JMM Correspondence

Phospholipid catabolism by gut microbiota and the risk of cardiovascular disease

The role of phospholipids in the development of cardiovascular disease (CVD) is unclear, in contrast to current knowledge about triglycerides and lipoproteins. To address this gap in our knowledge, a study by Wang *et al.* (2011) described a metabolomic analysis of serum samples from human CVD patients using liquid chromatography–mass spectrometry (LC–MS), and identified trimethylamine *N*-oxide (TMAO), a choline metabolite, as a possible marker of the risk of developing CVD.

Phosphatidylcholine (PC), a major component of both plant and animal plasma membranes and especially abundant in foods such as fish, eggs and milk, is a major dietary source of choline, which is converted to trimethylamine (TMA) by the microflora. TMA is converted to TMAO by at least one liver enzyme [hepatic flavin-containing monooxygenase 3 (FMO3)]. Wang *et al.* (2011) noted that when apolipoprotein E-deficient ($Apoe^{-/-}$) mice, which are prone to develop atherosclerosis, were fed a normal diet (control group) or a choline-enriched diet (experimental group), the latter group, as expected, developed atherosclerotic symptoms. Interestingly, administering broad-spectrum antibiotics to $Apoe^{-/-}$ mice in the experimental group prior to choline feeding counteracted this effect. It was therefore suggested that the catabolism of PC by the gut microbiota could increase the risk of developing CVD. However, the study did not indicate what bacterial effectors could be responsible for the production of choline from dietary PC. It has been suggested that bacterial phospholipase D (PLD), which can directly hydrolyse PC to phosphatidic acid and choline, could be this effector (Loscalzo, 2011), but the genes encoding such enzymes in the gut microbiota remain unidentified.

Bacteroidetes and *Firmicutes* are the predominant microbial phyla found in the human intestine (Eckburg *et al.*, 2005). Of the *Bacteroidetes*, it is known that *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* (Gram-negative anaerobes) are the predominant species, with the latter being chosen as a model symbiont in studies investigating host–microbiota interactions (Moore & Holdeman, 1974; Xu & Gordon, 2003). The genomes of both species have been sequenced, and are predicted to encode several putative phospholipases that may be assigned to one of two categories – the PLD and the patatin-like phospholipase families (see Table 1).

Early biochemical studies indicated that both *B. thetaiotaomicron* and *B. fragilis* exhibit phospholipase activity (James & Robinson, 1975), though the underlying genes remain unknown. The PLD-like enzymes (Table 1) contain two HKD domains that are shared by other members of the PLD family such as cardiolipin synthases, phosphatidylserine synthases and some endonucleases and helicases (Ponting & Kerr, 1996). Note that the enzymes listed in this category in Table 1 are actually annotated as cardiolipin synthases in the Comprehensive Microbial Resource, but none of these have been cloned or otherwise functionally characterized. However, given the strong conservation of HKD domains within the PLD family, it is difficult to predict enzyme activity accurately on the basis of protein similarity. For example, after sequencing the genome of *Rickettsia conorii*, a protein designated RC1270 was originally annotated as an 'unknown protein' (Ogata *et al.*, 2001). Subsequent biochemical testing of recombinant RC1270 demonstrated PLD activity, but phylogenetic clustering showed close matches with proteins from other bacteria that were variously annotated as cardiolipin synthases in *Buchnera aphicolada*, *Mycoplasma pulmonis* and *Pseudomonas putida*, but as PLD in *Arcanobacterium haemolyticum*, *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans* and *Photobacterium damselae* (Renesto *et al.*, 2003, in which this protein is designated 'RC127'). In another instance, the protein HP0190 encoded by gastric pathogen *Helicobacter pylori* strain 26695, and its homologues in strains J99 and HPAG1 that are more than 90 % identical at the protein level, have been annotated as 'conserved hypothetical secreted protein', 'putative cardiolipin synthase' and 'conserved hypothetical secreted protein', respectively. Interestingly, inactivation of HP0190 homologues in two other strains of *H. pylori* (J166 and 7.13) resulted in mutants having an attenuated phenotype in synergistic haemolysis tests and ERK1/2 activation assays in co-culture with a gastric cell line, when compared to the parental strains (Sitaraman *et al.*, 2012). This indicates that HP0190 homologues, in the tested strains at least, may possess membrane-damaging (lipolytic) activity.

