39,44] In addition, the presence of a peroxisome proliferator response element (PPRE) is important for the regulation of *FABP1*, as well as other genes involved in metabolism.[29,33,37,45] Treatment with hypercholesterolaemic and hyperlipidaemic drugs (statins and fibrates) upregulates *FABP1* expression in the liver.[44,46] This mechanism is important to consider in diseases in which metabolic homeostasis is altered, such as non-alcoholic fatty liver disease, metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM) and alcoholic liver disease.

L-FABP is abundantly expressed in the liver, accounting for up to 5 per cent of the total cytosolic protein. The liver isoform of the FABP family of proteins is unique, attributed to its ability to bind multiple ligands at once. It has a larger solvent-accessible core (440 $Å^3$) in comparison to

M-FABP (330 Å^3), H-FABP (323 Å^3), A-FABP (310 Å^3) or I-FABP (234 Å^3);[47] this allows for more diverse binding to substrates, further indicating a unique functionality by comparison with other FABP family members. X-ray crystallography and NMR analysis of both apo-and holo-isoforms of L-FABP have revealed differences in the structural orientation of the protein in both states.[47,48] When oleate occupies both binding sites, site-specific perturbations are found near the linker between αII and βB, between strands βC and βD and βE and βF, as well as other areas within the binding cavity.[48] In other words, it appears as though the alpha-helix 'cap' and the β-turn of sheets E and F fold inwards to encapsulate the binding pocket. The orientation of the first FA/ligand, in the designated primary binding site, is in a bent or U-shaped conformation, with the carboxyl group interacting with Arg122, Ser39 and Ser124.[47] Other amino acids in contact with the FA are Ile41, Phe63, Glu72, Thr73, Thr93 and Thr102.[47] The second binding site is more heavily involved in electrostatic interactions, interacting with Leu28, Gly32, Ile35, Ile53, Tyr54, Gly55, Lys57, Met113 and Arg122.[47] The FA extends with its carboxylate group at the opening of the binding pocket and is exposed to the aqueous environment. The molecular dynamics of ligand dissociation from L-FABP has been investigated using computer simulations but is still largely speculative. Three potential 'portals' for FA exit are proposed, located at: the αII and βC/βD loop (portal I); βG/βH and βE/βF loops and the C-terminal end of αI and N-terminal end of the αII/αI loop (portal II); and at the bottom of the cavity (portal III).[49] The first binding site is considered the 'high affinity' site (with K_ds ranging from 4–60 nM) and the second site is the 'low affinity' site (with K_ds ranging from 0.3–12 μM for FA and FA-CoAs).[11,39,50–52] L-FABP also binds intermediates of FA oxidation (fatty acyl-carnitines) and glyceride synthesis (1-oleoylglycerol), as well as lysophospholipids, cholesterol, BAs, prostaglandins, lipoxygenase products, retinoids, heme and bilirubin (for extensive review refer to Atshaves *et al.*[39]). For larger ligands, such as BAs, the stoichiometry (1:1) and affinity are both significantly reduced.[53] In addition to possessing affinity for endogenous substrates, L-FABP also binds to a variety of xenobiotic drugs, including beta-blockers (atenolol, nadolol), non-steroidal anti-inflammatory drugs (ibuprofen, ketorolac), fibrates (benzafibrate, clofibrate, gemfibrozil) and benzodiazepines (diazepam, lorazepam) with affinities in the nano- to micromolar range.[54]

The high level of expression, binding properties and function in regulating a variety of cellular processes (inflammation, immunity, metabolism and energy homeostasis) demonstrate the importance of L-FABP. Therefore, it would be detrimental if inactivation and/or loss of this protein occurred. Gene knockdown studies in mice have revealed a significant impact on metabolic regulation and weight gain when challenged with high-fat or high-cholesterol-containing diets.[55–57] To date, a highly conserved c.340A > G missense mutation in exon 3 of the human *FABP1* gene has been identified, which results in a Thr → Ala substitution at position 94 (T94A).[58] Carriers for this single nucleotide polymorphism (SNP) were identified in a subset of French Canadian subjects, and had higher baseline plasma-free FA levels, lower body mass index (BMI) and a smaller waist circumference than T94 homozygotes. When challenged with fenofibrate, subjects were at increased risk for exhibiting higher plasma triglyceride (TG) levels (≥ 2.00 mmol/L). The T94A mutant was later found to be associated with quantitative traits associated with MetS, cardiovascular disease and T2DM (elevated fasting TG and LDL-cholesterol content).[59] The functional consequences of this SNP on L-FABP binding have not been investigated, but can be inferred—based on the location of the mutation—to affect binding in the cavity of the protein.

FABP2 (I-FABP)

The human *FABP2* gene is located at Chr 4q28-q31.[60] Three conserved domains of 14 bp are located in the 5′-flanking region of *FABP2* which specifically bind to nuclear proteins.[6,60] In mice,

one of these conserved 14-bp domains (5′-TGAACTTTGAACTT-3′) was found to be necessary for establishing and maintaining its region-specific expression along the duodenal-to-colonic axis of perpetually renewing gut epithelium; this same domain is found in other genes transcribed in the intestine. Nucleotides between −1178 and −277 have been promote expression of *FABP2* in the ileum and colon, whereas nucleotides between −277 and −185 suppress expression in these regions.[61] Two regulatory elements, the CCAAT and TATA box sequences, are also found in the promoter region of the *FABP2* gene.[15] Several more elements are found within *FABP2* and include cyclic adenosine monophosphate (cAMP)-responsive elements (24-bp region), which bind colonic nuclear factors; a C/EBP binding site; and HNF4 and apolipoprotein regulatory protein-1 (ARP-1) binding sites.[29,6,62]

