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Aberrations in circulating ceramide levels are associated with poor clinical outcomes across localised and metastatic prostate cancer

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Abstract

Background—Dysregulated lipid metabolism is associated with more aggressive pathology and poorer prognosis in prostate cancer (PC). The primary aim of the study is to assess the relationship between the plasma lipidome and clinical outcomes in localised and metastatic PC. The secondary aim is to validate a prognostic circulating 3-lipid signature specific to metastatic castration-resistant PC (mCRPC).

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Patients and methods—Comprehensive lipidomic analysis was performed on pre-treatment plasma samples from men with localised PC (*N*=389), metastatic hormone-sensitive PC (mHSPC) (*N*=44), or mCRPC (validation cohort, *N*=137). Clinical outcomes from our previously published mCRPC cohort (*N*=159) that was used to derive the prognostic circulating 3-lipid signature, were updated. Associations between circulating lipids and clinical outcomes were examined by Cox regression and latent class analysis.

Results—Circulating lipid profiles featuring elevated levels of ceramide species were associated with metastatic relapse in localised PC (HR 5.80, 95% CI 3.04–11.1, $P=1\times10^{-6}$), earlier testosterone suppression failure in mHSPC (HR 3.70, 95% CI 1.37–10.0, $P=0.01$), and shorter overall survival in mCRPC (HR 2.54, 95% CI 1.73–3.72, $P=1\times10^{-6}$). The prognostic significance of circulating lipid profiles in localised PC was independent of standard clinicopathological and metabolic factors ($P<0.0002$). The 3-lipid signature was verified in the mCRPC validation cohort (HR 2.39, 95%CI 1.63–3.51, $P=1\times10^{-5}$).

Conclusions—Elevated circulating ceramide species are associated with poorer clinical outcomes across the natural history of PC. These clinically actionable lipid profiles could be therapeutically targeted in prospective clinical trials to potentially improve PC outcomes.

INTRODUCTION

Lethal prostate cancer (PC) remains a global challenge with 359,000 associated deaths (2018)[1]. Therapies such as novel anti-androgens, taxanes, PARP inhibitors and targeted radioisotopes have significantly increased survival in metastatic disease[2]. However, long term control and cure of lethal PC will require therapeutic approaches that address multiple hallmarks of cancer such as the neoplastic epithelium, the tumor microenvironment and systemic metabolic factors including lipid metabolism, all of which contribute to cancer progression and treatment resistance[3].

Epidemiological and molecular studies strongly indicate that perturbations in lipid metabolism contribute to the development of aggressive PC. For example, obesity is associated with higher rates of relapse after local therapy and PC-specific mortality[4]. Enhanced *de novo* lipogenesis in PC is well-documented and has underpinned recent efforts to target lipogenesis clinically[5,6]. Elevated circulating triglycerides are associated with increased risk of recurrence after radical prostatectomy, but elevated circulating cholesterol was only associated with recurrence in men with dyslipidemia[7] despite the association of statin usage and improved PC outcomes[8–10], suggesting that the beneficial effects of statin in PC is not due to its cholesterol-lowering effects. The association of other circulating lipids with PC outcomes is under-studied, despite the presence of over 500 unique lipid species in blood[11] and the emerging role of other lipids in cancer pathogenesis.

Recently, our group undertook comprehensive plasma lipid profiling in men with metastatic castration-resistant prostate cancer (mCRPC), demonstrating that higher levels of sphingolipids such as ceramide and sphingomyelin species were associated with shorter overall survival (OS)[12]. However, the study raised further questions such as whether the prognostic lipidomic profile is only present in the mCRPC stage of PC. Ceramides are well-known for their inflammatory and metabolic dysfunction in diabetes

and cardiovascular disease[13], where elevated plasma ceramides is associated with increased risk of cardiovascular death independent of other commonly used lipid markers[14] . There is now evidence suggesting that exogenous sphingolipids can alter PC cell metabolism and promote PC growth[15].

We hypothesise that circulating lipid profiles that include ceramides are associated with poor prognosis and therapeutic resistance, and these profiles are metabolically actionable through drug and lifestyle interventions. The main aim of this study is to comprehensively profile the circulating lipidome across the natural history of PC spanning localised PC, metastatic hormone-sensitive PC (mHSPC) and mCRPC. The secondary aim is to validate our previously published prognostic mCRPC lipid signature[12] in an independent mCRPC cohort.

PATIENTS AND METHODS

Study population

Plasma samples of localised PC were collected from fasted patients prior to radical prostatectomy at the Royal Adelaide Hospital (Adelaide, South Australia) from 2005 to 2016, by the Australian Prostate Cancer BioResource. Plasma samples from mHSPC patients (prior to testosterone suppression) and mCRPC patients (prior to first line chemotherapy) were sourced from an advanced PC biomarker registry at the Mayo Clinic (Rochester, MN, United States) from 2009 to 2014[16]. This mCRPC cohort is referred to as the ‘mCRPC validation cohort’. Plasma collection methods are described in Supplementary S1.1.

