Multimodal Chemical Imaging of Amyloid Plaque Polymorphism Reveals Aβ Aggregation Dependent Anionic Lipid Accumulations and Metabolism

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Running Title: Hyperspectral Chemical Imaging of Amyloid Plaque Pathology

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ABSTRACT

Amyloid plaque formation constitutes one of the main pathological hallmarks of Alzheimer's disease (AD) and is suggested to be a critical factor driving disease pathogenesis. Interestingly, in patients that display amyloid pathology but remain cognitively normal, Aβ deposits are predominantly of diffuse morphology suggesting that cored plaque formation is primarily associated with cognitive deterioration and AD pathogenesis. Little is known about the molecular mechanism responsible for conversion of monomeric Aβ into neurotoxic aggregates and the predominantly cored deposits observed in AD. The structural diversity among Aβ plaques, including cored/compact- and diffuse, may be linked to their distinct Aβ profile and other chemical species including neuronal lipids. We developed a novel, chemical imaging paradigm combining matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) and fluorescent amyloid staining. This multimodal imaging approach was used to probe the lipid chemistry associated with structural plaque heterogeneity in transgenic AD mice (tgAPPSwe) and was correlated to Aβ profiles determined by subsequent laser microdissection and immunoprecipitation-mass spectrometry. Multivariate image analysis revealed an inverse localization of ceramides and their matching metabolites to diffuse and cored structures within single plaques, respectively. Moreover, phosphatidylinositols implicated in AD pathogenesis, were found to localize to the diffuse Aβ structures and correlate with Aβ1-42. Further, lysophospholipids implicated in neuroinflammation were increased in all Aβ deposits. The results support previous clinical findings on the importance of lipid disturbances in AD pathophysiology and associated sphingolipid processing. These data highlight the potential of multimodal imaging as a powerful technology to probe neuropathological mechanisms.

Alzheimer's disease (AD) pathology is characterized by morphologically heterogeneous extracellular aggregates consisting of amyloid-β (Aβ) peptides together with intracellular neurofibrillary tangles

formed by aggregated truncated and hyper-phosphorylated tau protein (1). Several lines of evidence suggest that Aβ aggregation is the key pathogenic event in AD, which is the central tenet of the amyloid cascade hypothesis (2). Hence, the main focus in AD research and drug development has been on Aβ plaque pathology (2). The structural diversity, especially the main types cored and diffuse plaques, among these deposits has been linked to their Aβ isoform composition, as well as Aβ orientation during the formation of cross β-sheet rich fibrils (3). Here soluble, prefibrillar oligomers and protofibrils with distinct β-sheet structures that act as intermediates of mature fibrils are suggested to play the key role in synaptic dysfunction and neurodegeneration (3,4). However, little is known about the molecular mechanism responsible for the conversion of the monomeric Aβ peptide into these neurotoxic aggregates. In addition to Aβ isoform composition, the morphological heterogeneity among Aβ plaques may be related to differences in plaque associated lipids (5,6). Indeed, neuronal lipids have been implicated in Aβ plaque pathology by controlling trafficking and activity of membrane-bound proteins involved in Aβ production, as well as by modulating the aggregation propensity of Aβ peptides (7). In addition, the ε4 variant of the apolipoprotein E (*APOE*) gene that encodes the E4 isoform of apoE, a lipid transporter protein, was identified as the major genetic risk factor for sporadic AD (8). These observations strongly suggest that aberrant lipid homeostasis is tightly linked to Aβ pathogenesis in AD.

For probing in situ pathochemical changes, including spatial lipid alterations, advanced chemical imaging techniques, such as imaging mass spectrometry (IMS) are required (9,10). Using IMS allows to generate spatial intensity distribution maps of molecular species in complex biological tissues (10). In particular, matrix assisted laser desorption/ionization (MALDI)-based IMS has been repeatedly demonstrated to be well suited for imaging neuronal lipids, including both sphingolipids and phospholipids, as well as neuropeptides, in mammalian brain tissue (9). Here, MALDI-IMS has e.g. been used to measure plaque specific lipid localizations (6,11,12), and Aβ peptide truncation patterns (13) in transgenic AD mouse models. Transgenic AD mice carrying the Swedish mutation (K670N, M671L) under the Thyr1 promoter ($tgAPP_{Swe}$) are a suitable candidate for probing structural plaque heterogeneity as these mice display both compact cored and diffuse plaques at 18 months (14). Differences in Aβ fibril tertiary structures can be elucidated using luminescent conjugated oligothiophenes (LCOs) (15). These fluorescent amyloid probes, have been shown and verified through antibody staining to differentially recognize mature fibrillar and immature protofibrillar Aβ aggregates. (16,17) Moreover, these probes vary in their spectroscopic, electro-optic properties, allowing for hyperspectral fluorescent imaging for annotation of structural heterogenic Aβ pathology (16-18).

In the current study, we report a novel multimodal chemical imaging paradigm based on histology-compatible MALDI imaging mass spectrometry of neuronal lipids (12) along with hyperspectral fluorescent amyloid imaging using differential LCO staining. The methodology facilitated the investigation of spatially confined lipid changes associated with morphologically heterogeneous amyloid plaque pathology in 18 months old $tgAPP_{Swe}$ mice.

Results

Histology-compatible MALDI IMS enables subsequent hyperspectral imaging for Aβ plaque structural heterogeneity delineation

MALDI-IMS was multiplexed with differential fluorescent amyloid staining for hyperspectral imaging of mature Aβ fibrils, revealed by q-FTAA staining, and immature protofibrillar Aβ intermediates as visualized by h-FTAA (16,19). Indeed, the acquired IMS data showed very good co-localization of lipid signal with Aβ pathology as visualized by LCO staining (Figure 1A-F) and verified by immunohistochemistry (Supplemental Figure S-1). Here, acquisition of hyperspectral emission profiles at different excitation wavelengths, and linear un-mixing revealed structure specific emission profiles based on the respective LCO binding (Figure 1G). In alignment with previous results, the here observed Aβ pathology exhibited distinct LCO encoded optical properties as revealed by spectral delineation of plaque area cross-sections (16,17,20). Specifically, two major sub populations of plaques were identified and annotated. Here, one subpopulation of plaques exhibited spatial spectral variation with a blue shift (q-FTAA) in the center of the plaque and a diffuse fibrillary corona that exhibited red emission profile (h-FTAA) and was hence referred to as *cored* plaques (Figure 1H,I). The other plaque subpopulation that was morphologically predominantly diffuse fibrillary in nature, maintained mostly unchanged red emission profile (h-FTAA) in their line scan profile, and were collectively classified as *diffuse* Aβ deposits (Figure 1J,K). A preferential binding of either q- or h-FTAA is dependent on the size

of available binding pockets within the plaques fibrils, where h-FTAA also binds diffuse amyloid aggregates including diffuse plaques and well as the diffuse periphery of compact cored deposits. Such distinct spectral properties have been attributed to the conjugated polymer backbone of the LCOs, which allows a high degree of conformational freedom (15). Due to the hydrophobic nature of the Aβ aggregation intermediates, differences in lipid species localizing to structurally heterogeneous Aβ deposits should be expected.