This intestinal isoform of the FABP family mediates fat absorption through binding and intracellular trafficking of free LCFAs. It is highly expressed throughout the intestine, with the highest levels in the distal portion of the organ. L-FABP and Il-FABP are also expressed in different segments of intestine; therefore, it is difficult to determine the individual contributions of each protein in the uptake, trafficking and metabolism of FA.[1,63] Comparison of their binding properties (stoichiometry, specificity and affinity) reveals strong differences between all three FABPs expressed in the intestine.[63] Nevertheless, it can be inferred that I-FABP actively participates in dietary lipid metabolism due to its high specificity and selectivity for LCFAs. Although L-FABP and I-FABP bind saturated LCFAs with relatively the same affinity (but at different stoichiometric ratios), I-FABP exhibits lower affinity for unsaturated LCFAs.[64,65] In addition, the binding cavity of I-FABP is much smaller than that of L-FABP, which contributes to the lower binding ratio (1:1). Similar to L-FABP, the orientation of bound FA is in a slightly bent conformation, with its hydrocarbon tail extending toward the alpha helices (α_I-α_{II}) and the carboxylate group of the FA buried in the cavity and interacting with Arg106, Gln115 and two ordered water molecules.[9] Through the use of amide 15N relaxation and 1H exchange NMR experiments, it has been determined that the majority of residues do not undergo conformational changes between apo- and holo-I-FABP.[66] Residues Val26-Asp35, Ser52-Arg56 and Ala73-Thr76 (αII and the βC-βD and βE-βF turns) undergo significant conformational change upon ligand binding, however, forming a 'cap' over the FA. In addition to binding LCFAs ($K_d = 120$ nM for palmitate), I-FABP binds lipophilic drugs with lower affinity ($K_d = 62.5$ μM for ibuprofen; $K_d = 56.0$ μM for benzafibrate).[67,68]

Extensive studies have been performed on *FABP2* gene variants better to understand their possible contributions to metabolic diseases. Several SNPs in the *FABP2* gene have been identified, but only one has been associated with the pathologies attributed to MetS.[69–75] Gene variants include seven alleles (including the wild-type allele and trinucleotide repeats of 10–15 consecutive ATT sequences) in a microsatellite region of intron 2.[76,77] Three SNPs have been found—two being silent variants occurring in the coding regions of the gene (T → C at codon 71 and A → G at codon 118).[70] The third polymorphism in human *FABP2* is located at codon 54 (Ala → Thr) and is a missense variant associated with dyslipidaemia, insulin resistance and obesity.[69–71] Frequencies for the alanine-encoding (Ala54) and threonine-encoding (Thr54) *FABP2* alleles of 0.71 and 0.29 have been found in a population of 760 Pima Indians (which has the highest reported prevalence of T2DM).[70] Similar frequencies (0.69 and 0.31) were observed in 56 Caucasian DNA samples. In this study, Thr54 homozygotes had a higher mean two-hour plasma insulin concentration during an oral glucose tolerance test than did Ala54 homozygotes. Functional effects of the SNP were assessed with recombinant protein and were shown to reduce the affinity for oleate and arachidonate from 191 and 179 nM to 381 and 380 nM, respectively.[70] Based on these studies, it has been inferred that *FABP2* is not a major 'diabetes gene'; however, it may be associated with the propensity for T2DM. Association studies have also been performed with the Ala54Thr SNP and parameters of obesity and cardiovascular risk

factors in a group of 264 Spanish obese individuals.[78] Allelic frequencies for *Ala54/Thr54* heterozygotes and *Thr54/Thr54* homozygotes were 34.8 per cent and 7.2 per cent, respectively, and were significantly associated with elevated levels of C-reactive protein. In contrast to previously published reports, a newer study has failed to associate the Ala54Thr SNP with anthropometric and biochemical parameters of MetS in 760 obese subjects.[79] Another *FABP2* variant was found to be located in the 3′ non-coding region, where GCGCA → GCACA.[80] It is believed that this SNP does not affect the gene product, but little is understood about the sequences that flank coding regions of genes.[72] Of the evidence provided here, it is clear that more studies need to be conducted further to understand if gene variants of *FABP2* significantly contribute to pathologies associated with metabolic disorders.

FABP3 (H-FABP)

Human *FABP3* is located at Chr 1p33-p31, whereby distinct tissue distribution is regulated by concise promoter regions within the gene.[81] A 0.4 kb promoter region specifies expression of the protein in muscle, while elements further upstream in the 1.2 kb promoter are required for highly enriched expression of H-FABP in the heart. Multiple transcription factor binding sites are present within the promoter, including E box (CATGTG; consensus CANNTG) and TATA boxes. Full promoter activity is controlled via a CArG-like motif (5′-CTT CCT ATT TCG GGA GC-3′) that functions similar to SREs. The promoter also contains a potential response element for ERRα, while a PPAR response element is present in conserved regions of the *FABP3* gene.[37,81,82] Other motifs in the 5′-flanking region of porcine *FABP3* have been identified, including AP-1, AP-2, C/EBP, mammary active factor and Stat-5 binding sites.[83]