Clinical outcomes of the Phase 1 and 2 cohorts of mCRPC patients from Australian hospitals in our previous lipidomic profiling study[12] were updated after longer follow-up, and combined for increased statistical power in the analysis of progression-free-survival. This combined cohort is referred to as the ‘mCRPC discovery cohort’.

All participants provided written informed consent for blood collection and research (Royal Adelaide Hospital Human Ethics Committee 041011f; Mayo Clinic Institutional Review Board 09–1889; Royal Prince Alfred Hospital Human Research Ethics X19-0320; Australian-New Zealand Clinical Trials Registry ACTRN12607000077460).

Plasma lipidomic analysis

Lipidomic analysis of plasma samples was performed by liquid chromatography-mass spectrometry (LC-MS)[17] (Supplementary Information S1). The mHSPC and mCRPC samples were analysed together, approximately 2 years after the localised PC samples, using a different LC-MS instrument with a larger coverage of lipids (Figure 1).

Statistical analyses

The association of lipid levels (log2 of pmol/ml) with clinical outcome was determined by univariable Cox regression (R package survival, v2.41-3). Unique lipid profiles were identified by latent class analysis (LCA) of the levels of prognostic lipids categorised into quartiles (R package poLCA v1.4.1)[18] (Supplementary Information S1.7). T-tests assessing

lipid levels between the different lipid profiles were performed with R package multtest v2.24.0. Cox regression and t-test P-values<0.05 were considered as statistically significant.

RESULTS

Localised PC

The localised PC cohort consisted of 389 men, of whom 10% developed metastatic relapse at a median follow-up of 7.5 years (first quartile[Q1] 6.1, third quartile[Q3] 9.0) (Table 1, Figure 1).

Metastasis-free survival is an established surrogate for OS in localised PC unlike biochemical relapse[19], thus we focused on metastatic relapse as the endpoint. The levels of 90 lipids were significantly associated with metastatic relapse (Figures 1 & 2A, Supplementary List A). The top 20 significant lipid species mainly consisted of ceramide, acylcarnitine, alkenylphosphatidylethanolamine and alkylphosphatidylethanolamine (Figure 2A, Supplementary List A). Elevated levels of ceramide, acylcarnitine and triacylglycerol species; and lower levels of alkenylphosphatidylethanolamine and alkylphosphatidylethanolamine species were associated with a shorter time to metastatic relapse (Figure 2A).

LCA of the 90 prognostic lipids classified the cohort into six groups with distinct lipid profiles, referred to as Profile L1 to L6 (Figure 2B). These groups had different clinical outcomes – men with Profile L2 had the highest rate of metastatic relapse (Hazard Ratio [HR] 5.80, 95% CI 3.04–11.1, $P=1\times10^{-6}$), whereas men with Profile L3 had the lowest rate of metastatic relapse (Figure 2B). Men with Profile L2 had significantly higher plasma levels of the prognostic species of ceramide, dihydroceramide, acylcarnitine and triacylglycerol, and lower levels of alkenylphosphatidylethanolamine and alkylphosphatidylethanolamine species than other men (Figure 2C, Supplementary List A). Interestingly, the lipid profile of men with Profile L2 was the inverse of men with Profile L3, who had the lowest rate of metastatic relapse (Figure 2C).

The association of lipid profiles with metastatic relapse was independent of standard clinicopathological factors ($P=6\times10^{-6}$, Model 1 in Table 2) and key metabolic indicators ($P=2\times10^{-4}$ when modelled with diabetes, Model 2 in Table 2; $P\leq3\times10^{-5}$ when modelled with other indicators in Supplementary Information S2). The addition of the lipid profile to a Cox regression model of Gleason Score and pathological stage (Concordance Index [C-index] 0.83, $P=2\times10^{-22}$) improved the model's predictive ability (C-index 0.86, $P=4\times10^{-25}$). The lipid profile also improved the discriminatory value of the American Joint Committee on Cancer's TNM staging, where men with Stage 3 cancer (highest TNM stage in the cohort) had a higher metastatic relapse rate if they had Profile L2 (HR 6.30, 95% CI 3.12–12.7, $P=2\times10^{-6}$)(Supplementary Figure S3.1).

mHSPC

The mHSPC cohort consisted of 44 men, of whom 64% developed resistance to testosterone suppression at a median follow-up of 4.4 years (Q1=3.1, Q3=6.8)(Table 1, Figure 1).

The levels of 77 lipids were significantly associated with testosterone suppression failure (Figures 1 & 2D, Supplementary List B). The top significant lipid species mainly consisted of ceramide, diacylglycerol and triacylglycerol. Higher levels of the ceramide species and lower levels of diacylglycerol and triacylglycerol species were associated with early testosterone suppression failure (Figure 2D, Supplementary List B).