Cortical Aβ plaques display distinct hyperspectral profiles that reflect unique neuronal lipid composition

Given the multivariate nature of the IMS data that co-localize with fluorescent imaging data (Figure 1F), spatial segmentation using hierarchical clustering (bisecting k-means, Figure 1L) and Principal Component Analysis (PCA) (Figure 1M,N) was performed, in order to reveal histologically relevant localization patterns of distinct lipid species. In detail, multivariate analysis revealed the presence of typical spectral pseudo clusters that might outline chemical similarities across histological features and more specifically structurally heterogeneous Aβ aggregation features. While PCA, extracts the highest variance in the IMS dataset and provides a variable representation that directly reflects sample representation, it does not directly maximize the separation between groups of samples and often omits weak signals unless normalized and centered. Further, due to the bidirectional loading values, interpretation of PCA score images is not always straight forwards. In contrast, spatial segmentation using hierarchical clustering, pairs the variables based on their degree of similarity. The resultant pseudo-objects, i.e. clusters, reflect homogenous groups of variables, with dominating within-group similarities that can be interpreted with help of hierarchical cluster tree.

Inspection of the individual PCA score images and their corresponding loading plots (Figure 1M,N; Supplementary Figures S-2) allowed for identification of four distinct lipid distribution patterns in both imaging mode. In negative ion mode, PCA revealed lipid localizations to either the center of cored plaques (Figure 1M) or localization to diffuse plaques and the diffuse periphery of cored plaques, respectively (Figure 1N). Further, two distinct pattern of plaque associated lipid decrease were observed, including a general decrease for all plaques (Supplementary Figure S-2A, PC2) as well as with selective depletion at the center of cored plaques (Supplementary Figure S-2C, PC6).

Further, image analysis using hierarchical clustering revealed distinct lipid pseudo-clusters corresponding to prominent Aβ pathology. In negative ion mode, this identified the diffuse periphery of cored-plaques as well as entirely diffuse plaques belong to the same cluster and coincide with the histological annotation visualized by the q-/h-FTAA staining (Figure 1L). The MALDI-IMS spectral data of individual plaque ROI were categorized based on these hyperspectral emission profiles into either diffuse or cored plaques, as well as centers of cored deposits. The ROI data from cortex were extracted for each of the biological replicates (n=3, 10-15 diffuse- and 10-15 cored plaques per animal). ROI spectral data were processed by means of peak picking and data binning and analyzed with OPLS-DA and two class, paired t-statistics ($p<0.05$) in order to identity lipid species in between the different plaque ROI. Here models for diffuse plaques and the centers of the cored plaques, as well as diffuse plaques and whole cored plaques allowed separation, with strong cross validation matrices as indicated in the corresponding score plots (Supplementary Figure S-3A,B). The associated S-plot (Supplementary Figure S-3C,D) and loading vector plot (Supplementary Figure S-3E,F) for each of the models revealed significant underlying neurochemical differences. Inspection of the loading data and subsequent univariate statistics revealed distinct Aβ plaque morphology dependent patterns of both phospholipids and sphingolipids. Interestingly, comparison of entire cored deposit ROIs against diffuse plaque regions, and core plaque centers against diffuse coronal structures of cored deposits, revealed similar variables to be responsible for the separation in both cases.

Plaque morphology-independent monosialo-ganglioside (GM) accumulation and sulfatide depletion

Inspection of the loading data and subsequent univariate statistics revealed a general plaque associated distribution of monosialo-gangliosides (GM) with C18:0 and C20:0 fatty acid (FA) moieties, irrespective of plaque morphology. Here, a particularly pronounced localization to plaques was observed for GM2, and GM3, though no differences were observed between diffuse regions and centers of cored deposits (Figure 2A-E).

For various, a-series gangliosides (GD1a, GM1, GM2, and GM3) that are most prominent in the CNS,

alternating concentration changes have been linked to AD pathology. (21) Here tissue levels of GM2 and GM3 species were generally found to be increased in multiple brain regions in both human AD and tgAPP$_{Swe}$ mice. (22-24) which is well in line with the present data.

GM2 and GM3 accumulations were found in lysosomal storage disorders (25), suggesting impaired ganglioside hydrolysis and lysosomal degradation to be associated with the AD plaque pathology. Indeed, such AD-associated defects in endolysosomal pathways have been shown in gene expression studies of postmortem human brains (26) and genome-wide association studies (GWAS) (27), further reinforcing this hypothesis.

This suggests that while GMs might be involved in the Aβ pathology, their role appears to be general and independent of the Aβ plaques morphology. In the present study, MALDI and LCO imaging further identified a general decrease of sulfatides (ST, Figure 2F-I) and their hydroxylated isoforms (ST-OH, Supplementary Figure S-4) at Aβ plaques. The majority of sulfatides in the central nervous system (CNS) are present in myelinating oligodendrocytes. (28) The observed ST depletion pattern likely reflects general sulfatide catabolism and demyelination associated with Aβ plaque pathology.

Phospho-ceramides and ceramide monohexosides display a plaque core-specific accumulation pattern

Similar to gangliosides and sulfatides, a plaque pathology associated increase irrespective of plaque phenotype was observed for ceramide species (Figure 3A,B,F). In contrast, a morphology-dependent localization pattern selectively to the center region of cored plaques as outlined by complementary hyperspectral LCO emission profiles was observed for corresponding ceramide metabolite species. These compounds included ceramide monohexoside (HexCer, Figure 3C) as well as ceramide-1-phosphate (CerP, Figure 3D) and ceramide phospholipid conjugates including phosphoethanolamines (PE-Cer, Figure 3E) (Supplementary Figure S-5). The observed selective localization of these ceramide derivatives to the core region indicates an association of ceramide metabolism with plaque maturation from diffuse- to cored plaques.

