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Sphingolipidomics analysis of large clinical cohorts. Part 1: Technical notes and practical considerations

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ABSTRACT

Lipids comprise an exceptionally diverse class of bioactive macromolecules. While quantitatively abundant lipid species serve fundamental roles in cell structure and energy metabolism, thousands of structurally-distinct, quantitatively minor species may serve as important regulators of cellular processes. Historically, a complete understanding of the biological roles of these lipids has been limited by a lack of sensitive, discriminating analytical techniques. The class of sphingolipids alone, for example, is known to consist of over 600 different confirmed species, but is likely to include tens of thousands of metabolites with potential biological significance. Advances in mass spectrometry (MS) have improved the throughput and discrimination of lipid analysis, allowing for the determination of detailed lipid profiles in large cohorts of clinical samples. Databases emerging from these studies will provide a rich resource for the identification of novel biomarkers and for the discovery of potential drug targets, analogous to that of existing genomics databases. In this review, we will provide an overview of the field of sphingolipidomics, and will discuss some of the challenges and considerations facing the generation of robust lipidomics databases.

1. MS technology in lipidomics

Mass spectrometry (MS), a technique that characterizes ionized molecules based on their mass-to-charge ratios, has been widely used for the identification and quantitative analysis of specific lipid species for the past three decades. Prior to this advancement, lipid measurements relied on comparatively crude chromatography techniques that allowed only for the characterization of lipid classes rather than for the precise quantification of unique molecular species. As a result, MS has made it possible to determine detailed lipid profiles, or "lipidomes." These lipidomes are becoming increasingly precise as novel MS techniques continue to improve in both sensitivity and discriminatory capability. Continued improvements in this technology are driven by the profound complexity of the composition of the lipidome. To date, more than 30,000 distinct molecular species of lipids have been identified, which represents a fraction of the theoretical variants. Despite technical advancements, the complement of specific lipids that, due to practical limitations, can be accurately quantified in high-throughput format ranges in the hundreds. Mirroring advancements in genomics analyses, MS techniques will continue to improve, soon reaching the ability to provide near-complete lipidomes. While other review articles in this issue and elsewhere describe the state of the art of MS in detail [1–3], this review addresses practical aspects of MS analysis of sphingolipids, i.e. the "sphingolipidome." (see Table 1)

2. Sphingolipid structural diversity

Sphingolipids represents one of the eight major lipid classes, whose diverse members all share a common structure termed the sphingoid backbone – a long carbon chain with a nitrogenous head group (Fig. 1). The most common sphingoid base is sphingosine, canonically defined as an 18-carbon chain dihydroxylated at positions 1 and 3 and with a double bond at position 4 [4]. The

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Table 1
Sphingolipid classes and species.

Sphingolipid Classes	LIPID MAPS Classification	Number of Entries[a]	Number of Species in Plasma[b]	Examples of Species
Sphingoid bases and derivatives	SP01	88	6	Sphingosine (d18:1) Sphinganine (d18:0) S1P (d18:1)
Ceramides	SP02	185	41	Cer (d18:1/24:0) C1Ps
Phosphosphingolipids	SP03	328	101	SM (d18:1/24:1)
Glycosphingolipids	SP05, 06, 07, 08	3734	56	GlcCer (d18:1/22:0) Gangliosides
Others	SP04, 09, 00	22	–	
Total	-	**4357**	**204**	

Abbreviations: Cer, ceramide; SM, sphingomyelin; GlcCer, glucosylceramide.
[a] Number of entries refer to those on the LIPID MAPS Structural Database (LMSD), and includes both observed and computationally generated structures [5,7].
[b] Number of species refer to those identified by Quehenberger and others in human plasma standard reference material [12]. Numbers do not include all detectable sphingolipids (e.g. complex ceramides) due to technical limitations on quantification.