LCA of the 77 prognostic lipids classified the cohort into three groups with distinct lipid profiles referred to as Profile H1 to H3 (Figure 2E). Men with Profile H2 had the shortest time to testosterone suppression failure, whereas men with Profile H1 had the best outcome (Profile H2 versus H1: HR 3.70, 95% CI 1.37–10.0, P=0.01). Men with Profile H2 had significantly higher levels of the prognostic species of ceramide, sphingomyelin and acylcarnitine; and lower levels of prognostic species of deoxyceramide and triacylglycerol than men with Profile H1(Figure 2F).

mCRPC

The mCRPC validation cohort consisted of 137 men, of whom 51% subsequently received first-line chemotherapy and 20% were still alive at the end of the study period (Table 1). The median follow-up time was 26 months (Q1=14, Q3=46).

The levels of 275 lipids were significantly associated with OS (Figures 1 & 2G, Supplementary List C). The top 20 significant lipids mainly consisted of species of ceramide, sphingomyelin and acylcarnitine, where higher levels of these lipids were associated with shorter OS (Figure 2G, Supplementary List C).

LCA of these 275 prognostic lipids classified the cohort into three groups with distinct lipid profiles referred to as Profile C1 to C3. Men with Profile C2 had the shortest OS compared to the other groups (HR 2.54, 95% CI 1.73–3.72, $P=1\times10^{-6}$, Figure 2H). The levels of the ceramide, sphingomyelin, ganglioside, hexosylceramide and acylcarnitine species were significantly higher for men with Profile C2 compared to the other groups, whereas the levels of the species of deoxyceramide and triacylglycerol were lower in the poor prognostic group (Figure 2I). The mCRPC lipid profiles were independently associated with OS when modelled with age and BMI ($P=1\times10^{-5}$, Supplementary Table S4.1), or with PSA or alkaline phosphatase ($P\leq1\times10^{-4}$, Supplementary Table S4.2).

External validation of a prognostic 3-lipid signature in mCRPC

The association of the lipid profiles of the mCRPC validation cohort with OS is consistent with that of our previously described mCRPC discovery cohort, where plasma levels of sphingolipid species of ceramide, sphingomyelin, ganglioside and hexosylceramide were associated with shorter OS[12]. The circulating 3-lipid signature of poor prognosis (ceramide(d18:1/24:1), sphingomyelin(d18:2/16:0), phosphatidylcholine(16:0/16:0))(Figure 3A) previously derived from our mCRPC discovery cohort[12], was re-analysed with additional follow-up and retained prognostic ability (HR 2.80, 95% CI 1.89–4.13, $P=1\times10^{-6}$, Figure 3B). Furthermore, all patients had received docetaxel as first-line mCRPC therapy and those with the 3-lipid signature had a shorter time to PSA progression (HR 1.67, 95% CI 1.14–2.44, P=0.01, Figure 3C).

Importantly, the 3-lipid signature was also associated with shorter OS in the independent, external mCRPC validation cohort (HR 2.39, 95% CI 1.63–3.51, P 1×10^{-5}, Figure 3D). Patients with the 3-lipid signature had higher levels of sphingolipids including ceramide species, similar to the mCRPC discovery cohort (Supplementary Figure S4.1). Ceramide(d18:1/24:1) alone was comparable to the 3-lipid signature (HR 3.2 (95% CI 1.88–5.40, P 4×10^{-5}) on univariable analysis (Supplementary Table S4.3), but the 3-lipid signature performed better in the prediction of 1 year survival (ROC analyses in Supplementary Figure S4.2). Post-treatment progression data was not available for the mCRPC validation cohort so PSA progression-free survival was not assessable.

Significant circulating lipids across all PC phases

Acylcarnitine and ceramide species were the only lipids associated with poorer outcomes in all phases of PC progression. Ceramide is of particular interest as dysregulation of ceramide metabolism has been implicated in cancer and other pathological conditions[20]. Interestingly, higher levels of several other circulating sphingolipids which can be linked to ceramide such as sphingomyelin, hexosylceramide, and ganglioside species (Figure 3E), were associated with worse outcomes in mHSPC and mCRPC but not localised PC (Figure 2).

The number of ceramide species identified as prognostic differed between localised PC, mHSPC and mCRPC although all ceramide species had the same direction of association – elevated ceramides were associated with poorer clinical outcomes (Figure 3F). Nevertheless, five prognostic ceramides were common between all three disease phases and included ceramide(d18:1/24:1), which is part of our previously published and now validated prognostic 3-lipid signature for mCRPC.

Overall, these findings suggest that aberrations in sphingolipid metabolism are associated with aggressive PC, beginning with ceramide metabolism in localised PC and progressively encompassing the metabolism of other sphingolipids over the natural history of the disease.