Poly unsaturated fatty acid (PUFA)-conjugated phospholipids localize to Aβ pathology in a morphology dependent fashion

Anionic phospholipid species have previously been implicated in AD plaque pathology. In the present IMS study, plaque-specific accumulations of PI and PA phospholipids with distinct localization patterns were observed (Figure 4). We found that different anionic phospholipids and lysophospholipid species, including anionic PIs and PAs as well as LPI and LPA species displayed a characteristic accumulation to Aβ plaque pathology. This plaque associated PL localization pattern was predominantly observed for palmitic and stearic acid conjugates with either polyunsaturated arachidonic acid (AA) or docosahexaenoic acid (DHA) residues (Figure 4A-J). Here, anionic AA- and DHA-conjugated PIs, including PI(16:0/20:4), PI(18:0/20:4) as well as PI(16:0/22:6) and PC(18:0/22:6), showed a high degree of accumulation to the corona of the cored deposits and the diffuse plaques, which was more pronounced for the AA conjugates (Figure 4A,B,E,F).

Further, AA containing PA conjugates, including PA(16:0/20:4) and PA(18:0/20:4), were found to localize to plaques (Figure 4I,J,L). This strongly suggests that both the respective conjugated FA moiety as well as the backbone structure of the respective phospholipid species affect the distribution pattern around Aβ deposits. In addition to the observed PI and PA species, corresponding C16 and C18 fatty acid containing lysophosphatidylinositols (LPI) and lysophosphatidic acids (LPA) species exhibited a plaque associated distribution pattern. Here LPI 16:0 (Figure 4C), LPA 18:0 (Figure 4K) and LPA 16:0 (Supplementary Figure 7) exhibited a distinct localization pattern to Aβ plaque pathology, independent of morphology. In contrast, LPI C18:0 was found increased in diffuse Aβ fibrils (Figure 4G, H).

Amyloid beta 40/42 ratio changes associated with plaque morphology

To correlate the here observed lipid changes to amyloid plaque peptide chemistry, we performed Multiplexed, fluorescent staining with q-FTAA, h-FTAA and Anti- Aβ1-16 (6E10) was performed to confirm Aβ identity of LCO stained deposits in tgSwe animals. Plaque specific Aβ isoforms within each hyperspectrally delineated plaque population were characterized using laser microdissection and Aβ immunoprecipitation followed by MALDI mass spectrometry (IP-MS). Here, cored plaques that exhibited more q-FTAA staining showed significantly increases Aβ1-40 levels relative to Aβ1-42 as compared to diffuse plaques stained solely by h-FTAA (Figure 5).

Discussion

Amyloid pathology has been identified as the critical inducer of AD pathogenesis. Aβ plaques consist of β-sheet rich fibrils formed by differentially truncated Aβ peptide isoforms (29). Consequently, morphological heterogeneity, such as diffuse and cored, mature plaques have been linked to structural transitions of different Aβ isoforms during the aggregation process (30,31). However, plaque annotation and characterization as done by pathologists is a biased procedure with discrete output values. It is therefore of central relevance to probe and annotate the chemical composition and structure of a plaque in a quantitative way with high spatial resolution.

The here employed a hyperspectral fluorescent amyloid staining paradigm based on LCOs was used to generate plaque structure specific values corresponding to the degree of Aβ aggregation. We further set out to identify chemical correlates associated with Aβ plaque polymorphism. Here, the first experiments included LCO based hyperspectral annotation and IP-MS analysis of subsequently laser microdissected plaque species that identified a decrease in Aβ42/40 ratio for cored plaques compared to diffuse deposits (Figure 5). Cored plaques contain relatively smaller levels of Aβ42, due to a significant increase in Aβ40 deposition. This further suggests that the increase in structural complexity as indicated by q-FTAA staining is most prominently manifested in the core and due to increased amount of Aβ1-40 at the core, respectively.

Indeed, in line with these results, previous immunohistochemical efforts for characterizing plaque heterogeneity both in transgenic AD mice (32-34) and in human AD (35) revealed prominent Aβx-40 immunoreactivity within the core structure of cored plaques. In contrast Aβx-42 was found to stain mostly the corona as well as diffuse deposits, which is also well in line with our observations. Given that Aβ1-42 has been shown to rapidly form oligomers and subsequently fibrils, as compared to other C-terminally truncated peptides (36), the higher levels of Aβ1-42 in diffuse deposits as observed here likely reflect an essential role of these peptide species in the initial seeding of Aβ aggregation and early stages of plaque formation.

While Aβ peptide dynamics are central to plaque formation, a large focus in current AD research lies on identifying other plaque associated chemical correlates that might be essentially relevant in understanding Aβ aggregation and Aβ plaque polymorphism. Indeed, lipid species have been implicated in Aβ plaque pathology by controlling trafficking and activity of membrane-bound proteins involved in Aβ production, as well as by modulating the aggregation propensity of Aβ peptides (7). Therefore, in addition to Aβ isoform composition, Aβ plaque polymorphism may be related to differences in plaque associated lipids (5,6).

In present study, a novel multimodal imaging paradigm was established combining orthogonal chemical imaging techniques for probing chemical (MALDI-IMS of lipids) and structural properties (LCO staining) in situ with respect to spatial and histopathological context (Figure 1). Previously, combination of MALDI imaging and fluorescent imaging was demonstrated to be challenging particularly for peptide imaging due to laser ablation induced tissue distortion effects (12,13). These limitations were however successfully overcome for MALDI imaging of lipid species and immunofluorescent staining on the same tissue array (12).

The pathochemical data observed in the current study, argue for morphology specific sphinogolipid and anionic phospholipid alterations. Among sphingolipids, a general localization of GM2 and GM3 gangliosides as well as a general depletion of sulfatides was observed for all Aβ plaques irrespective of their morphology (Figure 2, Supplementary Figure S-4). Interestingly, sulfatide depletion was accompanied by a localized increase in the corresponding ceramide backbone species (Figure 5). AD implicated sulfatide deficiency has been attributed to neurodegeneration and demyelination, respectively. Mechanistically, plaque associated ST degradation has been suggested to involve low density lipoprotein (LDL) receptor-mediated endocytosis of ST containing ApoE associated lipoproteins, which become target to lysosomal degradation. (5,37) Indeed, the increased production of Aβ in transgenic mice has been shown to require an increase in apoE-mediated clearance (38), which further implicates an interplay of apoE, sulfatides and Aβ for both amyloid aggregation and clearance.