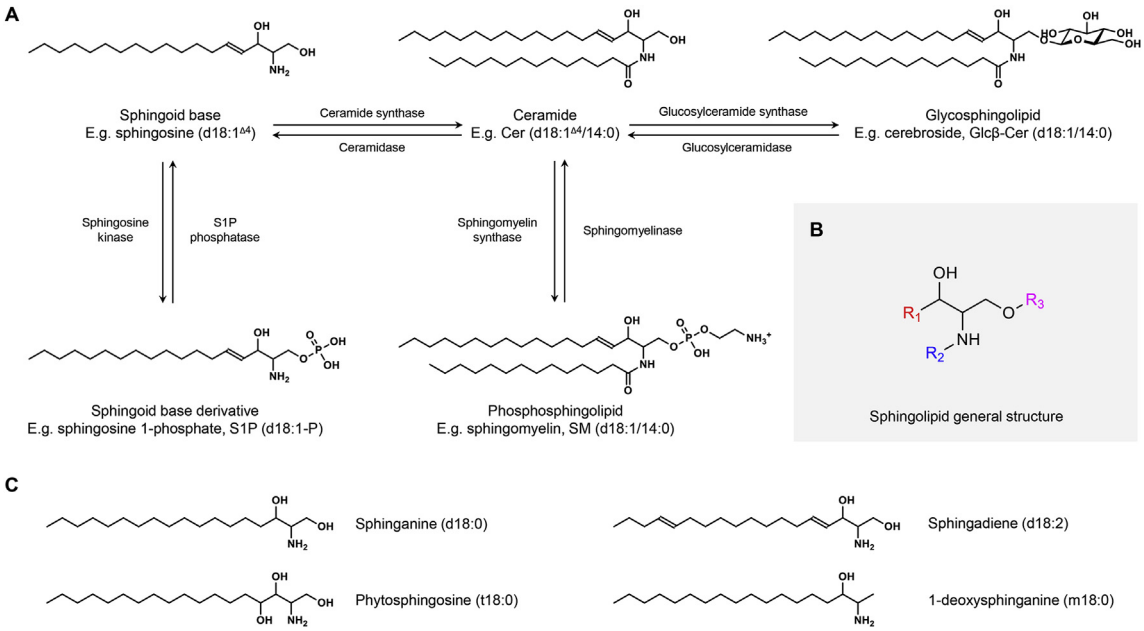

Fig. 1. An overview of sphingolipid structural diversity. (A) Major sphingolipid classes are metabolically linked by enzyme-mediated pathways. (B) Sphingolipids share a sphingoid base structure where R_1 can be alkyl chains of differing length, structure and saturation, R_2 either H or acyl chains, and R_3 either H, phospho- or glyco-groups. (C) Structures of alternative and more uncommon sphingoid bases. Abbreviations: Cer, ceramide; SM, sphingomyelin; Glc, glucose.

structural diversity of sphingolipids can originate from variations of a sphingoid base at three distinct parts: in the parent alkyl chain, at the N-linked group, and at the head group (Fig. 1B). Consequently, sphingolipid species can be organized into the following major subclasses according to their modifications: (i) sphingoid bases and simple derivatives, (ii) ceramides (N-acylated sphingoid bases), (iii) phosphosphingolipids (ceramides with phosphodiester-linked head groups, including sphingomyelins), (iv) glycosphingolipids (also complex ceramides, containing glycosidic-linked head groups) and (v) other groups (e.g. phosphonosphingolipids) [5–7]. A comprehensive classification is detailed by the LIPID MAPS Consortium, which to date includes 1181 curated species [6]. Members of these various categories are also metabolically linked in enzyme-mediated pathways (Fig. 1A) [8].

While sphingosine accounts for the sphingoid backbones of the majority of the sphingolipids, there is great variation in sphingoid bases in terms of chain length, saturation, branching, and hydroxyl groups. To capture these differences, a standardized shorthand has since been developed in sphingolipidomics – "m", "d" or "t" denote one, two or three hydroxyl groups respectively, followed by the number of carbons, the number of double bonds and optionally their positions in superscript [9]. The prototypical sphingosine for instance is d18:$1^{\Delta 4}$. Other sphingoid bases underlying sphingolipid diversity include sphinganines (also called dihydrosphingosines, which are fully saturated as opposed to sphingosine, e.g. d18:0), sphingadienes (which have two double bonds, e.g. d18:$2^{\Delta 4,14}$), phytosphingosines (additionally hydroxylated at position 4, e.g. t18:0) and deoxysphingosines (no hydroxyl group at position 1, e.g. m18:0) (Fig. 1C) [5,10,11]. Furthermore, variable chain length in the sphingoid bases also have been observed, notably 16-carbon species in plasma and heart tissue, a wide range of 16–26 carbon members in skin, and more rarely odd-numbered variants including 17 and 19 carbons [12,13]. An important bioactive group of simple sphingoid base derivatives are the sphingosine 1-phosphates (S1P), which have been identified as ligands for a family of G protein-coupled receptors (GPCRs) named S1P receptors 1 through 5 ($S1P_1$-$S1P_5$) [14,15].

In ceramides and its higher-order derivatives, the complexity is further multiplied by the more than 500 possible N-acyl-, phospho- and glyco-headgroups, resulting in hypothetically tens of

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