DISCUSSION

The key finding of this study is that elevated circulating ceramide levels are associated with poor outcomes across the key timepoints of PC progression from localised PC to mHSPC to mCRPC. Patients with elevated ceramide levels are more likely to have metastatic relapse, therapeutic failure (testosterone suppression/docetaxel chemotherapy) and shorter OS, while our previously published prognostic 3-lipid signature for mCRPC was successfully validated in an external independent cohort. The lipidomic assay used in this study has been extensively utilised and validated in cardiovascular disease and diabetes, identifying plasma lipidomic signatures that predict cardiovascular events in high-risk patients and the general population, and are now driving changes in the treatment of cardiac patients[17,21,22]. Importantly, the 3-lipid signature in mCRPC described in our study is potentially actionable using existing metabolic drugs that target ceramide metabolism.

Circulating sphingolipids are mainly derived from the liver, transported in lipoprotein pools[23], and can be increased by systemic inflammation[24]. However, some circulating sphingolipids may originate from the tumour as PC cells express the relevant biosynthetic

enzymes (The Cancer Genome Atlas) of which some are associated with poorer PC outcomes (Supplementary Information S5)[25]. Exosomes secreted by PC cells are also enriched in sphingolipids[26].

One hypothesis is that ceramides may also be promoting aggressive PC via their metabolite, sphingosine-1-phosphate (S1P). S1P is produced by a series of enzymatic reactions involving acid ceramidase and sphingosine kinase (SPHK) which are reported to have high expression/activity in PC cancers[27,28]. Furthermore, elevated SPHK gene expression in localised PC is associated with disease progression (Supplementary Information S5.2). S1P can promote cancer cell proliferation, survival and metastasis; and regulate lymphocyte trafficking by acting on S1P-specific receptors present on immune cells and cancer cells[29]. Mice lacking SPNS2, the lymphoid tissue-specific transporter of S1P, have reduced metastases[30]. Drugs that inhibit S1P production have anti-cancer properties, such as SPHK inhibitors[31], of which ABC294640 completed a Phase I trial for advanced solid tumours (NCT01488513) and is undergoing Phase IIA clinical trials for cholangiocarcinoma (NCT03377179).

Aberrant ceramide metabolism in PC could also be modulated by targeting the metabolic environment of the host. High-fat feeding increases circulating ceramides[32], and promoted inflammation and metastasis through S1P signalling in a breast cancer mouse model[33]. Importantly, this metabolic state can be pharmacologically normalised; cardiovascular and obesity studies demonstrate that elevated circulating ceramides can be decreased using cholesterol-lowering drugs (statins and PCSK9 inhibitors)[34,35] and exercise[36]. In summary, targeting ‘host’ or tumour sphingolipid metabolism are both clinically feasible approaches and may improve the outcome of PC patients.

The tumour-promoting effect of obesity is attributed to circulating metabolic and inflammatory mediators associated with the chronic inflammatory state of adipose tissue[3]. However, the type of circulating lipids appears to be an important mediator, as we found that lipid profiles were independently associated with clinical outcomes when modelled with BMI. This suggests that a subset of men with PC, irrespective of their obesity status, have a metabolic signature that affects their cancer outcome.

The association of circulating sphingolipids with poorer clinical outcomes have also been reported recently by two metabolomic studies on localised PC. Clendinen *et al* (2019) profiled the levels of 450 lipids in pre-radical prostatectomy serum samples from 40 patients with biochemical recurrence and 40 in remission, and found that ceramide levels were increased in those with biochemical recurrence[37]. Snider *et al* (2020) performed metabolomic analysis on plasma from 159 treatment-naïve men and found that circulating levels of glycosphingolipids, ceramides and sphingomyelins were increased in men with more aggressive cancer as defined by Gleason grade, PSA levels and tumour stage[38]. The total number of lipids profiled was not specified. Interestingly, one of the significant sphingolipids identified from both Clendinen *et al* and Snider *et al* was Cer(d18:1/24:1), which was prognostic in all our cohorts and part of the prognostic 3-lipid signature. Overall the findings of these studies are consistent with ours, which show that perturbations in ceramide metabolism is associated with aggressive prostate cancer. The strength of our study

as opposed to these is that we had sufficient follow-up to define metastasis-free survival as the endpoint, which was demonstrated by the ICECaP working group as the only surrogate endpoint associated with prostate-cancer specific survival[19]. The limitations of our study are the small size of the mHSPC cohort, and the limited availability of clinicopathological and metabolic data for some of the metastatic PC cohorts. Furthermore, there were some minor differences in the panel of lipid species profiled across all three cohorts due to improvements in analytical methodology where a larger number of lipids were profiled in the metastatic cohorts compared to localised PC. Thus, direct comparisons of certain lipid species could not be made, such as the ceramide species with different isoforms of the sphingoid backbone. Our prognostic 3-lipid signature is only applicable to mCRPC and not prognostic in the localised and mHSPC cohorts (data not shown), which is not suprising as the signature was originally derived from a mCRPC cohort[12]. The biology of prostate cancer will change through treatment and progression, thus some changes in the lipidomic profiles over the course of the disease are expected. The development of specific and accurate prognostic lipid signatures for localised and mHSPC will require future validation cohorts.