Along with sulfatide depletion, we observed an increase in ceramide species that localized characteristically to all Aβ plaques (Figure 5). For a majority of neurodegenerative diseases, dysregulated apoptosis associated with elevated ceramide levels has been described, including for AD (39,40), where several ceramide species were found increased in vulnerable regions of post mortem AD brain (39-41),

as well as in transgenic AD mice ($tgAPP_{ArcSwe}$ and $tgAPP_{Swe}$) (6,12). Elevated ceramide levels have been tied to changes in sphingolipid metabolism controlling enzymes, including neutral- and acid sphingomyelinase (nSMase, aSMase) (40-43) Indeed, partial inhibition of aSMase was shown to result in reduced Aβ plaque deposition (43) as well as reduced ceramide production and neurotoxicity (40), which further suggests that sphingomyelin degradation to ceramides plays a pivotal role in AD pathology.

Similarly, elevated ceramide levels can also originate from increased galactocerebroside metabolism as well as sulfatide depletion, respectively. In line with this, a characteristic elevation of galactocerebrosides (GalCer) was previously demonstrated in $tgAPP_{Swe}$ mice and the prefrontal cortex in human AD brain (23).

Indeed, in the present study, along with a general depletion of sulfatides in plaques, increased HexCer levels were observed at the core of cored plaques (Figure 3C). This indicates an altered ceramide glycosphingolipid catabolism in the center of cored deposits, associated with plaque maturation into cored deposits. Further, a distinct phosphorylation of ceramides to CerP and PE-Cer was observed at the core structure (Figure 3D,E; Supplementary Figure S-5). Both PE-Cer and CerP have been proposed to suppress ceramide induced apoptosis (44). In mice, PE-Cer was shown to be produced by all three isoforms of bifunctional sphingomyelin synthesizing enzymes (45), and has been suggested to prevent apoptosis due to imbalance in ceramide homeostasis during sphingolipid biosynthesis (46,47). CerP on the other hand, produced through ceramide kinase (CERK) mediated ceramide phosphorylation, displayed anti-apoptotic properties and ensures cell survival (48). Therefore, a presence of CerP and PE-Cer could indicate cellular defense mechanisms that prevent apoptosis at the site of Aβ plaque maturation and core formation.

Along with the observed sphingolipid alterations, the present study revealed an Aβ plaque specific accumulation of AA and DHA PUFA-conjugated, anionic phospholipids. DHA and particularly AA are essential precursors in eicosanoid synthesis underlying microglial and astroglial mediated inflammatory response mechanisms (49). The observed Aβ pathology specific accumulation of AA and DHA containing PI and PA species to likely reflects their involvement in immune response at the site of plaque formation. Similarly, to PUFA-containing phospholipids, a characteristic localization of corresponding anionic lysophospholipids (LPA, LPI) that are also implicated in neuroinflammation was observed. Lysophospholipids are degradation products of phospholipids by cytosolic phospholipase A2 (PLA_2), which has been shown to regulate neuroinflammation and neurodegeneration in AD, where PLA_2 hyperactivity was most prominent in the vicinity of Aβ plaques (50,51).

While PA and LPA displayed a general plaque associated increase irrespective of plaque morphology, some PI and LPI species were found to localize specifically to the diffuse aggregates. Interestingly, the here observed PI(18:0/20:4) species (Figure 5E) as well as the corresponding lysophosphoinositol (LPI 18:0, Figure 5G) are display the same fatty acid configuration as the most common form of the associated phosphoinositolbiphosphate (PIP2) species. PIP2 is a prominent secondary messenger integral to maintain neuronal and synaptic functions (52). PIP2 has been implicated in proteopathy by being decreased upon Aβ oligomerization via reduced phosphatase activity, yielding more PI or increased phospholipase C (PLC) activity yielding the corresponding diacylglyceride (DAG) (53,54). Indeed, a previously described specific increase in AD brain tissue levels of the corresponding DAG 38:4 species, resulting from PIP2(38:4) cleavage (23), suggests a critical involvement of these particular inositol species in neuroinflammatory processes and plaque pathology, respectively. The relevance of PI and LPI species is further highlighted as serum levels of LPI 18:0 were recently found as prognostic markers for AD, identifying patients with mild cognitive impairment (MCI) that will convert to AD (55) similar to Aβ42 (and Aβ42/40) levels in CSF, as commonly used in clinical practice (56). This further supports the hypothesis that the here observed lipid species do indeed reflect AD specific brain pathology and indicates a prominent role in neurotoxic aggregation Aβ mechanisms.

Furthermore, the relevance of anionic, neuronal phospholipids including PAs and PIs in particular, has gained great attention in AD research as recent genome-wide association studies (GWAS) have identified a several risk genes for developing sporadic AD that encode various proteins associated with functional lipid biomechanisms including triggering receptor expressed on myeloid cells 2 (TREM2), ATP-binding cassette, subfamily A, member 7 (ABCA7) and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1 (SHIP1) (57). TREM2 is a lipid-sensing microglia surface receptor that recognizes anionic- and

zwitterionic lipid species (58) and was functionally implicated in AD pathology as it was shown to mediate early microglia response and limits neuritic damage of Aβ since TREM2 knock-out mice were found to display accelerated Aβ accumulation, plaque pathology, and defects in microglia activity (59).

Interestingly, the here observed PA and PI lipids were found to be potent TREM2 ligands, which ties the present observations to previous data on TREM2's role in AD pathology as TREM2 lipid sensing was found to sustain the microglial response in AD (58).

The observed differences in plaque morphology associated anionic phospholipid and lysophospholipid distributions can therefore be attributed to highly complex mechanism, mediated through TREM2 signaling based microglial activation induced neuroinflammatory processes associated with Aβ plaque formation.

