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REVIEW

Ceramides in cardiovascular disease: emerging role as independent risk predictors and novel therapeutic targets

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Abstract

Ceramides are bioactive lipid mediators involved in apoptosis, inflammation, and fibrosis. This narrative review provides a concise overview of the emerging role of ceramides in cardiovascular disease with an emphasis on atherosclerotic vascular disease and heart failure, suggesting the potential use of ceramides in risk stratification and as putative therapeutic targets. Recent developments based on observational evidence and genetic associations, including Mendelian randomization studies in humans, are summarized and put into context with experimental evidence for the role of ceramides in human and animal models of disease. Emerging scores composed of ceramides and phosphatidylcholines that are based on the length and desaturation of the N-acyl chains are discussed in the light of novel data demonstrating age- and sex-specific differences. Also reviewed is the structural heterogeneity of the sphingoid bases, including non-conventional sphingolipids that are increasingly recognized for their importance in health and disease. Lastly, novel targets and potential modalities for tissue-specific transfer of drugs are discussed.

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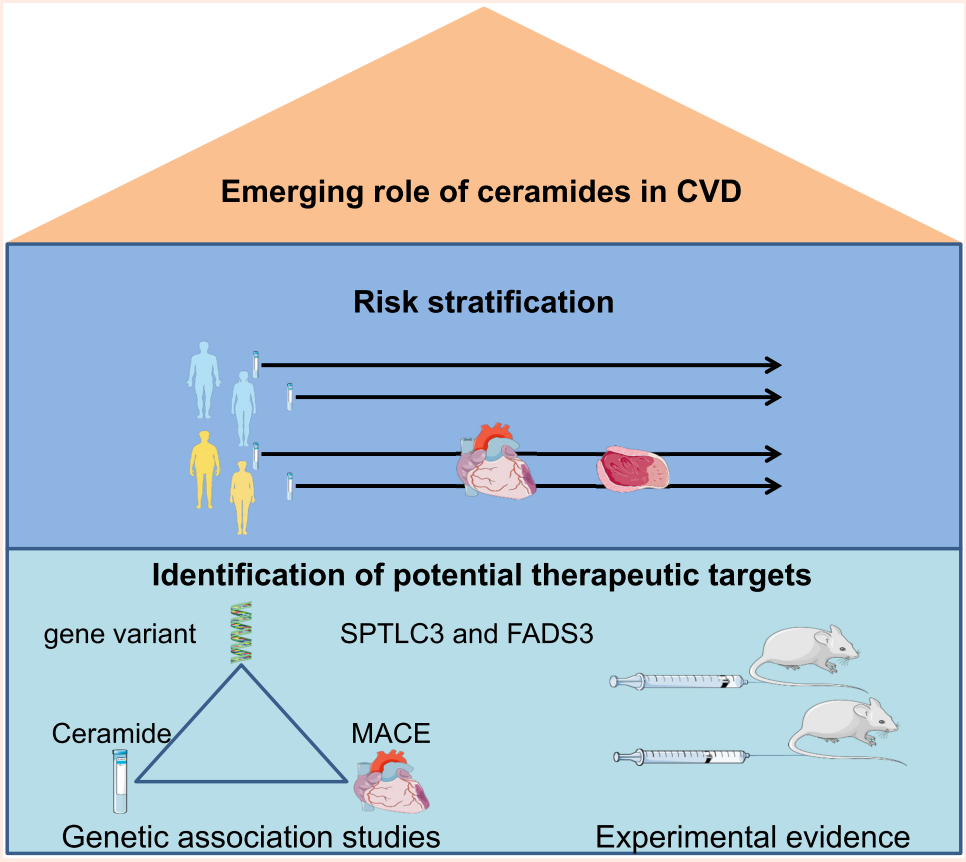
Graphical Abstract



Ceramides are increasingly recognized as important bioactive lipid mediators in cardiovascular disease (CVD). In this narrative review, we focus on atherosclerotic cardiovascular disease with its acute clinical manifestation of myocardial infarction and heart failure. We summarize current evidence on ceramide plasma levels or myocardial content as risk markers for major adverse cardiac events and CVD. Genetic association studies, and especially genome-wide association studies of plasma ceramide levels and incident CVD, including Mendelian randomization studies, provide further evidence regarding a potential causal role of ceramides in CVD. Based on experimental data, we provide an overview of drug targets that may have the potential for translation to the clinic. MACE, major adverse cardiac events; CVD, cardiovascular disease; SPTLC3, serine palmitoyltransferase long-chain base subunit 3; FADS 3, fatty-acid desaturase 3.

Keywords Ceramides • Atherosclerotic vascular disease • Heart failure • Risk stratification • Therapy

1. Introduction

Plasma levels of cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides are used for risk prediction in various guideline-recommended algorithms for the estimation of risk for atherosclerotic cardiovascular diseases (ASCVDs)[1] as a causal factor. LDL-C has even become the main therapeutic target in the management of patients with coronary artery disease (CAD).[2] However, the limited diagnostic efficacy of current clinical risk assessment tools as well as the considerable number of cardiovascular events occurring in individuals with optimal control of risk factors, including LDL-C values at target, suggest the presence of as yet untargeted causal risk factors.[3] Among them, the so-called residual lipidic risk is mostly connected with triglyceride-rich lipoproteins and lipoprotein(a) which became the target of advanced drug development programmes.[4] However, it is important to note that plasma and lipoproteins contain thousands of lipid species some of which may play a role in the pathogenesis of atherosclerosis beyond cholesterol.[5] In particular, sphingolipids have emerged as potential pathogenic or protective molecules in cardiometabolic diseases.[6] Sphingolipids are structurally very diverse because they arise from the combination of different sphingoid bases with different N-acyl chains and headgroups.[7] Moreover, sphingolipids are at the metabolic crossroad of fatty acids and amino acids and, indirectly, also carbohydrates, as glycolysis generates precursors of amino acids which are used for the synthesis of sphingoid bases.[7] There is increasing evidence that sphingolipid species and, in particular, ceramides are risk markers of various cardiometabolic diseases. Their plasma concentrations and derived scores are emerging as promising biomarkers for the identification and stratification of cardiovascular risk.[8,9] Although not endorsed by guidelines and not reimbursed by health insurance, several clinical laboratories have started to offer these scores as part of their clinical workup.[10]

Here, we present a critical review of the existing literature that not only addresses the evidence for the use of ceramides as a risk marker but also their causal role in disease and hence their suitability as a therapeutic target.

2. Structure, function, and metabolism of ceramides

Ceramides and other sphingolipids are usually quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS).[11] They are typically

described by the number of C-atoms and double bonds of the N-linked fatty acid: for example, C16:0 or C24:1 ceramides. This description ignores the heterogeneity of the sphingoid backbone. Indeed, only two-thirds of plasma sphingolipids are built on the canonical d18:1(4E) backbone with one double bond between the 4th and 5th C-atoms.[11,12] The other 3rd are atypical sphingolipids including, for example, deoxy-, C16-, and C20-sphingolipids. Hence, a complete description of a typical ceramide comprising a fully saturated fatty acid with 22 C-atoms reads Cer(d18:1(4E)/C22:0). Conversely, Cer(d16:0/C24:0), Cer(d18:2(4E,14Z)/C16:0, and Cer(m18:1(4E)/C22:0) are examples of atypical sphingolipids (*Figure 1*).