CONCLUSION

Elevated circulating ceramide species are associated with poorer clinical outcomes across the natural history of PC, from localised PC to mHSPC to mCRPC. Furthermore, our previously published prognostic mCRPC 3-lipid signature was validated in an independent mCRPC cohort. Precision oncology is commonly used to describe genomic-driven treatment, however, based on our data there is a case for personalised metabolic therapy in conjunction with standard-of-care to facilitate the optimal therapeutic environment. Prospectively-designed clinical trials with ceramide-targeting therapy using the lipid signature to guide treatment decisions in metastatic PC are now warranted to demonstrate its clinical utility and potentially improve patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CONFLICT OF INTEREST

A.Azad: Consultant - Astellas, Janssen, Novartis; Speakers Bureau - Astellas, Janssen, Novartis, Amgen; Honoraria - Astellas, Janssen, Novartis, Tolmar, Amgen, Pfizer, Telix; Scientific Advisory Board - Astellas, Novartis, Sanofi, AstraZeneca, Tolmar, Pfizer, Telix; Research Funding - Astellas, Merck Serono, Bristol Myers Squibb (institutional), Astra Zeneca (institutional), Aptevo Therapeutics (institutional), Glaxo Smith Kline (institutional), Pfizer (institutional), MedImmune (institutional), Astellas (institutional), SYNthorx (institutional), Bionomics (institutional), Sanofi Aventis (institutional), Novartis (institutional). L.Horvath: Research funding - Astellas; Travel sponsorship - Janssen, Pfizer; Honoraria - Imagion Biosystems. All remaining authors have declared no conflicts of interest.

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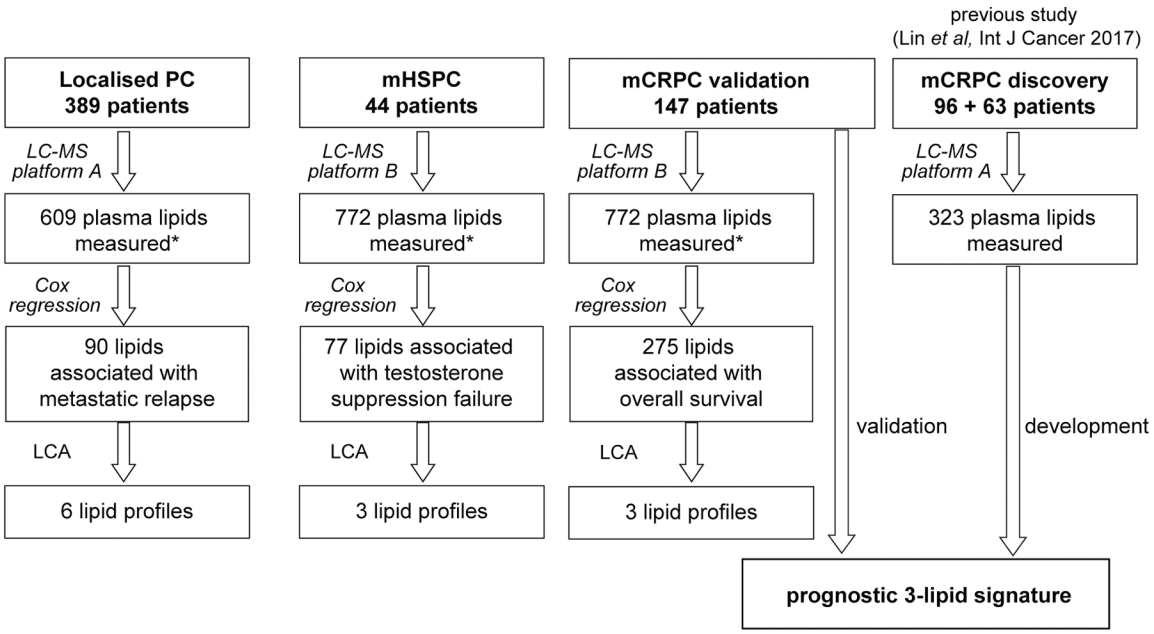

LC-MS platform A: Agilent 1200 LC, Applied Biosystems API 4000 QTRAP MS
LC-MS platform B: Agilent 1290 LC, Agilent 6490 QQQ MS

*512 lipids are common between localised PC, mHSPC and mCRPC

Abbreviations: PC, prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; mCRPC; metastatic castration-resistant prostate cancer; LC-MS, liquid chromatography-mass spectrometry; LCA, latent class analysis

FIGURE 1.
Prostate cancer study cohorts and analysis strategy.