In summary, the here presented results highlight the potential of high resolution MALDI-IMS when combined with complementary chemical imaging methods such as fluorescent LCO imaging based differentiation of the structural amyloid aggregation state. This allowed to elucidate chemical differences in structurally heterogenic plaque populations in AD pathology. This approach revealed a clear involvement of phospholipids and ceramide metabolites in formation of morphologically heterogeneous plaque morphology. Such morphological difference in plaque pathology as outlined by differential lipid distribution, emphasize the need for chemical analysis of Aβ pathology in order to shine further light on neurotoxic plaque formation and maturation that are critical to AD pathogenesis.

Experimental Procedures

Chemicals and Reagents

All chemicals for matrix and solvent preparation were pro-analysis grade and obtained from Sigma-Aldrich/Merck (St. Louis, MO), unless otherwise specified. TissueTek optimal cutting temperature (OCT) compound was purchased from Sakura Finetek (AJ Alphen aan den Rijn, The Netherlands).

Tissue Preparation

18 months old tgAPP$_{Swe}$ mice (n=3, 3 male), were investigated (DNr #C17⁄ 14 at Uppsala University). The brains were dissected (<3min post-mortem) and snap frozen. Frozen tissue sections (12μm) were thaw mounted onto indium thin oxide (ITO) coated, conductive glass slides. 1,5-diamino naphthalene (1,5-DAN) MALDI matrix was deposited via sublimation as described elsewhere. (6,12)

MALDI-Imaging

IMS was performed on an UltrafleXtreme MALDI TOF/TOF (Bruker Daltonics, Bremen, Germany) as described previously. (12) Briefly, a mass range of 300−2000 Da was analyzed in negative ion mode at 10 μm (n=1) and 30 μm (n=2) spatial resolution. MALDI- MS/MS (LIFT) was performed directly in situ (Supporting Figure S-8) (6,12) and lipids were identified by database listed fragment ions (www.lipidmaps.org).

Immunohistochemistry

Following MALDI analysis, sections were fixed in EtOH and rehydrated in PBS, and simultaneously stained with q- and h-FTAA (3 μM, 1.5μM). Stained sections were washed in PBS, milliQ water, and finally dried. For antibody labeling, 6E10 diluted (1:500) in dilution buffer was incubated overnight at 4°C, and secondary antibody (Alexa Fluoro 647) was used for visualization. Hyperspectral imaging was performed using LSM 710 NLO laser-scanning microscope equipped with a 34-channel QUASAR detector (Zeiss, Göttingen, Germany). The images were processed with Zen 2011 (Zeiss) and ImageJ. The 500nm/540nm emission ratio was used for correlation analysis as described before (16), in-house developed macro for ImageJ was used for assignment on plaque population.

Data Processing and Analysis

All MALDI were calibrated externally using batch processing. Image analysis of the IMS data was performed in SciLS (v2014, Bruker). LCO images were aligned with the imaging data followed by Principal component analysis (PCA) and hierarchical clustering based spatial segmentation (bisecting k-means) to identify characteristic lipid distributions and for region of interest (ROI) annotation based on the hyperspectral emission profiles assigned into to diffuse plaques, cored plaques, and centers of the cored plaques. ROI average spectra were exported, baseline subtracted followed by peak binning. Orthogonal Projection to Latent Structures by Partial Least Squares−Discriminant Analysis (OPLS-DA) was performed on the binned ROI data using SIMCA (v. 14.0, Umetrics, Umeå, Sweden) followed by univariate

comparisons between the groups using paired, two tailed t-test. ($p<0.05$).

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Author Contributions: W.M. and J.H. conceived and designed the study. I.K. performed all MALDI imaging and MALDI MS/MS experiments. W.M. performed all IHC, LCO and microscopy and IP-MS experiments. W.M. and J.H. analyzed and interpreted the data and W.M., I.K., S.N., L.G., K.P.R.N., P.H., K.B., H.Z. and J.H. discussed the data and wrote the manuscript.

References

1. Scheltens, P., Blennow, K., Breteler, M. M., de Strooper, B., Frisoni, G. B., Salloway, S., and Van der Flier, W. M. (2016) Alzheimer's disease. *Lancet (London, England)* **388**, 505-517
2. Masters, C. L., Bateman, R., Blennow, K., Rowe, C. C., Sperling, R. A., and Cummings, J. L. (2015) Alzheimer's disease. *Nature reviews. Disease primers* **1**, 15056
3. Scheidt, H. A., Morgado, I., Rothemund, S., Huster, D., and Fandrich, M. (2011) Solid-state NMR spectroscopic investigation of Abeta protofibrils: implication of a beta-sheet remodeling upon maturation into terminal amyloid fibrils. *Angew Chem Int Ed Engl* **50**, 2837-2840
4. Haass, C., and Selkoe, D. J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* **8**, 101-112
5. Han, X. (2007) Potential mechanisms contributing to sulfatide depletion at the earliest clinically recognizable stage of Alzheimer's disease: a tale of shotgun lipidomics. *Journal of neurochemistry* **103 Suppl 1**, 171-179
6. Kaya, I., Brinet, D., Michno, W., Syvanen, S., Sehlin, D., Zetterberg, H., Blennow, K., and Hanrieder, J. (2017) Delineating Amyloid Plaque Associated Neuronal Sphingolipids in Transgenic Alzheimer's Disease Mice (tgArcSwe) Using MALDI Imaging Mass Spectrometry. *ACS chemical neuroscience*
7. Di Paolo, G., and Kim, T.-W. (2011) Linking Lipids to Alzheimer's Disease: Cholesterol and Beyond. *Nat. Rev. Neurosci.* **12**, 284-296
8. Liu, C.-C., Kanekiyo, T., Xu, H., and Bu, G. (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106-118
9. Hanrieder, J., Malmberg, P., Lindberg, O. R., Fletcher, J. S., and Ewing, A. G. (2013) Time-of-flight secondary ion mass spectrometry based molecular histology of human spinal cord tissue and motor neurons. *Analytical chemistry* **85**, 8741-8748
10. McDonnell, L. A., and Heeren, R. M. A. (2007) Imaging mass spectrometry. *Mass Spectrometry Reviews* **26**, 606-643
11. Hong, J. H., Kang, J. W., Kim, D. K., Baik, S. H., Kim, K. H., Shanta, S. R., Jung, J. H., Mook-Jung, I., and Kim, K. P. (2016) Global changes of phospholipids identified by MALDI imaging mass spectrometry in a mouse model of Alzheimer's disease. *Journal of lipid research* **57**, 36-45
12. Kaya, I., Michno, W., Brinet, D., Iacone, Y., Zanni, G., Blennow, K., Zetterberg, H., and Hanrieder, J. (2017) Histology-Compatible MALDI Mass Spectrometry Based Imaging of Neuronal Lipids for Subsequent Immunofluorescent Staining. *Analytical chemistry*