Sphingolipids serve as key constituents of cell membranes and plasma lipoproteins and play an important role as lipid mediators. Ceramides are either synthesized *de novo* or formed by the breakdown of complex sphingolipids.[6,11] The classical *de novo* synthesis starts with the condensation of serine with palmitoyl-CoA by serine-palmitoyl-CoA transferase (SPT) to form the typical C18-sphingoid backbone of sphingolipids, including ceramides. However, SPT also uses alanine and glycine as well as shorter or longer and even branched acyl-CoAs as the substrates and thereby generates atypical sphingolipids.[11] Ceramide synthases (CerS) condensate the sphinganine with fatty acids of different chain lengths and saturation. The ceramides form the backbone of complex sphingolipids such as sphingomyelins, hexosylceramides, and, thereof, the more complex glycosphingolipids. Subsequent metabolism of ceramides via ceramide kinases, ceramidases, and sphingosine kinases (SK) results in the formation of ceramide-1-phosphate, a key regulator in inflammation, and sphingosine-1-phosphate (S1P), a bioactive intermediary metabolite that undergoes irreversible degradation by S1P lyase (*Figure 2*).

Disturbances in the biosynthesis or catabolism of ceramides are associated with or even cause chronic diseases, including ASCVD, cardiomyopathy, type 2 diabetes mellitus (T2DM), metabolic dysfunction-associated steatotic liver disease (MASLD), peripheral neuropathies, and amyotrophic lateral sclerosis.[6,13] The multifaceted effects of ceramide synthase family members 1–6 (CERS1-6) are summarized by Choi *et al.*[6] which emphasize the evidence for increased ceramide synthase 6 (CERS6) activity in a range of pathologies. While glycosphingolipids undergo enzymatic degradation in the endo-/lysosomal compartment only, sphingomyelins are degraded in lysosomes and the plasma membrane by acid and neutral sphingomyelinases, respectively.[14,15] Of note, however, acid sphingomyelinase can also be secreted and hence degrade sphingomyelins but also other phospholipids of circulating lipoproteins.[16] Disturbances in the lysosomal degradation of complex sphingolipids cause sphingolipidoses such as Niemann–Pick disease A and B, Fabry–Anderson disease, or Gaucher disease. Their pathologies, affecting frequently the central nervous system but also many other organs including the heart, are caused by the intracellular accumulation and cytotoxicity of the non-degraded sphingolipids or aberrant metabolites rather than by any reduction of ceramides.[15] Conversely, the inhibition of neutral sphingomyelinase 2 has become a therapeutic target, as it affects ceramide levels and, thereby, membrane structure, extracellular vesicle formation, cell survival, and inflammation.[14,17] Ceramides are key mediators in cellular energy metabolism,[18] including in the heart,[19] and serve as intracellular nutrient sensors.[20] Ceramides exert deleterious effects on the heart and vessels that involve apoptosis, inflammation, and fibrosis.[6] It is generally thought that ceramides exert deleterious effects on the heart and vessels that involve apoptosis, inflammation, and fibrosis.[6] However, this appears to partially depend on whether the exposure of cells is acute or chronic. Chronic exposure to ceramides formed intracellularly either by *de novo* synthesis or by the lysosomal breakdown of complex sphingolipids appears to exert mainly damaging effects. Conversely, a part of extracellular ceramides exposed to cells is rapidly processed by ceramidases and sphingosine kinase residing in the plasma-membrane residing enzymes. The resulting S1P counteracts several adverse effects of ceramides so that the balance between the intracellular levels of ceramide and extracellular abundance of S1P determines the fate of the cell. This situation has been summarized as the Cer-S1P rheostat.[21] *Table 1* summarizes the potentially pro- and anti-atherogenic effects of ceramides and S1P on endothelial cells, smooth muscle cells, monocytes, and macrophages as well as lymphocytes. Several animal studies showed that genetic or pharmacological interference with S1P generation and degradation by sphingosine kinase and S1P lyase, respectively, the S1P-binding protein apolipoprotein M (apoM), or S1P receptors affect the development of atherosclerosis. Most but not all studies support the anti-atherogenic role of S1P. One important reason for the discrepancies is the interaction of S1P with five different S1P receptors that elicit partially different downstream signalling cascades (*Table 1*).[22–26]

In plasma, sphingolipids including ceramides are transported by lipoproteins. Most of them are present in all lipoprotein classes, although some are predominant in specific classes, for example, ceramides and hexosylceramides in LDL,[27,28] deoxysphingolipids in very LDLs (VLDL), sphingomyelins and C16-sphingolipids in LDL and HDL,[27,29] or S1P in HDL due to its binding to apoM.[27,30] Interestingly, the concentration of sphingomyelins relative to phosphatidylcholines (PC) determines the aggregability and proteoglycan binding of LDL and, hence, atherogenicity of LDL. In animal models, dietary and pharmaceutical interventions that lower this ratio as well as inhibition of secretory acid sphingomyelinase also reduce LDL aggregation and atherosclerosis.[31,32]

2.1 Observational evidence

2.1.1 Association of ceramide accumulation or plasma levels with cardiometabolic diseases

2.1.1.1 ASCVD

Early work identified distinct ceramide signatures (particularly ceramide d18:1/16:0) in atherosclerotic plaques of patients with CAD that were associated with vulnerable plaque features as assessed by intravascular imaging (necrotic core and lipid core burden index).[33] These in turn were associated with adverse cardiovascular events in the ATHERO-REMO study,[33] which confirmed prior data from studies of carotid plaques showing that ceramide content was associated with markers of plaque inflammation and instability.[34] Initial hypothesis-free lipidomic analyses have revealed a distinct set of lipids potentially qualifying as useful prognostic markers for ASCVD.[35–37] Further analysis of large cohorts of patients with CAD (chronic and acute) demonstrated that certain ceramide signatures [ratio of Cer(d18:1/16:0)/Cer(d18:1/24:0) in plasma improved risk stratification for cardiovascular death even after adjustment for other lipid parameters including LDL-C, clinical risk scores such as GRACE, and statin treatment.[8] This finding was confirmed in other cohorts of chronic and acute CAD patients, demonstrating an independent predictive value of plasma ceramide ratios for adverse cardiovascular events beyond clinical risk factors.[38–40]

Recent work showed an improved prediction of cardiovascular disease (CVD) mortality in patients with CAD risk stratification when ceramides were combined with PC to form a specific ceramide–PC risk score (CERT2).[9] The original CERT1 score consisted of three single ceramides [Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1)] and three ceramide/ceramide ratios [Cer(d18:1/16:0)/Cer(d18:1/24:0), Cer(d18:1/18:0)/Cer(d18:1/24:0), and Cer(d18:1/24:1)/Cer(d18:1/24:0)].[8] The refined CERT2 score is based on one ceramide/ceramide ratio [Cer(d18:1/24:1)/Cer(d18:1/24:0)], two ceramide/PC ratios [Cer(d18:1/16:0)/PC(16:0/22:5) and Cer(d18:1/18:0)/PC(14:0/22:6)], and a single PC ratio [PC(16:0/16:0)].[9] The CERT2 score improved the prediction of cardiovascular death and was independent of established clinical risk factors and biomarkers in patients with acute coronary syndrome.[41] In patients with peripheral artery disease, this risk score (CERT) enabled long-term prediction of adverse clinical events.[42] Another ceramide–PC combination [Cer(d18:1/16:0)/PC(16:0/22:5) ratio] in plasma extracellular vesicles was associated with major adverse cardiovascular events during 3 years of follow-up in cerebrovascular ASCVD (after carotid endarterectomy).[43]

In primary prevention of ASCVD, distinct ceramide signatures were identified that were significantly associated with adverse cardiovascular events in apparently healthy individuals of the FINRISK cohort[44,45] and in individuals with hypertension.[46] The authors showed improved net risk classification by the CERT2 score comprising Cer16:0, Cer18:0, Cer24:1, and Cer24:0 if combined with a self-derived risk score (that does not