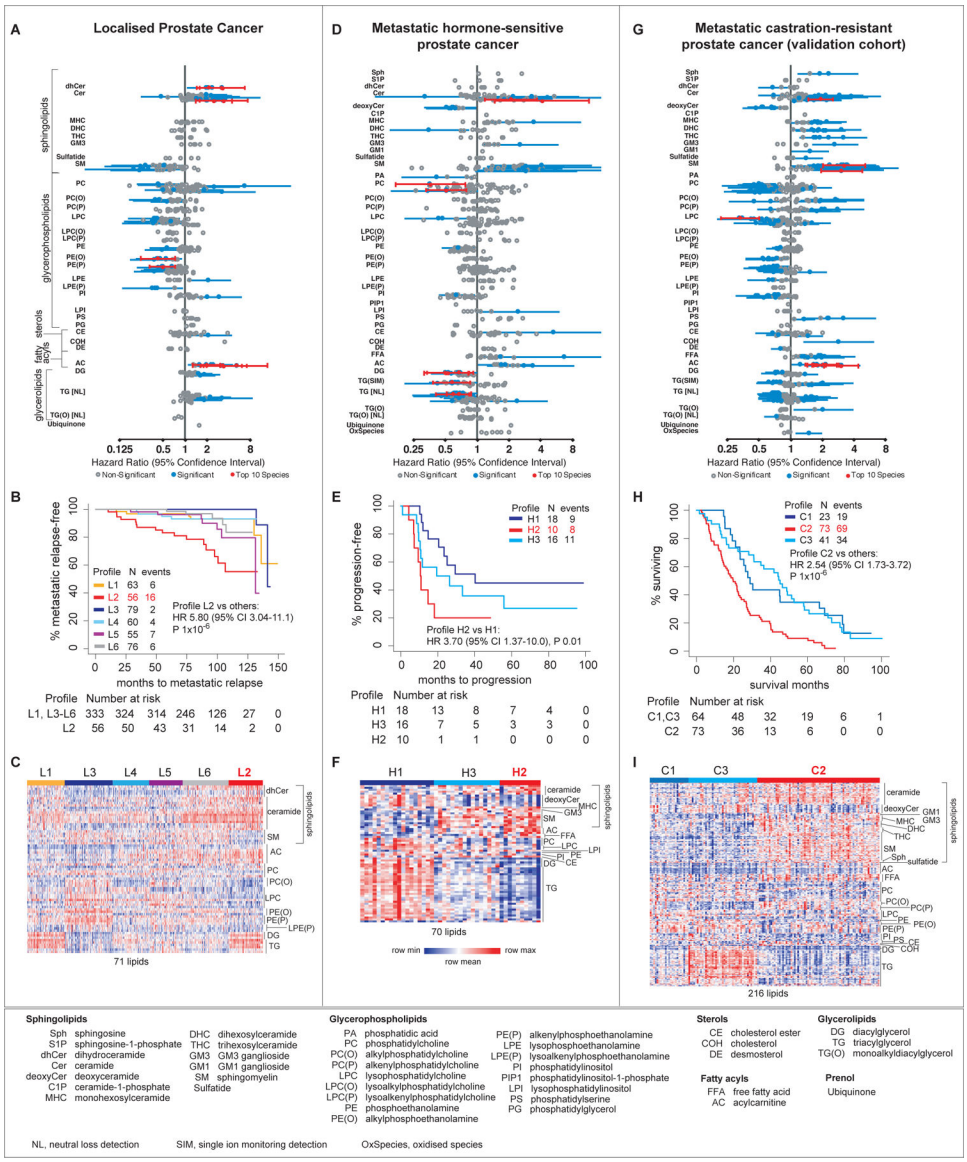

FIGURE 2. Association of plasma lipids with clinical outcomes in localised PC, mHSPC and mCRPC.

Forest plots of the hazard ratios of levels of plasma lipids (A,D,G); Kaplan-Meir curves (B,E,H) and heatmaps (C,F,I) of lipid profiles associated with clinical outcomes. The heatmaps show the levels of prognostic lipids that were significantly different between the profile with the worse outcome compared to the other profiles, except for mHSPC where the comparison is between Profile H2 and H1.