13. Carlred, L., Michno, W., Kaya, I., Sjovall, P., Syvanen, S., and Hanrieder, J. (2016) Probing amyloid-beta pathology in transgenic Alzheimer's disease (tgArcSwe) mice using MALDI imaging mass spectrometry. *Journal of neurochemistry* **138**, 469-478
14. Philipson, O., Lord, A., Gumucio, A., O'Callaghan, P., Lannfelt, L., and Nilsson, L. N. (2010) Animal models of amyloid-beta-related pathologies in Alzheimer's disease. *FEBS J* **277**, 1389-1409
15. Nilsson, K. P. (2009) Small organic probes as amyloid specific ligands--past and recent molecular scaffolds. *FEBS Lett* **583**, 2593-2599
16. Nystrom, S., Psonka-Antonczyk, K. M., Ellingsen, P. G., Johansson, L. B., Reitan, N., Handrick, S., Prokop, S., Heppner, F. L., Wegenast-Braun, B. M., Jucker, M., Lindgren, M., Stokke, B. T., Hammarstrom, P., and Nilsson, K. P. (2013) Evidence for age-dependent in vivo conformational rearrangement within Abeta amyloid deposits. *ACS Chem Biol* **8**, 1128-1133
17. Nilsson, K. P., Aslund, A., Berg, I., Nystrom, S., Konradsson, P., Herland, A., Inganas, O., Stabo-Eeg, F., Lindgren, M., Westermark, G. T., Lannfelt, L., Nilsson, L. N., and Hammarstrom, P. (2007) Imaging distinct conformational states of amyloid-beta fibrils in Alzheimer's disease using novel luminescent probes. *ACS Chem Biol* **2**, 553-560
18. Rasmussen, J., Mahler, J., Beschorner, N., Kaeser, S. A., Hasler, L. M., Baumann, F., Nystrom, S., Portelius, E., Blennow, K., Lashley, T., Fox, N. C., Sepulveda-Falla, D., Glatzel, M., Oblak, A. L., Ghetti, B., Nilsson, K. P. R., Hammarstrom, P., Staufenbiel, M., Walker, L. C., and Jucker, M. (2017) Amyloid polymorphisms constitute distinct clouds of conformational variants in different etiological subtypes of Alzheimer's disease. *Proc Natl Acad Sci U S A* **114**, 13018-13023
19. Klingstedt, T., Blechschmidt, C., Nogalska, A., Prokop, S., Haggqvist, B., Danielsson, O., Engel, W. K., Askanas, V., Heppner, F. L., and Nilsson, K. P. (2013) Luminescent conjugated oligothiophenes for sensitive fluorescent assignment of protein inclusion bodies. *Chembiochem* **14**, 607-616
20. Ellingsen, P. G., Nystrom, S., Reitan, N. K., and Lindgren, M. (2013) Spectral correlation analysis of amyloid beta plaque inhomogeneity from double staining experiments. *J Biomed Opt* **18**, 101313
21. Yanagisawa, K. (2007) Role of gangliosides in Alzheimer's disease. *Biochimica et biophysica acta* **1768**, 1943-1951
22. Barrier, L., Ingrand, S., Damjanac, M., Rioux Bilan, A., Hugon, J., and Page, G. (2007) Genotype-related changes of ganglioside composition in brain regions of transgenic mouse models of Alzheimer's disease. *Neurobiology of aging* **28**, 1863-1872
23. Chan, R. B., Oliveira, T. G., Cortes, E. P., Honig, L. S., Duff, K. E., Small, S. A., Wenk, M. R., Shui, G., and Di Paolo, G. (2012) Comparative Lipidomic Analysis of Mouse and Human Brain with Alzheimer Disease. *Journal of Biological Chemistry* **287**, 2678-2688
24. Pernber, Z., Blennow, K., Bogdanovic, N., Mansson, J. E., and Blomqvist, M. (2012) Altered distribution of the gangliosides GM1 and GM2 in Alzheimer's disease. *Dementia and geriatric cognitive disorders* **33**, 174-188
25. Dufresne, M., Guneysu, D., Patterson, N. H., Marcinkiewicz, M. M., Regina, A., Demeule, M., and Chaurand, P. (2017) Multimodal detection of GM2 and GM3 lipid species in the brain of mucopolysaccharidosis type II mouse by serial imaging mass spectrometry and immunohistochemistry. *Analytical and bioanalytical chemistry* **409**, 1425-1433
26. Ginsberg, S. D., Alldred, M. J., Counts, S. E., Cataldo, A. M., Neve, R. L., Jiang, Y., Wuu, J., Chao, M. V., Mufson, E. J., Nixon, R. A., and Che, S. (2010) Microarray analysis of hippocampal CA1 neurons implicates early endosomal dysfunction during Alzheimer's disease progression. *Biological psychiatry* **68**, 885-893
27. Naj, A. C., Jun, G., Beecham, G. W., Wang, L. S., Vardarajan, B. N., Buros, J., Gallins, P. J., Buxbaum, J. D., Jarvik, G. P., Crane, P. K., Larson, E. B., Bird, T. D., Boeve, B. F., Graff-Radford, N. R., De Jager, P. L., Evans, D., Schneider, J. A., Carrasquillo, M. M., Ertekin-Taner, N., Younkin, S. G., Cruchaga, C., Kauwe, J. S., Nowotny, P., Kramer, P., Hardy, J., Huentelman, M. J., Myers, A. J., Barmada, M. M., Demirci, F. Y., Baldwin, C. T., Green, R. C., Rogaeva, E., St George-Hyslop, P., Arnold, S. E., Barber, R., Beach, T., Bigio, E. H., Bowen, J. D., Boxer, A., Burke, J. R., Cairns, N. J., Carlson, C. S., Carney, R. M., Carroll, S. L., Chui, H. C., Clark, D. G., Corneveaux, J., Cotman, C. W., Cummings, J. L., DeCarli, C., DeKosky, S. T., Diaz-Arrastia, R., Dick, M., Dickson, D. W., Ellis, W.