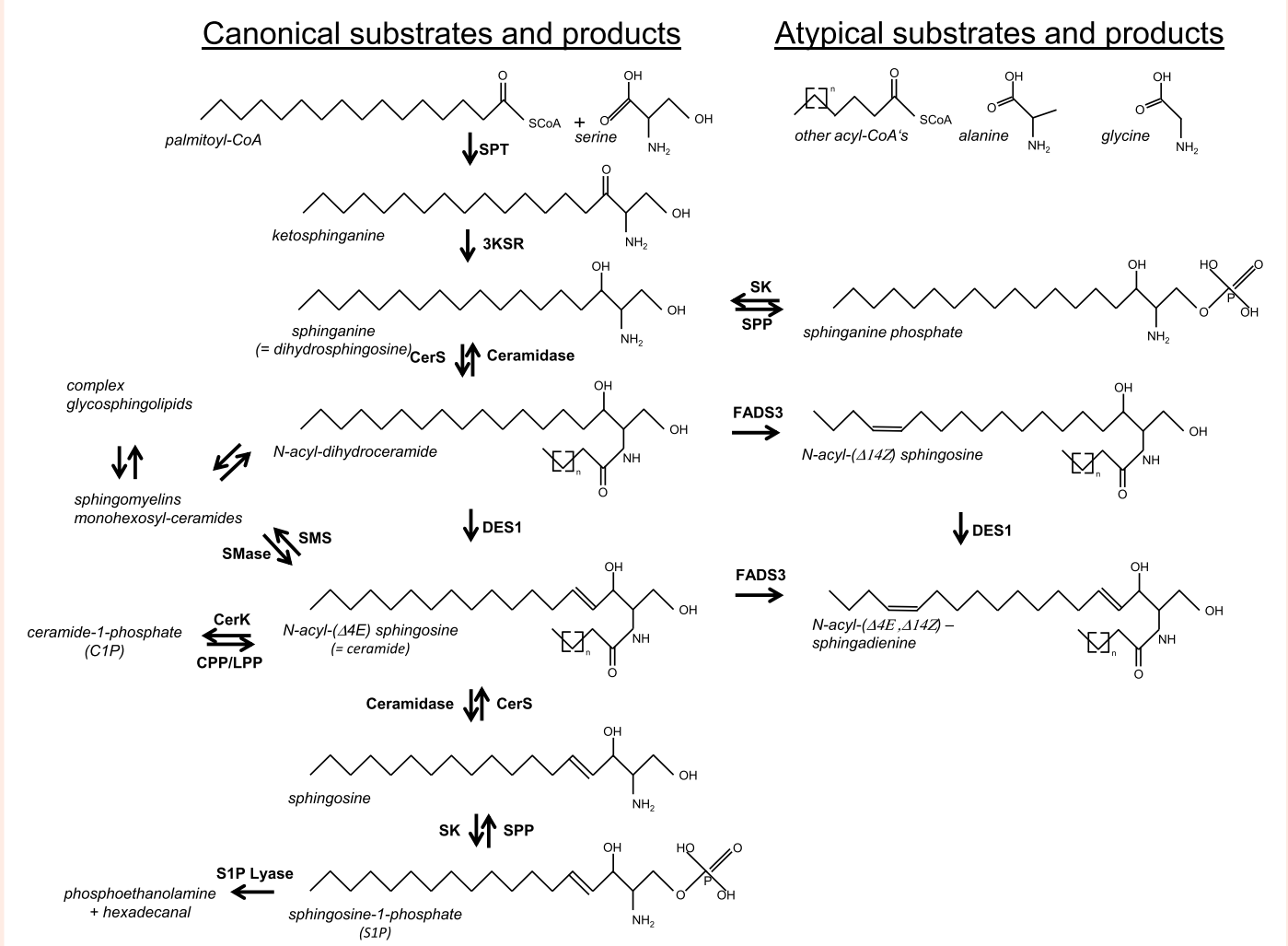


Figure 1 Pathways involved in the biosynthesis and degradation of ceramides. The left side of the figure shows the metabolism of canonical sphingoid bases. The 1st step can vary by the use of alanine or glycine instead of serine by SPT and gives rise to 1-deoxy- or 1-desoxymethylsphingoid bases, respectively, that due to the missing hydroxyl group cannot be metabolized to complex sphingolipids (sphingomyelins, glycosphingolipids) nor degraded via formation of sphingosine-1-phosphate. SPT can also use other acyl-CoAs in addition to palmitoyl-CoA, especially when SPT contains the SPTLC3 subunit instead of the SPTLC2 subunit. Ceramides and sphingomyelins containing these shorter, longer, or branched sphingoid bases undergo normal metabolism but differ by lipoprotein distribution. Finally, FADS3 introduces a double bond at the 14Z position instead of the canonical E4 position formed by DES1. Enzymes: CerK, ceramide kinase; CerS, ceramide synthase; CPP, ceramide phosphate phosphatase; DES1, dihydroceramide desaturase 1; FADS3, fatty acid desaturase 3; LPP, lipid phosphate phosphatase; SK, sphingosine kinase; Smase, spingomyelinase; SMS, sphingomyelin synthase; SPP, sphingosine phosphate phosphatase; SPT, serine palmitoyltransferase; SPTLC1, serine palmitoyltransferase long-chain base subunit 1; 3KSR, 3-ketosphinganine reductase.

contain any lipid risk factor. They also described improved granularity of SCORE, i.e. the ancestor of SCORE2 which is nowadays recommended by ESC for risk assessment, however, without quantifying the prognostic performance of either SCORE alone or the combination with the CERT2 score. The same is true for the analysis of the CERT1 score in the FINRISK population, where the authors adjusted for the Framingham Score but did not compare areas under the receiver operating characteristic curve of the clinical score and the combined clinical/CERT1 score and did not calculate net-reclassification by the CERT1 score.[44,45]

In a primary prevention cohort, ceramide ratios were significantly associated with adverse cardiovascular events independently of LDL-C and conventional CAD risk factors.[10] Of note, a 12-point risk score combining the values from Cer(16:0), Cer(18:0), Cer(24:1), and the ratios with Cer(24:0)[47] based on the ceramide score evaluated in secondary prevention[8] was shown in this community cohort of individuals with asymptomatic left ventricular hypertrophy to discriminate risk of stroke/myocardial infarction in a quartile-based approach of this score after adjustment for ASCVD, albeit at a moderate *C*-statistic of 0.67.[10]

Thus, the currently available data do not allow any conclusion about whether the CERT scores improve risk prediction in primary prevention beyond guideline-recommended risk scores. A prospective case–control analysis within the PREDIMED trial of the effect of a Mediterranean diet with olive oil or nuts vs. a control diet in individuals at high risk for CVD revealed that elevated plasma ceramide concentrations at baseline were significantly associated with incident adverse cardiovascular events during a median of 4.5 years.[48] Using a weighted sum score of concentrations of four ceramide species (Cer16:0, Cer22:0, Cer24:0, and Cer24:1), the association between baseline plasma ceramide concentrations and incident events was most pronounced in the control group but not in the two dietary intervention groups, suggesting an effect of the dietary intervention,