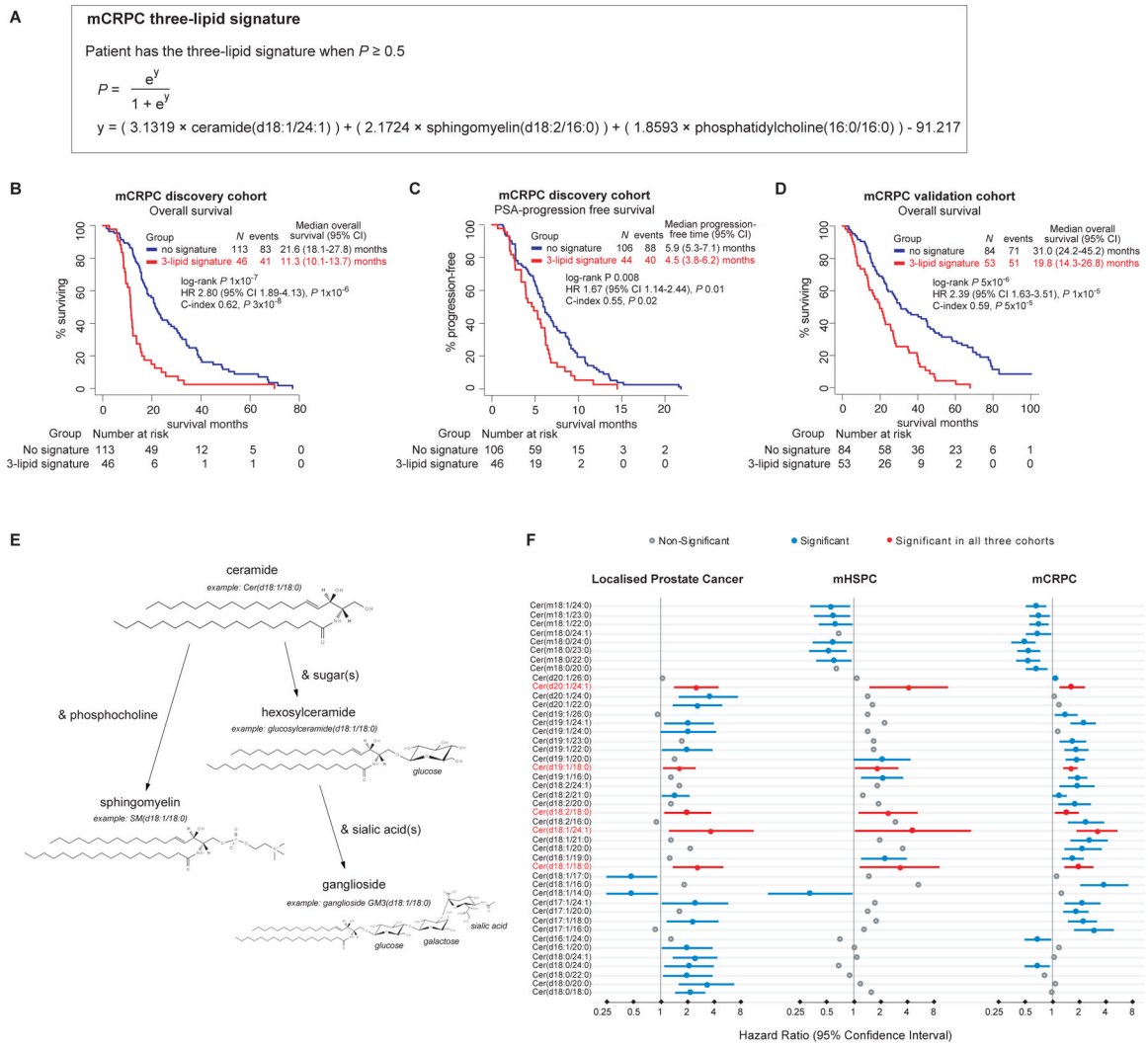

FIGURE 3. Prognostic mCRPC 3-lipid signature and ceramide species.

Formula of prognostic mCRPC 3-lipid signature derived in previous study of discovery cohort[12] (A); overall survival (B) and PSA progression-free curves (C) of discovery cohort classified by the 3-lipid signature; overall survival curves of validation cohort classified by the 3-lipid signature (D); metabolism of ceramide and other sphingolipids (E); forest plots of the hazard ratios of ceramide species that are prognostic in localised PC, mHSPC or mCRPC validation cohorts (F).

TABLE 1.

Clinical characteristics of prostate cancer cohorts (n.d, no data; n.a, not applicable).