The phospholipases containing patatin motifs are expected to exhibit phospholipase A activity based on precedent. Similar enzymes are also encoded by several species of pathogenic bacteria (Banerji & Flieger, 2004). For example, the enzyme PatD expressed by *Legionella pneumophila* exhibits lysophospholipase A and phospholipase A activities (Aurass *et al.*, 2009). The ExoU cytotoxin of *Pseudomonas aeruginosa* is known to possess both lipase and phospholipase A2 activities (Sato *et al.*, 2003). However, a product of phospholipase A activity on PC species – glycerophosphorylcholine diester – could subsequently serve as a substrate for microbial glycerophosphoryl diester phosphodiesterases (GDPDs), releasing choline. Putative GDPDs in the genomes of *B. thetaiotaomicron* and *B. fragilis* have also been identified and annotated (see Table 1).

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Table 1. Putative phospholipases and glycerophosphoryl diester phosphodiesterases encoded by *Bacteroides* species

Locus designations and annotation are given exactly as in the Comprehensive Microbial Resource (CMR, available at http://cmr.jcvi.org).

Organism	Protein family	Locus	CMR annotation
Bacteroides thetaiotaomicron VPI-5482	Phospholipase D	BT_2046	Putative cardiolipin synthetase
		BT_2382	Putative cardiolipin synthetase
		BT_3978	Putative cardiolipin synthetase
	Patatin-like phospholipase (probable phospholipase A)	BT_0303	Putative patatin-like phospholipase
		BT_0774	Conserved protein with a conserved patatin-like phospholipase domain
		BT_0896	Putative patatin-like phospholipase
		BT_1016	Conserved protein with a conserved patatin-like phospholipase domain
	Glycerophosphoryl diester phosphodiesterase	BT_0195	Glycerophosphoryl diester phosphodiesterase
		BT_0442	Glycerophosphoryl diester phosphodiesterase
		BT_0550	Putative glycerophosphodiester phosphodiesterase
		BT_3162	Glycerophosphoryl diester phosphodiesterase
		BT_4726	Glycerophosphoryl diester phosphodiesterase
		BT_4727	Glycerophosphoryl diester phosphodiesterase
Bacteroides fragilis YCH46	Phospholipase D	BF0746	Putative cardiolipin synthetase
		BF3733	Putative cardiolipin synthetase
	Patatin-like phospholipase (probable phospholipase A)	BF0519	Putative patatin-like phospholipase
		BF2408	Putative patatin-like phospholipase
		BF3111	Putative patatin-like phospholipase
		BF3807	Putative patatin-like phospholipase
	Glycerophosphoryl diester phosphodiesterase	BF2640	Putative glycerophosphodiester phosphodiesterase
		BF4444	Putative glycerophosphoryl diester phosphodiesterase
Bacteroides fragilis NCTC 9343	Phospholipase D	BF0675	Putative cardiolipin synthetase
		BF3522	Putative phospholipid biosynthesis-related protein
	Patatin-like phospholipase (probable phospholipase A)	BF4363	Putative lipase/esterase
		BF2490	Phospholipase (similar to BF2408 of YCH46)
		BF2948	Putative exported protein (similar to BF3111 of YCH46)
		BF3599	Hypothetical protein (similar to BF3807 of YCH46)
	Glycerophosphoryl diester phosphodiesterase	BF2662	Conserved hypothetical exported protein
		BF4242	Putative glycerophosphoryl diester phosphodiesterase (*ugpQ*)

Admittedly, this list is only a tentative one that is unlikely to be either definitive or complete, given the paucity of specific experimental data from *Bacteroides* with regard to lipases/phospholipases. It may, however, serve as a starting point in a search for bacterial effectors that could influence host phospholipid profiles. In the light of the foregoing account, the mouse model developed by Wang *et al.* (2011), i.e. germ-free *Apoe*$^{-/-}$ mice, affords experimenters the opportunity to test the functionality of putative bacterial effectors by using *B. thetaiotaomicron* strains that are mutated in one or more of the genes listed in Table 1. Then, mice colonized with mutant strains of *B. thetaiotaomicron* should exhibit reduced plasma TMAO levels, compared to those infected with the wild-type strain.

It is likely that *Bacteroides* enzymes hydrolyse dietary PC, contributing to the total choline/TMA load in the host. By the same token, ectopic and chronic expression of phospholipases by pathogens could be one of mechanisms underlying the apparent association of CVD with certain chronic infections with certain pathogens such as *Helicobacter pylori* (Patel *et al.*, 1995; Kountouras *et al.*, 2011), *Chlamydia* species (Saikku *et al.*, 1988; Patel *et al.*, 1995) and cytomegalovirus (Epstein *et al.*, 1996). Specific inhibitors of bacterial phospholipases, preferably non-absorbable through the gut, might alleviate excess TMAO levels, at least in the short-term.

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