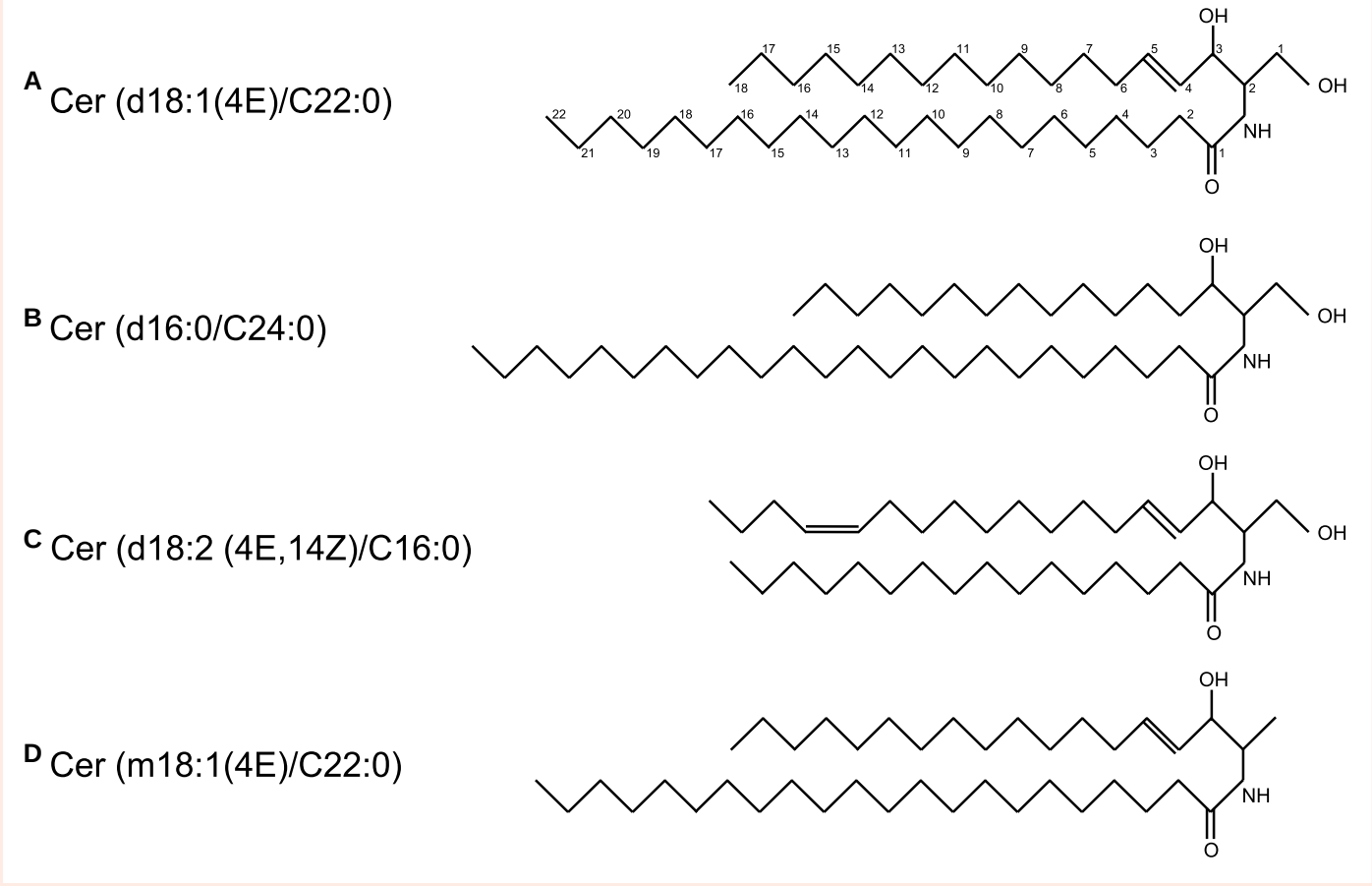


Figure 2 Examples of canonical and non-canonical ceramides. Structures and nomenclature of canonical and non-canonical ceramides differ according to the sphingoid base as well as the N-acyl chain. Canonical ceramides contain a C-18 sphingoid base with one double bond between the 4th and 5th C-atom[6,11] (*A*). Non-canonical ceramides (*B–D*) contain other sphingoid bases, for example, C16-sphingolipids (*B*), additional double bonds (*C*) or a lack thereof (*B*), or lack the hydroxyl group at the 1st C-atom necessary for canonical degradation (*D*). Variations in the length and structure of the N-acyl chain (= fatty acid) introduce additional variability.

although ceramide concentrations at short-term follow-up (1 year) were not different between the groups.[48]

Distinct age-related patterns were found for PC species 18:1;0_20:4;0, which increased in women and decreased in men with age, whereas PC species 16:0;0_22:6;0 persistently increased with age with no sex difference.[49] Plasma levels of ceramides species 42:2;2 (corresponding to Cer d18:1/24:1) were higher in middle-aged men, remaining stable with increasing age, whereas they increased with age in women, indicating a clear sex difference with ageing.[49] These data point to a potential bias of risk scores that combine ceramide and PC species which should be used only after age- and sex-specific stratification. Age and sex differences may also explain some of the discrepancies in the prognostic value for cardiovascular events of individual ceramide species (C16:0, C18:0, C24:1, C24:0), ratios of ceramides, and composite ceramide scores.[48]

The role of the sphingoid backbone heterogeneity in ASCVD has been little investigated. In a study of 349 subjects with chronic coronary syndrome and 126 fatal and nonfatal ASCVD events during a median follow-up of 7.7 years, d20:0 and d18:2 sphingolipids were associated with increased and decreased risks, respectively, after adjusting for traditional risk factors, lipid-lowering therapy, and coronary artery stenosis.[50] Similarly, the importance of the ceramide tertiary group has been little investigated with regard to its association with ASCVD. Glucosyl- and lactosylceramides are quantitatively the most prominent sphingolipids in atherosclerotic plaques of the aorta or the carotid arteries.[51] The analysis of 79 molecularly distinct sphingolipids in the plasma of 2627 Chinese Singaporeans found that higher levels of monohexosylceramides and sphingomyelins but not total ceramides were independently associated with 152 incident major cardiovascular events during more than 12 years of follow-up.[52] Cross-sectional analyses of an angiographic case–control study and the MESA cohort study revealed significant and independent associations of total sphingomyelin levels in plasma with the presence of CAD and higher coronary calcium score.[53,54] However, a longitudinal analysis of data from more than 6800 MESA study participants did not find any significant association of total sphingomyelin levels with 189 incident cardiovascular events during 5 years of follow-up.[55] In both the Strong Heart Family Study of 1930 unique American Indians and the Malmö Diet and Cancer-Cardiovascular Cohort of 3943 Caucasians, plasma levels of total sphingomyelins and ceramides were associated with total and cardiovascular mortality during mean follow-up periods of 17.8 and 23.7 years, respectively. Resolution into various sphingolipid species revealed significant associations of 11 and 8 of 15 sphingomyelins with total and cardiovascular mortality, respectively. There was a tendency for sphingomyelins formed on shorter carbon chain ceramides (e.g. SM34:0 or SM34:1) to show a stronger association than sphingomyelins with longer carbon chains (e.g. SM42:3 or SM44:2).[56] Likewise, in the Bruneck cohort study, SM34:2 was the sphingolipid showing the strongest association with 90 incident coronary events of 685 participants during a follow-up of 10 years.[45] The Cardiovascular Health Study, which followed 4612 participants for a median

Table 1 Contrasting effects of ceramides and sphingosine-1-phosphate on the function and fate of cells in the cardiovascular system