H-FABP constitutes 4–8 per cent of the cytosolic protein in mammalian heart, and is highly expressed in both cardiac and skeletal muscle and, to a lesser extent, in stomach, brain, lung and mammary gland.[84] An important function for H-FABP in skeletal muscle is to traffic FAs towards the mitochondria, where they undergo β-oxidation for energy expenditure. The presence of H-FABP in blood has been proposed as an early biochemical marker of acute myocardial infarction, as it is rapidly released from the myocardium into the circulation after cells are damaged.[85–87] It is also being considered as a cerebral spinal fluid marker for the fatal neurodegenerative disease, Creutzfeldt–Jakob disease.[88] H-FABP shares similar affinity for FAs with K_ds in the nanomolar range, with affinities for palmitate, oleate and arachidonic acid being 0.96 μM, 0.43 μM and 0.37 μM, respectively.[89] Additionally, H-FABP binds non-prostanoid oxygenated FAs (epoxyeicosatrienoic acids, hydroxyeicosatetraenoic acids and dihydroxyeiosatrienoic acids) with similar affinity ($K_d' = 0.05–14.2$ μM).[42] The conformation of a bound FA differs slightly from that with I-FABP, being orientated in a more confined U-shape, with the carboxylate group buried within the core of H-FABP.[90] In addition, different FAs (palmitic, oleic, elaidac and stearic acids) exhibit similar orientations in the crystallised holo-form.[90,91] An ordered network of hydrogen bonds allows for ligand binding, and involves the side chains of five residues of H-FABP (Arg126, Tyr128, Thr40, Arg106 and Thr53), as well as two water molecules with the carboxylate group of oleate. The portal region of H-FABP is similar to that of I-FABP, and is defined by the side chain residues of Val25, Thr29, Phe57, Ala75 and Asp76. It also has been postulated that lysine residues on the edge of this 'binding portal' add positivity to this region and may serve to draw the carboxylate group of FA toward the portal via electrostatic interactions. Solvent mapping of H-FABP has also highlighted a secondary portal region (Glu72, His93 and Arg106), which may act as the conduit for solvent efflux as a ligand either enters or exits the primary binding portal. Phe16 is also involved in van der Waals interactions with the bound ligand, and is hypothesised to be a key determinant for ligand specificity and affinity in H-FABP.[90] Mutation of this residue proved the importance of Phe16 in ligand binding.[92]

Initial characterisation of *FABP3* using southern hybridisation revealed multiple hybridising fragments of cDNAs in both humans and mouse, highlighting the presence of pseudogenes. An intronless *FABP3* gene (*FABP3P2*) has been identified in region 13q13-q14 and found to be 81 per cent homologous to *FABP3*; however, no cDNAs have been detected in human skeletal muscle or foetal brain libraries.[27] In addition to pseudogenes, several polymorphisms of *FABP3* have been identified and are presumed to contribute to traits associated with obesity, T2DM and MetS. In an attempt to screen for polymorphic markers and candidate genes for T2DM, 14 polymorphisms of *FABP3* have been identified.[93] These include two insertion/deletions, two short tandem repeats and ten SNPs (two in the promoter, nine within introns, two in the 3′-untranslated region and on the 3′ end of the gene). Among the identified polymorphisms found in 24 Korean DNA samples, five polymorphic sites (c.-530_-532delCTC, c.-345T > C, c.348 + 429(CA)9-18, c.246 + 1806G > C and c.634 + 483delT) were chosen for larger-scale screening in 669 individuals and found to have allele frequencies of 0.184 (c.-530_-532delCTC), 0.495 (c.-345T > C), 0.177 (c.246 + 1806G > C) and 0.139 (c.634 + 483delT), respectively. Logistics analysis between patients and normal subjects were performed and revealed the association of one insertion/deletion polymorphism at the 3′ end (c.634 + 483delT) of *FABP3* with an increased risk of T2DM. *FABP3* polymorphisms were also compared with five metabolic parameters (levels of cholesterol, TG, HDL, BMI and waist/hip ratio) and the only significant association, again, was with the 3′ end ins/del (c.634 + 483delT) SNP. These studies have shown only moderate associations between *FABP3* polymorphisms with the risk for T2DM and related phenotypes. Other association studies performed in Beijing-You chickens and pigs have related SNPs to intramuscular fat deposition and other fatness traits (back fat thickness and body weight).[94–96] A newly published report analysing postmenopausal osteoporosis in Hungarian women found a significant correlation between the homozygous recessive genotype of rs10914367 in the promoter region of *FABP3* and increased hip bone mineral density.[97] This polymorphism tags the first exon and intron of *FABP3*, which contain the transcription factor binding sites forkhead box protein P3 (GCCAAC), C/EBP-β (CCAA) and progesterone receptor isoforms A and B (AACACCA). These physical interactions between transcription factor binding sites are ablated in the case of the polymorphism, however. It is evident that only correlative studies have been performed with polymorphisms of the *FABP3* gene, and more detailed analyses need to be performed to clarify the contribution of genetic variations in the pathomechanisms of various diseases.

FABP4 (A-FABP)

FABP4 is located on Chr 8q21 and is regulated by a number of *cis*-acting regulatory elements and *trans*-acting nuclear factors in the 5′ flanking region of the genes that activates or represses transcription.[29,98,99] Two fat-specific elements (FSE) are found within the *FABP4* gene, including four copies (three direct and one inverted) of the 13 bp FSE1 (5′-GGCTCTGGTCATG-3′) and 15 bp FSE2 (5′-ACTCAGAGGAAAAG-3′).[22] The canonical TATA box is located 31 bp upstream of the transcription start site. Additionally, the gene has overlapping positive (AP-1) and negative (cAMP) regulatory elements, with a binding sequence for the trans-activating C/EBP upstream of these regulatory elements.[29] Further upstream of the 5′-flanking region of *FABP4* are five *cis*-acting adipocyte regulatory elements (ARE1, ARE2, ARE4, ARE6 and ARE7).[100] The ARE1 site targets a member of the nuclear factor (NF) 1 family and, when mutated, reduces the activity of the enhancer in adipocytes by 76 per cent. Both ARE2 and ARE4 are recognised by adipocyte regulatory factor 2 (ARF2), stimulating promoter activity in several cell types, while ARE6 and ARE7 stimulate promoter activity only in adipocytes and are bound by adipocyte regulatory factor 6 (ARF6).[29] *FABP4* also contains several functional PPREs approximately 5.5 kb upstream of the transcription start site, allowing for the transcriptional regulation of