G., Faber, K. M., Fallon, K. B., Farlow, M. R., Ferris, S., Frosch, M. P., Galasko, D. R., Ganguli, M., Gearing, M., Geschwind, D. H., Ghetti, B., Gilbert, J. R., Gilman, S., Giordani, B., Glass, J. D., Growdon, J. H., Hamilton, R. L., Harrell, L. E., Head, E., Honig, L. S., Hulette, C. M., Hyman, B. T., Jicha, G. A., Jin, L. W., Johnson, N., Karlawish, J., Karydas, A., Kaye, J. A., Kim, R., Koo, E. H., Kowall, N. W., Lah, J. J., Levey, A. I., Lieberman, A. P., Lopez, O. L., Mack, W. J., Marson, D. C., Martiniuk, F., Mash, D. C., Masliah, E., McCormick, W. C., McCurry, S. M., McDavid, A. N., McKee, A. C., Mesulam, M., Miller, B. L., Miller, C. A., Miller, J. W., Parisi, J. E., Perl, D. P., Peskind, E., Petersen, R. C., Poon, W. W., Quinn, J. F., Rajbhandary, R. A., Raskind, M., Reisberg, B., Ringman, J. M., Roberson, E. D., Rosenberg, R. N., Sano, M., Schneider, L. S., Seeley, W., Shelanski, M. L., Slifer, M. A., Smith, C. D., Sonnen, J. A., Spina, S., Stern, R. A., Tanzi, R. E., Trojanowski, J. Q., Troncoso, J. C., Van Deerlin, V. M., Vinters, H. V., Vonsattel, J. P., Weintraub, S., Welsh-Bohmer, K. A., Williamson, J., Woltjer, R. L., Cantwell, L. B., Dombroski, B. A., Beekly, D., Lunetta, K. L., Martin, E. R., Kamboh, M. I., Saykin, A. J., Reiman, E. M., Bennett, D. A., Morris, J. C., Montine, T. J., Goate, A. M., Blacker, D., Tsuang, D. W., Hakonarson, H., Kukull, W. A., Foroud, T. M., Haines, J. L., Mayeux, R., Pericak-Vance, M. A., Farrer, L. A., and Schellenberg, G. D. (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nature genetics* **43**, 436-441

28. Hirahara, Y., Wakabayashi, T., Mori, T., Koike, T., Yao, I., Tsuda, M., Honke, K., Gotoh, H., Ono, K., and Yamada, H. (2016) Sulfatide species with various fatty acid chains in oligodendrocytes at different developmental stages determined by imaging mass spectrometry. *Journal of neurochemistry*
29. Portelius, E., Bogdanovic, N., Gustavsson, M. K., Volkmann, I., Brinkmalm, G., Zetterberg, H., Winblad, B., and Blennow, K. (2010) Mass spectrometric characterization of brain amyloid beta isoform signatures in familial and sporadic Alzheimer's disease. *Acta neuropathologica* **120**, 185-193
30. Huang, T. H., Yang, D. S., Fraser, P. E., and Chakrabartty, A. (2000) Alternate aggregation pathways of the Alzheimer beta-amyloid peptide. An in vitro model of preamyloid. *J Biol Chem* **275**, 36436-36440
31. Jiang, D., Rauda, I., Han, S., Chen, S., and Zhou, F. (2012) Aggregation pathways of the amyloid beta(1-42) peptide depend on its colloidal stability and ordered beta-sheet stacking. *Langmuir* **28**, 12711-12721
32. Kuo, Y. M., Beach, T. G., Sue, L. I., Scott, S., Layne, K. J., Kokjohn, T. A., Kalback, W. M., Luehrs, D. C., Vishnivetskaya, T. A., Abramowski, D., Sturchler-Pierrat, C., Staufenbiel, M., Weller, R. O., and Roher, A. E. (2001) The evolution of A beta peptide burden in the APP23 transgenic mice: implications for A beta deposition in Alzheimer disease. *Mol Med* **7**, 609-618
33. Philipson, O., Hammarstrom, P., Nilsson, K. P., Portelius, E., Olofsson, T., Ingelsson, M., Hyman, B. T., Blennow, K., Lannfelt, L., Kalimo, H., and Nilsson, L. N. (2009) A highly insoluble state of Abeta similar to that of Alzheimer's disease brain is found in Arctic APP transgenic mice. *Neurobiology of aging* **30**, 1393-1405
34. Kawarabayashi, T., Younkin, L. H., Saido, T. C., Shoji, M., Ashe, K. H., and Younkin, S. G. (2001) Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J Neurosci* **21**, 372-381
35. Iwatsubo, T., Saido, T. C., Mann, D. M., Lee, V. M., and Trojanowski, J. Q. (1996) Full-length amyloid-beta (1-42(43)) and amino-terminally modified and truncated amyloid-beta 42(43) deposit in diffuse plaques. *Am J Pathol* **149**, 1823-1830
36. Ahmed, M., Davis, J., Aucoin, D., Sato, T., Ahuja, S., Aimoto, S., Elliott, J. I., Van Nostrand, W. E., and Smith, S. O. (2010) Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nature structural & molecular biology* **17**, 561-567
37. Cheng, H., Zhou, Y., Holtzman, D. M., and Han, X. (2010) Apolipoprotein E mediates sulfatide depletion in animal models of Alzheimer's disease. *Neurobiology of aging* **31**, 1188-1196
38. Zeng, Y., and Han, X. (2008) Sulfatides facilitate apolipoprotein E-mediated amyloid-beta peptide clearance through an endocytotic pathway. *Journal of neurochemistry* **106**, 1275-1286
39. Han, X., D, M. H., McKeel, D. W., Jr., Kelley, J., and Morris, J. C. (2002) Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *Journal of neurochemistry* **82**, 809-818