Cell type	Ceramides	Sphingosine-1-phosphate
Vasculature		
Endothelial cells	• Limit barrier function • Promote formation of reactive oxygen species • Inhibit nitric oxide production and thereby promote vasoconstriction and blood pressure increase	• Supports barrier formation by stabilizing interendothelial junctions • Promotes formation of NO and thereby endothelium-dependent vasorelaxation • Inhibits ICAM and VCAM expression and thereby leukocyte diapedesis • Inhibits transendothelial LDL transport but promotes transendothelial HDL transport • Promotes angiogenesis
Vascular smooth muscle cells	• Promote apoptosis	• Stimulates proliferation and migration
Monocytes and macrophages	• Promote foam cell formation by favouring aggregation and thereby uptake of LDL.	• Inhibits apoptosis • Inhibits foam cell formation by suppressing uptake of modified LDL through scavenger receptors and promoting cholesterol efflux
Heart		
Cardiomyocytes	• Positive inotropic effects • Promote apoptosis	• Negative inotropic effect • Suppresses apoptosis • Reduces ischaemia reperfusion injury
Cardiac fibroblasts	• Promote collagen production	• Pro- and antifibrotic effects depending on S1P receptor

Assembled from references[22–26]

of 10.2 years, C16-sphingomyelins and C16-ceramides showed equally strong and independent association with sudden cardiac death,[57] whereas the association of sphingomyelins and ceramides with longer carbon side chains were not statistically significant. In the subgroup of 597 diabetic subjects within the Strong Heart Family Study, long-chain C22-sphingomyelins were even inversely associated with the risk of CVD events.[58]

In summary, glucosyl- and lactosylceramides as well as sphingomyelins built on shorter-chain ceramides may serve as biomarkers of CVD risk. Of note, however, in the Bruneck study, triacylglycerols and cholesteryl esters with a low carbon number and double-bond content as well as phosphatidylethanolamine PE(36:5) had much stronger associations than any sphingolipid with incident CVD, such that no sphingolipid species became part of the lipidomic risk score derived from the data of the Bruneck cohort that includes TAG(54:2), CE(16:1), and PE(36:5).[45]

2.1.1.2 Heart failure

Distinct ceramide species combined in a score predicted incident heart failure in individuals at high risk for CVD (PREDIMED), which was validated in the EPIC-Potsdam cohort.[59] Increased plasma levels of ceramides were associated with clinical stages of heart failure and mortality.[60] The association of plasma ceramides and sphingomyelin (SM) species with incident heart failure was evaluated in the Cardiovascular Health Study, which demonstrated higher plasma levels of Cer-16 and SM-16 were associated with increased risk of heart failure and higher levels of Cer-22, SM-20, SM-22, and SM-24 with decreased risk of heart failure (no further information using ceramide terminology was provided by the authors).[61]

The data concerning tissue levels of ceramides in failing human hearts are inconsistent. For instance, increased ceramide levels were found in ischaemic end-stage hearts at the time of left ventricular assist device placement.[62] Conversely, metabolomic, proteomic, and transcriptomic evaluation of human hearts explanted for end-stage heart failure (due to dilated cardiomyopathy) compared with nonfailing donor hearts found reduced levels of ceramides in failing cardiac tissue that were likely attributable to low fatty-acid tissue content.[19] A recent case–control study that used proteomics, metabolomics, and lipidomics to compare myectomy samples from patients with hypertrophic cardiomyopathy and matched nonfailing donors as controls found reduced cardiac ceramide formation and lipid storage.[63] These differences are likely due to the distinct aetiologies, although differences in a sampling of tissue specimens may also impact results.

2.1.1.3 Hypertension and T2DM

In hypertensive patients at high cardiovascular risk, ceramides and a ceramide score (CERT) predicted incident adverse cardiovascular events during a mean follow-up of 2.3 years.[46] Furthermore, ceramide signatures are associated with incident T2DM in individuals at risk.[64–67] Interestingly the typical sphingosine (d18:1) and atypical sphingadienine (d18:2) backbones of sphingolipids show the opposite association with T2DM, namely positive and inverse, respectively.[68] Moreover, higher plasma levels of deoxysphingolipids formed by excessive use of alanine by SPT were found to be associated with both present and incident T2DM[69] as well as with MASLD.[70]

2.1.2 Monogenic diseases caused by variants of genes related to ceramide metabolism

The best-known disorders of sphingolipid metabolism are sphingolipidoses that result from deficiencies of enzymes that are essential for the lysosomal degradation of complex sphingolipids such as sphingomyelins (Niemann–Pick diseases A and B) or glycosphingolipids (e.g. Gaucher disease, Tay–Sachs disease, or Andersen–Fabry disease).

Anderson–Fabry disease is of special relevance in cardiovascular medicine as it leads to the accumulation of globotriaosylceramide [Gb_3] and related glycosphingolipids in the heart and thereby to left ventricular hypertrophy (LVH).[71] Anderson–Fabry disease is a rare monogenic X-chromosome-linked lysosomal storage disease with a high penetrance in males caused by variants of the *GLA* gene causing deficient or absent activity of α-galactosidase A.[72] This causes ubiquitous accumulation of glycosphingolipids, mainly Gb_3, also in the heart and this accumulation of glucosylceramides underlies the cardiomyopathy of patients with Anderson–Fabry disease. A recent report from a large Danish Fabry cohort including a cascade screening procedure conducted in family members revealed 115 patients in whom 24 different pathogenic α-galactosidase A *GLA* gene variants were identified. In leukocytes from affected males, enzyme activity of α-galactosidase A was very low to undetectable, plasma concentrations of Gb_3 were higher than the upper limit of the reference range.[73]

Table 2 Emerging patterns of ceramide associations with disease

Entity	Ceramide species	Positive association	Study cohort
ASCVD	Cer(d18:1/16:0)	Adverse CV events	ATHERO-REMO[33]
ASCVD	Cer(d18:1/16:0)/Cer(d18:1/24:0) ratio	CV death	Corogene, SPUM-ACS, and BECAC[8]
ASCVD	Cer(d18:1/24:1)/Cer(d18:1/24:0), Cer(d18:1/16:0)/PC(16:0/22:5),	CV death	WECAC, LIPID, KAROLA[9]
	Cer(d18:1/18:0)/PC(14:0/22:6), (PC 16:0/16:0) ratios	CV death	SOLID-TIMI 52[41]
		Adverse clinical events	Feldkirch PAD Cohort[42]
ASCVD	Cer(d18:1/16:0)/PC(16:0/22:5) ratio	Adverse clinical events	Athero-Express biobank[43]

ASCVD, atherosclerotic vascular disease; Cer, ceramides; CV, cardiovascular; dhCer, dihydro-ceramides; PC, phosphatidylcholines.

Variants of genes encoding enzymes in the biosynthesis or degradation of ceramides can also cause monogenic diseases, most of which affect the nervous system.[74] Variants in the SPTLC1 and SPTLC2 subunits of SPT are of special interest in the context of cardiometabolic diseases, as the resulting hereditary sensory neuropathy type 1 (HSAN1) clinically resembles diabetic neuropathy. Interestingly, the pathogenesis of HSAN1 is caused by the enhanced formation of deoxysphingolipids, which also occurs in diabetes.[74]