FABP4 by fatty acids, PPARγ, insulin and agonists of PPARγ, such as thiazolidinediones.[37,101,102]

Adipocyte FABP (aP2) is highly homologous to myelin P2 protein, and is significantly expressed in white and brown adipose tissue, monocytes and macrophages.[33] Total expression in adipocytes is proposed to be 1–3 per cent of the total cytosolic protein and is a known gene marker for the maturation of adipocytes (50-fold upregulation).[103] In addition to the chaperone-like activity responsible for integrating lipid signals and organelle responses, A-FABP interacts with HSL, potentially modulating its catalytic activity and integrating signalling networks that control inflammatory responses and lipid hormone production in adipocytes.[1,26] In macrophages, A-FABP also mediates inflammatory responses through the inhibitor of kappa kinase nuclear factor κB (IKK-NF-κB) and c-Jun N terminal kinase (JNK)-AP-1 pathways and attenuates cholesterol efflux through inhibition of the PPARγ–liver X receptor α (LXRα)–ATP binding cassette A1 (ABCA1) pathway.[1,104,105] Recently, A-FABP has been found to be expressed within the skeletal muscle fibres, but is much less abundant than H-FABP.[106] The significance of muscular expression is hypothesised to be for metabolic adaptation, based on the fact that mRNA expression of A-FABP is elevated in endurance-trained subjects. A-FABP appears specifically to bind LCFAs with high affinity ($K_d = 80$, 83, 57, 92 and 182 nM for stearate, palmitate, oleate, linoleate and arachidonate, respectively).[11] A-FABP also binds retinoic acid, but with lower affinity ($K_d = 50\ \mu M$).[107] Crystal structures have been solved for the apo-form and holo-forms liganded with oleate, stearate, palmitate, hexadecane sulphonate and arachidonate and modified with the reactive aldehyde 4-hydroxynonenal.[108–112] Upon ligand binding, both the surface area and cavity volume increase and changes in the topology of the electrostatic potential and surface area are observed around the ligand portal.[113]The stoichiometry of binding is similar to that of I-FABP and H-FABP (1:1), while the orientation of stearate is slightly bent and U-shaped for arachidonic acid. The hydrocarbon tail of bound FA (stearic and oleic acid) is located in the cavity formed by side chains of hydrophobic amino acids, with the first segment of the FA (C-4) making van der Waals contact with Cys117, the middle segment (C5–C14) interacting with Tyr19 and a salt bridge formed by Asp76 and Arg78, and the last segment (C15–C18) protruding towards the solvent in close proximity to Val32, Phe57 and Lys58.[110] Tyr19 is phosphorylated by the insulin receptor kinase, which has been shown significantly to reduce both the affinity for LCFA and the release of ligand, demonstrating the involvement of A-FABP in the lipogenic pathway.[111,114,115] The carboxylate group of the bound FA forms a network of hydrogen bonds and other electrostatic interactions with Arg126, Tyr128, and Arg106 through an intervening water molecule.[112] Structural differences induced from binding affect the portal region and involve the end of αII (Met35, Ala36, Lys 37 and Pro38) and Phe57. These changes are absent when arachidonic acid is the bound ligand because its orientation is entirely buried within the cavity, although additional amino acids are involved in binding (Ser53 and Pro38).

A-FABP is becoming a hot target in the area of drug development for T2DM, based on animal studies involving a mutated protein and the resulting observed protection from insulin resistance when fed a high-fat diet as compared with wild-type controls.[116,117] Additionally, significantly higher concentrations are secreted from adipocytes into the serum of overweight/obese patients than into serum of lean persons.[118] These serum concentrations of A-FABP also correlated positively ($p < 0.005$) with waist circumference, blood pressure, dyslipidaemia, fasting insulin and the homeostasis model assessment insulin resistance index. Components of MetS also were assessed ($p < 0.05$), suggesting that A-FABP is a central regulator of systemic insulin sensitivity. Polymorphisms of the *FABP4* gene have also been associated with resistance to obesity and other associated pathologies that include dyslipidaemia, insulin resistance, T2DM and cardiovascular disease. A multitude of SNPS (119 via SNPHunter[119] and 130 from the National Center