	Median [first quartile, third quartile] or number [% of cohort]			
	Localised	**mHSPC**	**CRPC discovery**	**CRPC validation**
Number of men	389	44	159	137
Median age, years	63 [59, 68]	67 [63, 78]	70 [64,75]	72 [67, 77]
Median baseline PSA, μg/l	7.0 [5.2, 9.2]	25 [7,43], 9% n.d	111 [40,407], 2% n.d	16 [4, 66], 5% n.d
Median alkaline phosphatase, U/l	n/a	98 [72,131], 23% n.d	135 [93,299], 4% n.d	92 [68, 131], 4% n.d
Median haemoglobin, g/l	n/a	139 [136,147] 93% n.d	125 [111,135], 2% n.d	127 [121,137], 69% n.d
Median lactase dehydrogenase	n/a	172 [166,196], 82% n.d	n.d	188 [159,223], 53% n.d
Median body mass index	27 [25, 30], 55% n.d	29 [26, 32] 11% n.d	27 [25, 31] 23% n.d	30 [27, 34], 3% n.d
Metastatic relapse	40 [10%]	n/a	n/a	n/a
Biochemical relapse	157 [40%]	n/a	n/a	n/a
Testosterone suppression failure	n/a	28 [64%]	n/a	n/a
Dead	0	24 [55%]	124 [78%]	122 [89%]
Gleason grade				
≤6	76 [20%]	6 [14%]	11 [7%]	15 [11%]
7	274 [70%]	10 [23%]	34 [21%]	49 [36%]
8	17 [4.4%]	9 [20%]	24 [15%]	23 [17%]
≥9	22 [5.6%]	16 [36%]	52 [33%]	41 [30%]
Unknown		3 [7%]	38 [24%]	9 [6%]
Pathological stage		n/a	n.a	n/a
PT1	0			
PT2	205 [53%]			
PT3	184 [47%]			
Extraprostatic extension	177 [46%]	n/a	n/a	n/a
Positive surgical margin	143 [37%]	n/a	n/a	n/a
Seminal vesicle invasion	45 [12%]	n/a	n/a	n/a
Metastasis	n/a			
Bone		34 [77%]	94 [59%]	120 [88%]
Visceral		0	59[37%](soft tissue)	10 [7%]
Unknown		10	13 [8%]	14 [10%]
mHSPC therapy	n/a		n/a	n/a
Testosterone suppression		44 [100%]		
With docetaxel		2 [5%]		
CRPC 1st line therapy	n/a	n/a		
Docetaxel			159 [100%]	66 [48%]
Docetaxel +/− atrasentan			0	3 [2%]
Docetaxel +/− OGX-11–011			0	1 [0.7%]
Cisplatin + etoposide			0	1 [0.7%]

	Median [first quartile, third quartile] or number [% of cohort]			
	Localised	**mHSPC**	**CRPC discovery**	**CRPC validation**
Carboplatin + etoposide			0	1 [0.7%]
Abiraterone			0	3 [2%]
Dexamethasone			0	1 [0.7%]
Sipuleucel-T			0	1 [0.7%]
CRPC 2nd line therapy	n/a	n/a		
Cabazitaxel			37 [23%]	9 [7%]
Enzalutamide			3 [2%]	14 [10%]
Abiraterone			44 [28%]	14 [10%]
Mitoxantrone			13 [8%]	1 [0.7%]
Radium 223			0	1 [0.7%]
Sipuleucel-T			0	2 [1%]
other			14 [9%]	0
Diabetes	12 [3.1%], 36% n.d	n.d	23 [14%], 4% n,d	n.d
Statin medication	81 [21%], 36% n.d	n.d	49 [31%], 4% n.d	n.d
Hypertension medication	119 [31%], 36% n.d	n.d	n.d	n.d

TABLE 2.

Cox regression analyses of the association of metastatic relapse with lipid profiles, metabolic factors and clinicopathological factors in the localised prostate cancer cohort.

Clinicopathological factor	case	event	Univariable cox regression		Multivariable cox regression			
					Model 1		Model 2	
			HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Lipid profile Profile L2 vs others	389	40	5.80 (3.04–11.1)	1×10^{-7}	4.79 (2.43–9.44)	6×10^{-6}	4.06 (1.94–8.56)	2×10^{-4}
Gleason score ≤7 vs >7	389	40	10.5 (5.38–20.3)	4×10^{-12}	6.29 (3.00–13.2)	1×10^{-6}	6.25 (2.87–13.6)	4×10^{-6}
P-stage PT2 vs PT3	389	40	14.3 (5.02–40.7)	6×10^{-7}	11.4 (3.91–33.3)	8×10^{-6}	5.63 (1.89–16.8)	0.002
Pre-operative PSA *	389	40	1.05 (1.02–1.07)	4×10^{-4}	1.02 (0.99–1.05)	0.2	-	-
Surgical margin Positive vs negative	389	40	1.72 (0.92–3.20)	0.09	-	-	-	-
Age *	389	40	1.07 (1.01–1.13)	0.01	-	-	-	-
Diabetes Yes vs no	251	33	5.25 (1.81–15.2)	2×10^{-3}	-	-	2.13 (0.67–6.73)	0.2
Statin usage Yes vs no	251	33	1.70 (0.85–3.37)	0.1	-	-	-	-
Hypertension Yes vs no	249	32	1.49 (0.74–3.00)	0.3	-	-	-	-

* continuous variable