2.1.3 Mendelian randomization to evaluate the causal role of ceramides in cardiometabolic diseases

2.1.3.1 ASCVD

In a pilot genome-wide association study (GWAS) of plasma levels of major sphingolipid species comprising sphingomyelins and ceramides in five large European cohorts, it was demonstrated that diversity in circulating sphingolipids is due to genetic variation.[75] Three of the identified genetic variants were associated with myocardial infarction in three German case–control studies: serine palmitoyltransferase long-chain base subunit 3 (*SPTLC3*); Class IV ATPase, type 10D (*ATP10D*), and fatty-acid desaturase 3 (*FADS3*). A recent GWAS study by McGurk *et al.*[76] identified gene variants that were significantly associated with plasma ceramides in a cohort of 999 members of 196 British Caucasian families. A gene variant in the *SPTLC3* gene locus was identified by these authors,[76] supporting the previously described GWAS association with plasma ceramides.[75,77] They further identified genetic associations of plasma ceramide levels with variants of *CD83*,[76] a member of the immunoglobulin superfamily of membrane receptors expressed by immune cells, in *SGPP1* encoding S1P phosphohydrolase 1, which was already previously identified,[75] and *DEGS1* encoding dihydroceramide (dhCer) desaturase 1 (DES1). A rare damaging variant of *DEGS1* impacts plasma ceramide levels.[78] Clinically, DEGS1 deficiency causes severe neurological impairment.[79] However, using two-sample Mendelian randomization in the CARDIoGRAMplusC4D and DIAGRAM cohorts, no evidence for a causal association with CVD or diabetes mellitus of the four ceramide-related gene variants identified (*SPTLC3*, *CD83*, *SGPP1*, and *DEGS1*) was found.[76] Major limiting factors of this analysis are the small size of the cohort and the absence of clinical information regarding lipid-lowering medication or hyperlipidaemia diagnoses. Sex-stratified genome-wide association analyses in the Finnish GeneRISK cohort, replicated in the EUFAM and FINRISK cohorts with available lipidomics and genetic data,[80] found no relevant contribution of genetic factors to sex differences and no impact of menopause on ceramide levels.[49] It is important to note that SPTLC3 serves as an alternative subunit of SPT, which, unlike SPTLC1 and SPTLC2, is not ubiquitously expressed. The expression of SPTLC3 instead of the more abundant SPTLC2 leads to the preferred use of longer or shorter and even branched acyl-CoA species instead of the canonical palmitoyl-CoA and thereby to the formation of shorter or longer and even branched sphingoid bases.[29,81] Fatty-acid desaturase 3 (FADS3) introduces a double bond at the atypical 14Z position instead of the canonical E4 position formed by DEGS1[82] (*Figure 1*). Hence, it may be that the structural heterogeneity of the sphingoid base rather than that of the N-acyl chain defining the ceramide species is important for the disease association of these genes.

2.1.3.2 ASCVD and T2DM

A recent GWAS[20] evaluated the effects of specific ceramides (Cer) and dhCer on the risk of developing T2DM and CVD in two case–control samples (T2DM and CVD) nested within the prospective EPIC-Potsdam cohort of healthy individuals. After multiple adjustments, the ceramide species Cer18:0, Cer22:0, dhCer20:0, and dhCer22:2 were significantly associated with higher T2DM risk, and Cer20:0 and dhCer26:1 with lower T2DM risk; Cer16:0 and dhCer22:2 were positively associated with CVD. Using a univariable, two-sample Mendelian randomization approach, the SNP-phenotype associations (SNP rs680379 in the *SPTLC3* gene locus—Cer 22:0 plasma concentration)[20] were compared with published GWAS on Cer 22:0 (EUROSPAN[75,77] and FHSOC[83]) and SNP-T2DM associations (SNP rs680379—T2DM) were drawn from DIAGRAM (T2DM).[84] This analysis demonstrated a higher T2DM risk in individuals with higher genetically predicted Cer 22:0 plasma levels, suggesting a causal role of the SNP rs680379 in the *SPTLC3* gene locus.[20]

A limitation of this study[20] was the lack of external GWAS data to validate the association of relevant SNPs with ceramides in independent cohorts. Another limitation was the lack of information on associations with sphingoid bases of atypical length that are produced by SPT containing the SPTLC3 instead of the usual SPTLC2 subunit, which may confound the abundance of Cer22:0.[85] *Table 2* summarizes studies with a common denominator of ceramide signatures in ASCVD associated with adverse clinical events.

2.1.3.3 Interventional studies

Currently, there is no drug available that selectively targets ceramides to be used in assessing the potential causal role of ceramides in ASCVD and heart failure. However, treatment with recombinant α-galactosidase A, a drug approved for use in patients with Anderson–Fabry disease, reduces microvascular endothelial deposits of Gb_3 in the heart.[86] Early initiation of enzyme replacement therapy with recombinant α-galactosidase A was shown to reduce left ventricular mass, improving myocardial function, and increasing exercise capacity.[87] In Anderson–Fabry disease, novel cardiac imaging surrogates may enable earlier initiation of therapy and improve therapeutic monitoring. Thus, non-contrast T1 mapping by cardiovascular magnetic resonance is an early imaging marker of cardiac manifestation. This enables discrimination of Anderson–Fabry disease in patients with LVH from other causes. T1 parameters were abnormal in 40% of subjects who did not have LVH,[88] with a significant negative linear relationship between lipid content and non-contrast T1 values.[89] Detection of early disease stages before the development of LVH was demonstrated upon adding global longitudinal strain analysis to T1 mapping, with characteristic imaging signs of cardiac mechanical dysfunction[90] and signs of microvascular dysfunction and altered atrial depolarization and ventricular repolarization intervals on electrocardiogram.[91]

Contemporary lipid-lowering therapy such as simvastatin[37] and rosuvastatin,[92] as well as proprotein convertase subtilisin/kexin type 9 (PCSK 9) inhibitor therapy,[93] were found to significantly reduce circulating ceramide levels. However, the effect size and the clinical relevance of this reduction remain unclear. It may simply be that ceramide levels are passively reduced

through enhanced catabolism of their carriers, i.e. LDL particles, through the upregulated LDL-receptor pathway in the liver. However, dietary interventions as well as PCSK9 inhibitors were also shown to modulate the self-aggregation and proteoglycan binding of LDL by changing the sphingomyelin and ceramide content of LDL.[31]

2.2 Experimental validation of the causal contribution of ceramides to disease and its potential therapeutic value

2.2.1 ASCVD

A recent lipidomic analysis found significant changes in the myocardial sphingolipid profile in the reparative phase after myocardial infarct injury; in particular, ceramide-1-phosphates were upregulated. Thus, cardiac-specific targeting of ceramide kinase has been reported to have therapeutic potential in ischaemic heart diseases.[94] Ceramides are critically involved in the pathogenesis of endothelial dysfunction, which precedes hypertension and atherosclerosis.[6] Cerebral microvascular dysfunction is a common feature after stroke. Transcriptomic analysis revealed shared alterations with increased expression of ceramide and S1P receptor signalling pathways when comparing murine stroke cerebral microvessels and the microvasculature in human stroke.[95] Furthermore, higher ceramide levels were found in microvessel samples from mice with stroke compared with sham controls.[95] Genetic ablation of NOGO-B (neurite outgrowth inhibitor) in a hyperlipidaemic atherosclerosis-prone knock-out model increased S1P levels while decreasing ceramide levels and therefore attenuated atherogenesis and atherosclerosis progression,[96] identifying NOGO-B as a novel target. Inhibition of SPT pharmacologically with myriocin[62,97–101] or by genetic ablation (SPTLC1)[62,102] inhibits the development of atherosclerosis, vascular dysfunction with hypertension and heart failure with reduced ejection fraction. However, gut toxicity associated with this drug impedes its use in the clinic, and genetic ablation of SPTLC2 produces lethal gut structural alterations.[103,104] Degradation of sphingomyelin by secretory sphingomyelinase generates ceramides and promotes aggregation and, consequently, subendothelial retention.[105,106] In a randomized clinical trial, the sphingomyelin content of LDL and LDL aggregation were increased and decreased by overfeeding of volunteers with saturated and unsaturated fat, respectively.[107] In an atherosclerosis-prone hyperlipidaemic mouse model, the knock-out of SM synthase (SMS) 2 decreased plasma levels of sphingomyelin and aortic tissue levels of ceramides as well as atherosclerosis.[108] Interestingly, the ablation of the SMS isoform 1 (SMS1) also reduced atherosclerotic lesions on a hyperlipidaemic background.[109] Thus, inhibition of sphingomyelin synthesis appears to be another interesting target for ASCVD prevention or treatment. However, also, the inhibition of the degradation of sphingomyelin by neutral sphingomyelinase 2 (nSMase2) or the genetic deficiency of this enzyme markedly decreases the development of atherosclerotic lesions in *Apoe*$^{-/-}$ mice by reducing inflammatory responses, although this occurs without causing any changes in plasma levels of lipids including ceramides and sphingomyelins.[17] Several animal studies showed that genetic or pharmacological interference with S1P generation and degradation by sphingosine kinase and S1P lyase, respectively, or the S1P-binding protein apoM or S1P receptors affects the development of atherosclerosis. Most but not all studies support the anti-atherogenic role of S1P.[110] Notably, chronically high S1P concentrations achieved through pharmacological inhibition of its degradation has been shown to result in hypercholesterolaemia and accelerated atherosclerosis in mice.[111] Thus, specific S1P receptors, notably S1P1, or apoM rather than total S1P abundance may be therapeutic targets for ASCVD.