for Biotechnology Information [NCBI] SNP database) have been identified, but only a few representative studies will be addressed in this overview. Five SNPs have been identified in Caucasian men and women, and include sequence variants at positions T-87C, C2600T, T2613C and an insertion of G at position 4265, which are outside the coding regions of the gene.[120] A silent variant, G4356C, was found on exon 4. Allele frequencies for these SNPs in 96 primarily Caucasian men and women were 4.69 per cent, 16.67 per cent, 0.52 per cent, 16.67 per cent and 0.52 per cent, respectively. The T-87C variant is located at the C/EBP binding site, and it was found that this mutation exhibited significantly reduced competitive-binding capacity ($\geq$50 per cent) for C/EBPα compared with the wild-type (WT) *FABP4* promoter. This variant also significantly impaired transcriptional activation of the promoter by C/EBPα in cultured cells. In a population cohort of 7,899 subjects, carriers for the T-87C variant (2.2 per cent allele frequency) had significantly lower fasting TGs and were associated with reduced risk for coronary heart disease and T2DM. Additionally, 1,247 subjects from Costa Rica (non-fatal myocardial infarction case-control study) were screened, and carriers for T-87C were found to have a 63 per cent reduction in adipose tissue A-FABP mRNA expression, indicating that T-87C is a loss-of-function allele *in vivo*. Two additional SNPs within the 5′ region of *FABP4*: A-376C and A-374C have been identified from 314 Hispanic and Caucasian males.[121] When A-376C and a SNP of PPARγ (Pro12Ala) were examined with measures of insulin sensitivity and body composition, the individual variants were not significantly associated; however, when both sites were considered as one model, they were significantly associated. *FABP4* variants have also been assessed in cases of childhood obesity, where the frequency of rs1054135 G/A was higher in obese compared with non-obese individuals and found to contribute to higher FABP4 levels. Meanwhile, rs16909233 G/A was found to be associated with insulin resistance in the context of obesity.[122] In contrast to fat-related pathologies, SNPs of *FABP4* have been analysed for contribution to the development of polycystic ovary syndrome (PCOS).[123] Three SNPs were identified in a population of Chinese women, where the genotype frequencies of two alleles (T-911C and G-737C) did not differ between PCOS and case control subjects. An insertion/deletion CA SNP at −798 to −799 bp was found to have higher frequencies in PCOS subjects, but was not associated with the clinical/biochemical parameters associated with PCOS.

FABP5 (E-FABP)

FABP5 is located at Chr 8q21.13, where putative response elements within the promoter region may be responsible for the wide level of expression observed throughout tissues. A more detailed analysis involving the mouse *FABP5* gene revealed promoter elements and transcription factor binding sites in the 5′ region.[124] The TATA box is at nucleotide (nt) −40 (5′-ATATAA-3′); a CCAAT box at nt −80 (5′-ATTGG-3′); and GC-rich regions at nt −110 (5′-GGGGCGG-3′). Putative binding sites for myogenic differentiation factor (MyoD), HNF1, C/EBPα and β, GATA binding protein 1 (GATA1) and myeloid zinc finger 1 (MZF1) exist within the promoter of *FABP5*. Additionally, PPREs present in the gene allow for functional interactions between PPARβ/δ.[38] To date, 15 known pseudogenes have been identified in humans, and are classified as *FABP5P1–15* (HGNC).

E-FABP is highly expressed in epidermal cells, but also in a plethora of other tissues, including mammary gland, brain, liver, kidney, lung, adipocytes, macrophages, tongue and testis.[1,33,36] It was first identified in human psoriatic keratinocytes (due to its significant upregulation) and then later in human epidermis.[125,126] It is difficult to decipher the exact functionality of the protein (in addition to its generic role of FA binding and trafficking) because all of these tissues also express additional FABPs. Gene ablation of A-FABP in adipocytes results in a significant upregulation of E-FABP expression, and ablation of both genes in mice suggests importance in systemic glucose and lipid homeostasis.[33,116,127] Similar to A-FABP, E-FABP

physically interacts with HSL when in the holoform, further characterising separate and distinct functions of FABPs.[127] It has also been hypothesised that E-FABP plays an important role in PPARβ/δ-mediated keratinocyte differentiation.[38] E-FABP differs from other members of the FABP/iLBP family in several ways. First, it displays a different electrophoretic mobility compared with FABPs and CRBPs of other tissues in the range pI = 6.2–6.4 for recombinant human E-FABP.[128] Secondly, it possesses six conserved cysteine residues, which is unusually high in FABPs, with Cys120 and Cys127 participating in a disulphide bond (the only reported case for the entire iLBP family).[128,129] Additionally, it has been found to complex with psoriasin (S100A7), a calcium-binding protein expressed in keratinocytes in psoriasis and in malignant breast epithelial cells.[130,131] The physiological ligand(s) of E-FABP are not completely known; however, the preferred ligand for E-FABP in recombinant protein binding assays is stearic acid ($K_d = 0.29$ μM) at a stoichiometric ratio of 1:1, while sites of unsaturation appear to reduce affinity ($K_d = 1.6$ μM and 1.73 μM for oleic acid and arachidonic acid, respectively).[128] X-ray crystallography has elucidated that interactions with bound ligands are similar to those of other FABP complexes, where the carboxylic headgroup forms salt bridges with Arg109 and Arg129 and hydrogen bonds with Tyr131.[128] Additionally, E-FABP exhibits the lowest conformational stability of FABPs in the presence of urea.[129]

Various studies have been conducted utilising models that involve either the ablation or overexpression of E-FABP, stressing an important contribution in the regulation of insulin responses inflammation, the water permeability barrier in the epidermis and even inducing a metastatic phenotype in rat mammary epithelial cells.[132–135] It has also become a serum marker for the association with MetS (independent of A-FABP), as it was found in a population of 497 Chinese subjects and correlated positively with parameters of adiposity, adverse lipid profiles, serum insulin, A-FABP and C-reactive protein levels, and was higher in subjects with MetS ($p < 0.001$ versus no MetS).[136] Polymorphisms in human *FABP5* have been identified in several studies. A 340G > C (Gly114Arg) variant of *FABP5* was found in a cohort of 285 Japanese autistic patients, but failed to make a genetic association with both autism and schizophrenia.[137] Phylogenetic analysis of the pig and human genomes shows a comparative map between these orthologues; seven polymorphisms of *FABP5* (g.1165G > T, g.1441G > A, g.3000T > G, g.3478G > A, g.3813A > G, g.4106C > T and g.4218C > T) were identified in Iberian x Landrace crosses and found to be associated with fatness traits.[138]