40. Cutler, R. G., Kelly, J., Storie, K., Pedersen, W. A., Tammara, A., Hatanpaa, K., Troncoso, J. C., and Mattson, M. P. (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 2070-2075
41. Filippov, V., Song, M. A., Zhang, K., Vinters, H. V., Tung, S., Kirsch, W. M., Yang, J., and Duerksen-Hughes, P. J. (2012) Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *Journal of Alzheimer's disease : JAD* **29**, 537-547
42. Katsel, P., Li, C., and Haroutunian, V. (2007) Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: a shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? *Neurochemical research* **32**, 845-856
43. Lee, J. K., Jin, H. K., Park, M. H., Kim, B. R., Lee, P. H., Nakauchi, H., Carter, J. E., He, X., Schuchman, E. H., and Bae, J. S. (2014) Acid sphingomyelinase modulates the autophagic process by controlling lysosomal biogenesis in Alzheimer's disease. *The Journal of experimental medicine* **211**, 1551-1570
44. Tafesse, F. G., Vacaru, A. M., Bosma, E. F., Hermansson, M., Jain, A., Hilderink, A., Somerharju, P., and Holthuis, J. C. (2014) Sphingomyelin synthase-related protein SMSr is a suppressor of ceramide-induced mitochondrial apoptosis. *Journal of cell science* **127**, 445-454
45. Ding, T., Kabir, I., Li, Y., Lou, C., Yazdanyar, A., Xu, J., Dong, J., Zhou, H., Park, T., Boutjdir, M., Li, Z., and Jiang, X. C. (2015) All members in the sphingomyelin synthase gene family have ceramide phosphoethanolamine synthase activity. *Journal of lipid research* **56**, 537-545
46. Bickert, A., Ginkel, C., Kol, M., vom Dorp, K., Jastrow, H., Degen, J., Jacobs, R. L., Vance, D. E., Winterhager, E., Jiang, X. C., Dormann, P., Somerharju, P., Holthuis, J. C., and Willecke, K. (2015) Functional characterization of enzymes catalyzing ceramide phosphoethanolamine biosynthesis in mice. *Journal of lipid research* **56**, 821-835
47. Vacaru, A. M., Tafesse, F. G., Ternes, P., Kondylis, V., Hermansson, M., Brouwers, J. F., Somerharju, P., Rabouille, C., and Holthuis, J. C. (2009) Sphingomyelin synthase-related protein SMSr controls ceramide homeostasis in the ER. *The Journal of cell biology* **185**, 1013-1027
48. Chalfant, C. E., and Spiegel, S. (2005) Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. *Journal of cell science* **118**, 4605-4612
49. Xu, D., Omura, T., Masaki, N., Arima, H., Banno, T., Okamoto, A., Hanada, M., Takei, S., Matsushita, S., Sugiyama, E., Setou, M., and Matsuyama, Y. (2016) Increased arachidonic acid-containing phosphatidylcholine is associated with reactive microglia and astrocytes in the spinal cord after peripheral nerve injury. *Scientific reports* **6**, 26427
50. Moses, G. S., Jensen, M. D., Lue, L. F., Walker, D. G., Sun, A. Y., Simonyi, A., and Sun, G. Y. (2006) Secretory PLA2-IIA: a new inflammatory factor for Alzheimer's disease. *Journal of neuroinflammation* **3**, 28
51. Sundaram, J. R., Chan, E. S., Poore, C. P., Pareek, T. K., Cheong, W. F., Shui, G., Tang, N., Low, C. M., Wenk, M. R., and Kesavapany, S. (2012) Cdk5/p25-induced cytosolic PLA2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration. *J Neurosci* **32**, 1020-1034
52. Di Paolo, G., and De Camilli, P. (2006) Phosphoinositides in cell regulation and membrane dynamics. *Nature* **443**, 651-657
53. Berman, D. E., Dall'Armi, C., Voronov, S. V., McIntire, L. B., Zhang, H., Moore, A. Z., Staniszewski, A., Arancio, O., Kim, T. W., and Di Paolo, G. (2008) Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. *Nature neuroscience* **11**, 547-554
54. Arancio, O. (2008) PIP2: a new key player in Alzheimer's disease. *Cellscience* **5**, 44-47
55. Mapstone, M., Cheema, A. K., Fiandaca, M. S., Zhong, X., Mhyre, T. R., MacArthur, L. H., Hall, W. J., Fisher, S. G., Peterson, D. R., Haley, J. M., Nazar, M. D., Rich, S. A., Berlau, D. J., Peltz, C. B., Tan, M. T., Kawas, C. H., and Federoff, H. J. (2014) Plasma phospholipids identify antecedent memory impairment in older adults. *Nature medicine* **20**, 415-418

56. Blennow, K., Mattsson, N., Scholl, M., Hansson, O., and Zetterberg, H. (2015) Amyloid biomarkers in Alzheimer's disease. *Trends in pharmacological sciences* **36**, 297-309
57. Rosenthal, S. L., and Kamboh, M. I. (2014) Late-Onset Alzheimer's Disease Genes and the Potentially Implicated Pathways. *Current genetic medicine reports* **2**, 85-101
58. Wang, Y., Cella, M., Mallinson, K., Ulrich, J. D., Young, K. L., Robinette, M. L., Gilfillan, S., Krishnan, G. M., Sudhakar, S., Zinselmeyer, B. H., Holtzman, D. M., Cirrito, J. R., and Colonna, M. (2015) TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell* **160**, 1061-1071
59. Wang, Y., Ulland, T. K., Ulrich, J. D., Song, W., Tzaferis, J. A., Hole, J. T., Yuan, P., Mahan, T. E., Shi, Y., Gilfillan, S., Cella, M., Grutzendler, J., DeMattos, R. B., Cirrito, J. R., Holtzman, D. M., and Colonna, M. (2016) TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *J. Exp. Med.* **213**, 667-675

FOOTNOTES

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The abbreviations used are: AD, Alzheimer’s disease; APP, amyloid precursor protein; Cer, ceramides; CerP, ceramide phosphate; IMS, imaging mass spectrometry; LCO, luminescent conjugated oligothiophenes; MS, mass spectrometry; MALDI, matrix assisted laser desorption/ionization (MALDI).

This article contains supplemental Figs S1-S8 and STAR methods.

Capsule

Background: Lipid dyshomeostasis has been linked to amyloid polymorphism and Alzheimer disease (AD) pathology.
Results: Hyperspectral imaging of tgAPPSwe mice revealed plaque morphology-specific changes in multiple bioactive lipids, some of which are phenocopied in AD mouse models.
Conclusion: Lipid anomalies observed in Abeta plaque pathology may be linked to AD pathogenesis, including endolysosomal dysfunction, microglial activation and neuroinflammation.
Significance: This study highlights the hypothesis-generating potential of multimodal hyperspectral amyloid and MALDI and its applicability to other diseases.