2.2.2 Heart failure

Recent data demonstrate a specific role for SPTLC3 in ischaemic cardiomyopathy: cardiomyocyte-specific depletion of SPTLC3 in mice attenuates oxidative stress, fibrosis, and hypertrophy under conditions of chronic ischaemia.[112] Furthermore, gain-of-function alteration of acid ceramidase via mRNA transfer mediates post-infarct cardioprotection and constitutes a promising novel therapeutic target.[113]

In obese patients with heart failure with preserved ejection fraction, bariatric surgery resulted in lower circulating ceramide levels, but this was not associated with echocardiographic parameters of functional improvement.[114] However, in heart failure with reduced ejection fraction, ceramides were found to play an important role. Increased ceramide levels were observed on endomyocardial biopsies from patients with failing hearts (heart failure with reduced ejection fraction), and ceramide levels were reduced upon left ventricular unloading using left ventricular assist device therapy.[62]

2.2.3 Valvular heart disease

Emerging evidence is consistent with the potential role of ceramides in valvular heart disease. In a mouse model of acid ceramidase deficiency mimicking the enzyme defect of Farber disease, a recent study reported structural and functional alterations suggestive of mitral valve dysfunction and a phenotype of heart failure with preserved ejection fraction. Compared with wildtype littermates these mutant mice had a profoundly increased ceramide content with a predominance of the C16:0 and C24:1, dhCer, monohexosylceramide, and dihexosylceramide species.[115] In a small observational study, myocardial ceramide content in the left ventricle was found to be higher in aortic stenosis patients with higher gradients referred for surgical aortic valve replacement.[116] Furthermore, CD172+ extracellular vesicles released upon hypoxia from cardiomyocytes from patients with aortic stenosis contain large amounts of ceramides that exert a positive inotropic effect on normoxic myocardium in culture. Of note, high circulating numbers of extracellular vesicles were found to be predictive of a good outcome after transcatheter aortic valve replacement.[117]

2.2.4 Atrial fibrillation

Experimental evidence using whole-cell patch-clamping and fluorescence imaging in rabbit pulmonary veins supports a role for ceramide-induced effects on heart rate and modulation of Ca^{2+} and Na^{+} ion currents and intracellular reactive oxygen species, suggesting a mechanism involving ceramides in supraventricular arrhythmias.[118] Conversely, lipidomic analysis of atrial myocardium in a subset of patients from a Swedish registry study who were admitted to a coronary care unit and subsequently referred for cardiac surgery showed a reduced content of triglycerides in patients with atrial fibrillation compared with those having sinus rhythm, whereas no difference was found with respect to the content of ceramides, PC, lysophosphatidylcholine, PE, sphingomyelins, free cholesterol, cholesterol esters, or diacylglycerols.[119] A prospective longitudinal study found that ceramides and sphingomyelins with palmitic acid were associated with an increased risk for incident atrial fibrillation, whereas ceramides and sphingomyelins with very long-chain saturated fatty acids were associated with reduced risk.[120] Altogether, a clear pattern of the role of ceramides in atrial fibrillation cannot be discerned at this stage.

2.2.5 T2DM

Inhibition of ceramide synthesis markedly improves glucose tolerance and prevents the onset of diabetes in obese rodents.[121] Universal genetic ablation of DES1 (coded by DEGS1) (*Figure 1*) or tissue-specific deletion in the liver and/or adipose tissue resolved hepatic steatosis and insulin resistance in mice.[122] The synthetic retinoid fenretinide (4HPR), recently identified to inhibit CerK,[123] blocks ceramide production partly by directly inhibiting DES1, which improves insulin sensitivity.[124] Replenishment of sirtuin 1 (SIRT1) was recently reported as a novel approach to counteract beta-cell dysfunction via inhibition of *de novo* ceramide synthesis; the mechanism involves inhibition of SPTLC1/2 upon SIRT1 binding and deacetylating TLR-4 in T2DM.[125] In this regard, it is noteworthy that deoxysphingolipids formed by the use of alanine instead of serine by SPT cause beta-cell apoptosis and inhibit insulin secretion.[126] *In vivo* glucose tolerance of mice and rats is disturbed by providing alanine in excess relative to serine.[127] In addition, genetic mouse models lacking S1P receptors or the S1P-bearing chaperone apoM are glucose intolerant due to impaired beta-cell function or reduced insulin sensitivity.[128]