FABP6 (Il-FABP)

FABP6 was originally identified in both pig and mouse, and termed gastrophin and ileal LBP, respectively.[139–141] Localisation of the human orthologue on Chr 5q23-q35 has revealed, like other FAPBs, several conserved elements that transcriptionally regulate *FABP6*.[142] The proximal promoters of human, rabbit and mouse *FABP6* consist of a highly conserved but imperfect inverted repeat of the nuclear receptor half-site sequence (5′-(G/A)GGTGAATAACCT-3′) separated by one nt (IR-1) at position −160/−148 bp.[143] The IR-1 allows for the farnesoid X receptor/retinoic acid receptor heterodimer to bind to the *FABP6* DNA sequence, where binding is activated in the presence of BAs. Therefore, the IR-1 motif within the *FABP6* promoter is a functional BA response element. A consensus element for the caudal-related homeobox factor 2 is present within *FABP6*.[144] A putative PPAR-binding site is located at the −198 bp position and −186 bp upstream of the transcription initiation site, where gene induction via benzafibrate is PPARα- or PPARβ/δ-dependent.[145] Additionally, an SRE has been identified at position −72/−62 (5′-GTGGGGTGAC-3′), which is highly homologous with an SRE-binding protein (SREBP) binding site (SRE-1) found in the promoters of the LDL receptor, HMG-CoA synthase and glycerol 3-phosphate acyltransferase.[146] A putative Sp-1 binding site (GC box) (5′-GGGGC-3′) has also been identified at positions −72/−62, proximal to the SRE, further demonstrating involvement in

cholesterol homeostasis. This is confirmed when mice are fed a high cholesterol-containing diet, where Il-FABP is hypothesised to be upregulated though the SREBP1c/LXR pathway, although no LXR response element-like sequence has been found in the *FABP6* promoter.

Il-FABP is highly expressed in the ileum, and to a lesser extent in the ovaries, placenta and adrenal gland.[142] The ileum plays a critical role in entero-hepatic circulation of BA, further demonstrating the importance for Il-FABP and as specificity for binding and trafficking BAs. Il-FABP also binds BA and FA (although with less affinity) and interacts with the ileal BA transporter protein at the cytoplasmic face of the ileocyte, to aid in uptake and trafficking of BAs.[147] The binding properties of Il-FABP are not yet well known and existing publications conflict as to whether one or two binding sites exist. Binding constants have not been calculated for mono-, di-, tri-hydroxylated, conjugated or unconjugated BAs, but Il-FABP exhibits significantly less affinity for palmitate and oleate than for L-FABP.[63,142] Comparisons of the binding of nine different conjugated and non-conjugated BAs to Il-FABP have been investigated by monitoring changes in intrinsic tyrosine fluorescence; affinities for ligands increased in the order of taurine-conjugated > glycine-conjugated > unconjugated BA.[148] The solution structure of human Il-FABP has been analysed using 2D- and 3D-homo and heteronuclear NMR techniques, revealing binding interactions and structural information.[149] Bound cholyltaurine is deeply buried within the core of Il-FABP and its flexible side chain is located near the FA portal as an entry region into the binding core. This study concluded that these interactions act at 1:1 protein to ligand binding ratios, and involve a large number of intermolecular nuclear overhauser effects (NOEs) between cholyltaurine and amino acids Trp49, Met59, Asn61, Phe63, Ile71, Phe79, Val83, Leu90, Val92, Phe94 and Tyr97. Interactions between photoaffinity-labelled rabbit Il-FABP and photoreactive analogues of cholyltaurine indicated the contact point of the 3-position of the cholyltaurine derivative to be at residues His100, Thr101 and Ser102 of Il-FABP.[150] The contact point for the negatively charged side chain of cholyltaurine was identified at Arg122, where blocking studies with phenylglyoxal further reduced binding affinity for the ligand. Titration calorimetry demonstrated a lower affinity for oleate ($Kd = 36\ \mu M$).[151] X-ray crystallographic studies with zebrafish Il-FABP revealed conformational changes between apo-and holo-Il-FABP bound with cholate, where the hairpin connecting strands βG-βH obtains a more open conformation upon ligand binding.[152] This study also showed that several cholate molecules can bind to hydrophobic patches on the surface of the protein, and that two to three cholate molecules can bind within the core of a single Il-FABP molecule. Ligand selectivity has also been investigated using glycocholic (GCA) and glycochenodeoxycholic (GCDA) acids; where each ligand occupies individual binding sites of human Il-FABP (when incubated individually with the protein), but when equimolar amounts of GCA and GCDA are mixed with protein, GCDA binds exclusively to site 1 and GCA to site 2.[153] It was concluded that this ligand selectivity is energetically favoured, and is governed by the presence/absence of a hydroxyl group at the C-12 position of the steroid ring system.

It has been established that Il-FABP exhibits high affinity for BA and must participate in the regulation of bile homeostasis in the intestine; therefore, it seems that factors that affect the expression/activity of this protein may significantly contribute to the pathogenesis of many diseases. In a fat assimilation study, a variant of *FABP6* (Thr79Met) was the most significant marker gene associated with T2DM.[154] The Met allele of the Thr79Met substitution showed decreased risk for T2DM in a nested case-control study of 192 incident T2DM subjects and 384 controls from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort. The associative relationship for risk of T2DM and the Thr79Met SNP was further investigated in a larger cohort of 543 incident T2DM cases (EPIC-Potsdam cohort) and a case control study including 939 T2DM cases from the Co-operative Health Research in the Region

Augsburg (KORA).[155] The overall frequency of the homozygous rare (*Met79/Met79*) allele was 16 per cent. This allele was significantly associated with a lower risk of T2DM ($p = 0.04$) when adjusted for age, sex, BMI and waist circumference in the EPIC-Potsdam cohort; however, this significance was not reached in the association studies with the KORA subject cohort ($p = 0.06$). When association studies were analysed in different BMI groups (BMI $<25\ kg/m^2$, BMI $25-30\ kg/m^2$, BMI $>30\ kg/m^2$), it was found that *Met79/Met79* obese individuals (BMI $>30\ kg/m^2$) were at 53 per cent (EPIC-Potsdam; $p = 0.02$) and 31 per cent (KORA; $p = 0.05$) reduced relative risk for diabetes compared with Thr-allele carriers (*Thr79/Thr79, Thr79/Met79*). When the Thr79Met SNP was analysed for associations with anthropometric and metabolic traits related to T2DM and lipid metabolism (glucose, insulin, cholesterol, triglycerides and lipoproteins) in 2,122 non-diabetic subjects, no significant differences in age- and sex-adjusted mean values were established.

FABP7 (B-FABP)

Human *FABP7* has been identified at Chr 6q22-q23, where expression of the transcript during foetal stages is thought to play an essential role in the development of the brain.[156] Regulatory elements of *FABP7* control gene expression and regional expression within the brain and central nervous system (CNS). A canonical TATA box is located 30 bp upstream of the transcription start site, along with a *Pax* gene consensus-binding sequence (5′-AATATATTTTCACTCACACTTCGTATC-3′) at −548 to −565.[157] Temporal and spatial *FABP7* expression in both radial and Bergman glial cells is regulated by a radial glial cell-specific element (RGE), and is located between 0.3 and 0.8 kb upstream of the transcription start site.[6,157] Within the same region, a Pbx/Pit-1/Oct/Unc-86 (POU) binding site for the CNS-specific Pbx proteins Pbx-1, Brn-1 and Brn-2 (5′-ATCAATCTC-3′) has been found at positions −370 to −362 bp.[158] This Pbx/POU binding site has also been shown to be a critical element for the expression of B-FABP in the CNS. A hormone response element has also been identified at −286 to −275. It has been established that transcriptional regulation of *FABP7 in vivo* is complex; in addition to the RGE, another region controls expression in the dorsal root ganglion and notochord (−800 bp to −1,200 bp) and a silencer required for suppression in the dorsal spinal cord (−1,200 bp to −1,600 bp) also has been identified.[157] However, two NF1 binding sites have been found at positions −35 to −58 bp and −237 to −260 bp in malignant glioma cell lines.[159]

Tissue expression of B-FABP is both spatially and temporally correlated with neuronal differentiation in many regions of the CNS, including the postnatal cerebellum, embryonic spinal cord and cerebral cortex.[160] In mice, high levels of expression have been demonstrated in developing and postnatal cerebellum (postnatal day 0−10), with lower levels in the young adult (day 20) and negligible expression in the adult.[161] B-FABP is also highly expressed in radial glial cells in the developing brain (preperinatal striatum) and at diminished levels in mature glia of the white matter. It is also noteworthy that neurones in the grey matter do not express B-FABP; however, both H-FABP and E-FABP are present.[36] The binding properties of B-FABP exhibit certain characteristics of other FABP family members. For example, affinities for oleic acid and arachidonic acid are ~0.4 μM and 0.25 μM, respectively in mouse B-FABP; by contrast, B-FABP does not bind palmitic acid or retinoic acid.[162] Additional binding studies revealed that B-FABP exhibits higher affinity towards FAs with longer chain lengths, with *n*-3 polyunsaturated FAs (docosahexaenoic acid [DHA], eicosapentaenoic acid [EPA] and α-einolenic acid) being the preferred ligands. Titration calorimetry binding studies of human B-FABP revealed affinities for monounsaturated *n*-9 FAs and long-chain polyunsaturated *n*-6 and *n*-3 FAs to be $K_d = 41\text{-}47$ nM, 115-206 nM and 27-53 nM, respectively.[163] Polyunsaturated FAs (DHA, EPA and arachidonic acid) are highly concentrated within membrane phospholipids in the brain, by comparison with other tissues, and may further explain how B-FABP functions in the brain during

the development of the CNS by ensuring the constant supply of FA during cellular maturation. Crystal structure studies have revealed the conformation of a bound oleic acid to be in the typical bent conformation within the binding pocket, at a 1:1 ratio.[163] The carboxylate group of bound oleate interacts with Try128, Thr53, Arg106 and Arg126. Additionally, Phe16 is thought to regulate ligand binding, and the side chains of residues around the outer surface (Try19, Met20, Leu23, Thr36, Pro38, Val40, Thr53, and Arg78) of the binding site appear to stabilise the folded conformation of the bound FA. By comparison with oleate, the conformation of the acyl chain of DHA adopts a more helical conformation, where the double bonds of DHA are in the *cis*-configuration and have rotational flexibility. The carboxylate moiety interacts with Try128 and Arg126, and the double bonds form extensive $\pi-\pi$ interactions with Phe104, Met115 and Phe16.