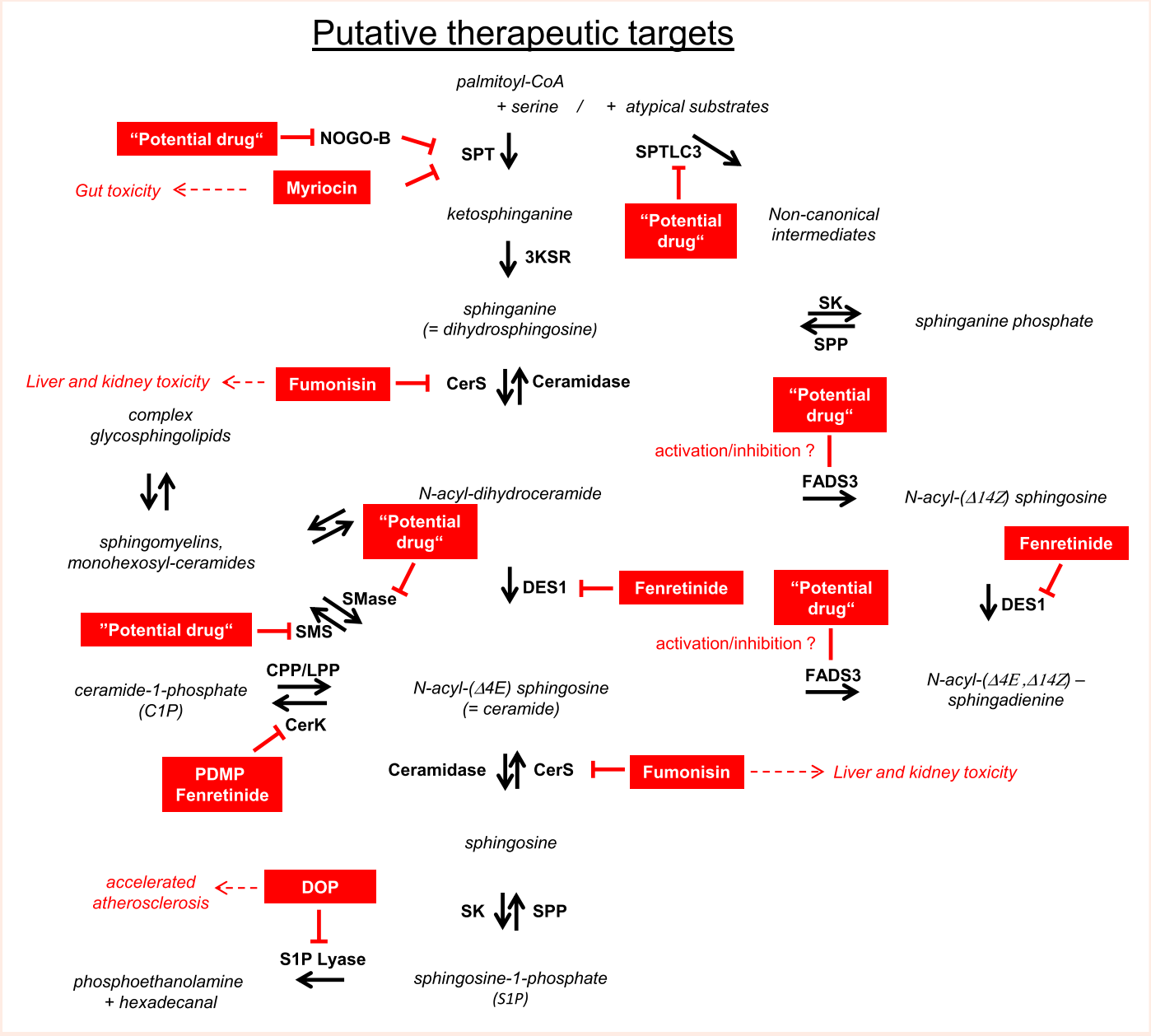


Figure 3 Improving risk stratification and exploiting putative therapeutic targets. Abbreviations for drugs, targets, and diseases: DOP, 4-deoxypyridoxine; Fenretinide (also known as 4HPR), 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; HSAN1, hereditary sensory neuropathy type 1; NOGO-B represents reticulon 4B, a growth inhibitor ('no-go').

3. Gaps in knowledge and unmet medical needs

Recent findings suggest that ceramide signatures have the potential to improve residual cardiovascular risk prediction, at least in patients with manifest CVD where the conventional lipid risk factors including LDL-C are not predictive biomarkers. In asymptomatic individuals (primary prevention), the prognostic relevance of ceramides beyond conventional risk factors and guideline-recommended clinical risk prediction tools has not been sufficiently investigated. The few reported studies did not fully adjust their statistical analyses for potentially confounding risk factors, notably the conventional lipid profile, and did not show whether ceramide scores improve state-of-the-art risk prediction by guideline-recommended algorithms such as SCORE2 or the Pooled Cohort Equation. Moreover, the interaction with triglyceride-rich lipoproteins and lipoprotein(a) should be investigated.

Therefore, and because specific treatment options are missing, we recommend against the use of ceramide scores in asymptomatic subjects. First, current ceramide tests have not been approved by the US Food and Drug Administration.[129] Second, current guidelines do not recommend assessing ceramides or ceramides scores, and neither do they recommend any risk-substratified LDL-C targets.[130] A level of 1.4 mmol/L is appropriate for all but those with progressing atherosclerosis despite having reached this target, so that 1.0 mmol/L is recommended. Hence, in the absence of therapeutic consequences, there is little argument to use ceramide scores for substratification of risk in secondary prevention. Currently, one indication may be the diagnostics of patients who have ASCVD in the absence of standard modifiable cardiovascular risk factors.[3]

Only a small set of ceramides has thoroughly been investigated thus far, and little is known beyond ceramide–PC combinations (ratios, scores) beyond the CERT1/2 scores. Notably, the much larger diversity of sphingolipids beyond the N-linked carbon, which is multiplied by the structural heterogeneity of the sphingoid base and the tertiary group, has not been sufficiently taken into consideration. In fact, in a case–control study of 462 individuals with familial CAD and 212 population-based controls, the combination of sphingolipidomics with machine learning led to the development of several scores that combine minor sphingolipid species [in particular dihydro-cer(d18:0/16:0), dihydro-cer(d18:0/18:1), dihydro-SM(d18:0/24:1), dihydro-SM(d18:0/22:0), SM(d18:1/18:0), cer(d18:1/18:0), and cer(d18:1/24:0)] and outperform the CERT1 score.[131] However, none of these scores was validated externally, especially not in any longitudinal study. The large prognostic potential of sphingoid base heterogeneity is indicated by the genetic associations of STPLC3, DEGS3, and FADS3 with ASCVD. Genetic variants of SPTLC3 and FADS3 are associated with CVD and diabetes, but these associations are not mediated by ceramides as defined by their N-fatty-acid composition and hence the CERT scores. Because SPTLC3 and FADS3 contribute to the formation of atypical sphingolipids differing by the structure of the O-linked fatty acid, i.e. the sphingoid backbone, we hypothesize that this heterogeneity rather than the heterogeneity of the N-fatty acid composition explains the association of SPTLC3 and FADS3 variants with disease. This hypothesis, however, needs to be tested by comprehensive analysis of sphingolipid heterogeneity in prospective cohort studies.

It will also be important to investigate whether the distribution among lipoproteins has any impact on the association of ceramides with disease, similar to LDL-C and non-HDL-C vs. HDL-C. In fact, in case–control studies on the lipidomics of HDL and non-HDL particles, lower sphingomyelin content in HDLs was associated with a higher risk of coronary heart disease.[132] In addition, the introduction of ceramides into the clinical routine requires international standardization of their measurement. An interlaboratory comparison of ceramide measurements by 31 laboratories in 19 countries found interlaboratory coefficients of variation of up to 14%. Biases between laboratories were reduced by the use of authentic, labelled standards and common standard reference material for calibration.[133]

Novel strategies of targeted pharmacotherapy are needed to address the risk mediated by ceramides. Targeting key enzymes in ceramide synthesis and metabolism with drugs offers a promising novel therapeutic approach. Among the major candidates are the six different isoforms of ceramide synthase (CERS1-6) with different tissue distribution and fatty acyl-CoA selectivity[6] and other enzymes such as SMS2 and nSMase2 whose inhibition decreased atherosclerosis in mouse models.[17,109] The genes and gene products that show association with disease, such as SPTLC3 and FADS3, may be interesting therapeutic targets for drug development (*Figure 3*).

Due to the ubiquitous expression of ceramides, a focus on tissue (heart)-specific therapies is of paramount importance for translation to the clinic. RNA-based therapies using specific vehicles for delivery to endothelial cells and/or cardiomyocytes hold great potential. Adeno-associated virus serotype 9-based strategies may also be considered, although they have the pitfall of immune reactions against the virus particle. Lipid nanoparticles targeted by integration of antibodies/lipids carrying specifically modified mRNA translation systems[134] constitute another approach.

4. Conclusions

Sphingolipids including ceramides have gained great interest both as biomarkers for cardiovascular risk assessment and therapeutic targets for the treatment or prevention of cardiometabolic diseases. Although already offered as a diagnostic service by some clinical laboratories, the clinical application of ceramide-based scores appears to be premature since there is limited evidence of clinical utility either in improving risk prediction beyond current guideline-recommended risk assessment tools or in influencing treatment decisions. Rather, the promising data from cohort studies, genetics, and experiments *in vitro* and *in vivo* call for more intensive translational research to exploit sphingolipids including ceramides for the diagnostics and therapy of cardiometabolic diseases.

Authors' contributions

R.K., A.L., A.v.E., and H.D. contributed to the conception of the work. R.K., B.L., W.M., and A.v.E. contributed to the interpretation of data for the work. R.K., A.L., and A.v.E. drafted the manuscript. All authors critically revised the manuscript and gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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Data availability

This review is based on data provided in the pertinent references.

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