Utilising gene knockdown studies in mice,[164] B-FABP has been shown to have effects on brain development, emotion, learning and memory. Although *B-FABP*$^{-/-}$ mice showed no gross morphological or histological alterations in the brain, distinct behavioural differences were found in these mice.[33] These differences included enhanced anxiety and increased fear memory, decreased levels of DHA during the neonatal period, altered FA content in the amygdala of the adult brain (increased arachidonic and palmitic acids) and decreased *N*-methyl-D-aspartate receptor-mediated current to DHA.[164] In mice, *FABP7* was also traced using quantitative train loci analysis for impaired prepulse inhibition as an endophenotype for schizophrenia.[165] Additionally, a population-based genetic association study of 570 schizophrenics and 570 age/sex frequency-matched controls identified eight *FABP7* SNPs (SNP1 −4058T > C and SNP2 −2323G < A in the 5′-flanking region; SNP3 IVS1-63T > G in intron 1; SNP4 182C > T (Thr61Met) in exon 2; SNP5 IVS3-1047G > T and SNP6 IVS3-555G > T in intron 3; and SNP7 Ex4 + 810T > C and SNP8 Ex4 + 3890T > C in the 3′-flanking region of the gene). Significant associations were detected with the Thr61Met missense SNP4 ($p = 0.038$), but only in male cohorts. Following modelling studies, it was proposed that this SNP causes decreased binding of DHA and alters protein stability. Another *FABP7* SNP (376G > C, Val126Leu) was identified in 285 Japanese autistic individuals, but no genetic association of the SNP with autism and schizophrenia could be established.[137] A study assessing candidate genes for ethanol-preferring phenotypes in rodent models of human alcoholism uncovered that *FABP7* is differentially regulated by alcohol, although the expression pattern failed to segregate phenotypes that either prefer or avoid behavioural alcohol consumption.[166] Additionally, it has been shown that B-FABP is overexpressed in the brains of foetuses with Down's syndrome (DS), and *FABP7* is hypothesised to contribute to DS-associated neurological disorders.[167]

FABP8 (M-FABP)

FABP8 is located at Chr 8q21.3-q22.1. The 5′-flanking region contains two AP-1 binding sites, a candidate TATA element and two CAAT sequences.[13,29,168] Along with P_0 glycoprotein and myelin basic protein, M-FABP (peripheral myelin protein 2) is one of the major proteins comprising peripheral nervous system myelin (up to 15 per cent soluble protein).[29] It is also expressed in small amounts in the CNS myelin (spinal cord and brain stem).[169] Myelin is the multilamellar compacted membrane structure that surrounds and insulates axons and thus facilitates conduction of nerve impulses.[13] In addition to binding LCFA in Schwann cells, the function of M-FABP is largely unknown. It is hypothesised to stabilise myelin membranes by supplying LCFA and contributing to membrane biogenesis, which can be attributed to a high composition of lipid (75–80 per cent). In recombinant binding assays, it shared a similar affinity for LCFA as other FABP family members ($K_d = 0.62$, 0.31 and 0.37 μM for palmitate, oleate and arachidonate, respectively).[148] X-ray crystallography has revealed that residues Arg106, Arg126 and Tyr128 interact with the carboxyl group of bound oleic acid.[170] Computational docking

studies and fluorescence spectroscopy measurements have also demonstrated that cholesterol is a likely ligand for M-FABP *in vivo*.[169] More studies with this family member are required, as SNP associations with human diseases have not yet been established.

FABP9 (T-FABP)

FABP9 is another poorly understood member of the FABP family. It is located at Chr 8q21.13. T-FABP, or PERF15, is a major protein found in the inner acrosomal membrane and outer face of the nuclear envelope of mammalian sperm.[171] It shares the most homology with M-FABP (MP2), A-FABP and H-FABP; therefore, it is hypothesised that it shares similar binding affinities for LCFA. It is expressed during spermatogenesis and in mammalian testis.[172,173] It is known that the FA composition of sperm is related to fertility, so the proposed function of T-FABP has been hypothesised to protect sperm FA from oxidation, thus maintaining their ability to fertilise oocytes.[174] Other roles of T-FABP have been investigated with $FABP9^{-/-}$ mice, where gene-ablated mice had significant increases in sperm head abnormalities (8 per cent greater than WT), but still remained fertile.[175] The total lipid profile of the $FABP9^{-/-}$ mice did not change when compared with WT controls; however, it was proposed that FABP12 may share a compensatory role.

FABP12

The *FABP12* gene is the newest member of the FABP family and, to date, little information is available on the functional properties of the protein. *FABP12* is phylogenetically restricted; it has been identified in human, rat and mouse but no counterpart has been identified in chicken and zebrafish genomes.[18] It has been mapped to the same chromosomal region as *FABP4*, *FABP5*, *FABP8* and *FABP9* (8q21.13). Regulatory elements that control gene transcription have not been verified. FABP12 mRNA has been identified in the retina and testis at high levels, and to lesser extent in the cerebral cortex, kidney and epididymis of rat and mouse tissues. The transcript has also been identified in human retinoblastoma cells. Identification of the *FABP12* gene provides further evidence of the evolutionary divergences of the FABP protein family from a single ancestral gene, as it is hypothesised to have formed from a tandem gene duplication.

Conclusions

The *FABP* gene family embodies a group of diverse proteins which participate in a spectrum of tissue-specific pathways involved in lipid homeostasis. To date, nine putatively functional protein-coding *FABP* genes have been identified in humans. A newer member of the family, *FABP12*, has been less studied. The evolutionary conservation of these genes suggests that FABPs have diverse and highly specified roles in regulating the metabolism and actions of the ligands they bind. As we understand these functions more clearly, FABPs will be highlighted as important targets for drug development and therapy for many metabolic diseases.

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