

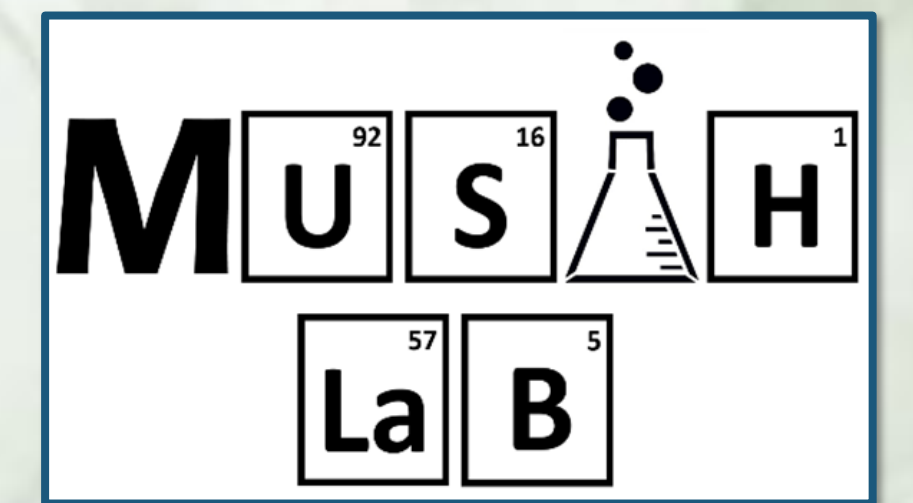
# Let Me Talk Your Ears Off: Determination of Chemical Markers

## of Ménière's Disease in Earwax

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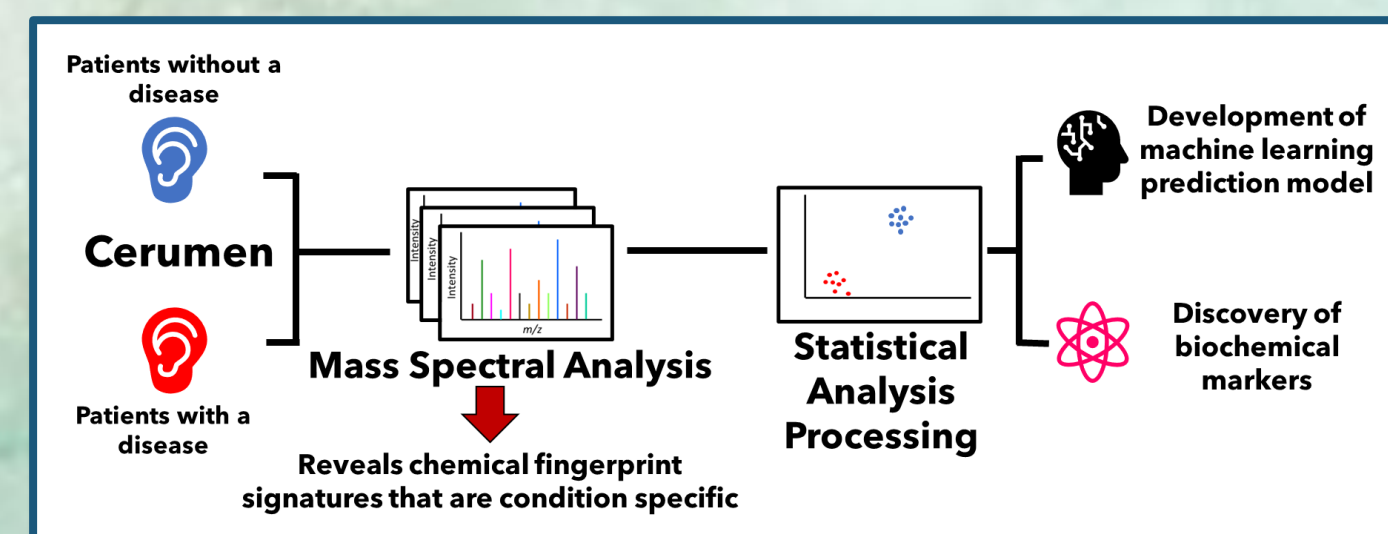
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An increasing trend in the field of medical diagnostics is to utilize non-traditional biological matrices that can be collected in a minimally invasive manner as reporters of disease. These can include tears, sweat, fingernails and cerumen, otherwise known as earwax. Earwax is a highly lipid-rich matrix that can potentially provide information on diseases associated with lipid dysregulation. One disorder that is being investigated is Ménière's disease. It is a disease of the inner ear that results in extreme vertigo and eventual hearing loss. The current approach to disease detection involves eliminating other potential disorders through "diagnosis by exclusion". As a result, its presence is extremely time consuming to determine, and thus a rapid and accurate method of diagnosis would be of high benefit. Earwax was collected at a clinic from confirmed Ménière's disease and non-Ménière's disease donors. The individual plugs were suspended in ethyl acetate and the mass spectrometric technique, direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) was used to acquire their chemical profiles. The acquired mass spectra were then subjected to multivariate statistical analysis to ascertain the chemical differences between the two groups. Earwax is reported to contain hundreds of compounds and thus the DART-MS-derived data were screened against a compiled list of the known earwax constituents contained in the literature. A heatmap rendering of the mass spectral data for the subset of masses that matched those of earwax constituents reported in the literature was created, and analysis of variance was performed to reveal which masses were within a 95% confidence limit. A total of 100 masses were found to be important for differentiating the Ménière's disease and non-Ménière's disease samples. When inspecting the bar graph renderings of these masses, it was concluded that a subset of fifteen were presence/absence markers that were only found in one of the two groups including cholesteryl stearate and a cholesterol derivative with the formula  $C_{28}H_{48}O$  that we detected in previous studies. A partial least squares (PLS) classification model built on the basis of these markers exhibited an 83.08% leave-one-out cross validation, and an external validation prediction accuracy of 85% when screening the cerumen of unknowns. The results revealed 15 potential compounds that could serve as reporters for Ménière's disease. This work demonstrates that: (1) earwax can serve as a viable and readily accessible matrix that can be analyzed to reveal a disease state; and (2) a rapid and accurate Ménière's disease diagnosis approach could be developed through interrogation of earwax using ambient ionization mass spectrometry.

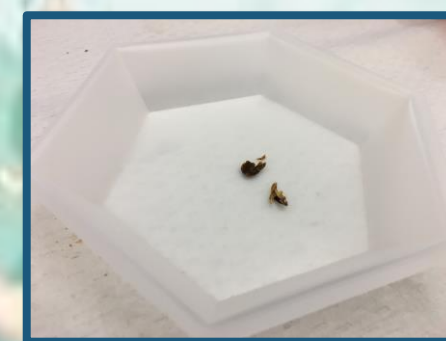
### INTRODUCTION

- Successful disease diagnosis often relies on the detection of changes in a biochemical profile.
- Earwax (cerumen) is a readily accessible biological matrix that contains some of the highest levels and greatest diversity of surface accessible excreted lipids.
- Ménière's disease is a disorder of the inner ear that causes vertigo and deafness.
- There is no known cause or cure of the disease and diagnosis is based on exclusionary criteria, and can be extremely time consuming to perform.
- By exploiting mass spectrometric techniques, disease-specific profiles, as well as diagnostic biomarkers can be discovered.



### EXPERIMENTAL

- Earwax extracted using ethyl acetate
- Unsaponified method to retain complete lipid profile
- 13 Non-Ménière's disease samples
- 13 Ménière's disease samples

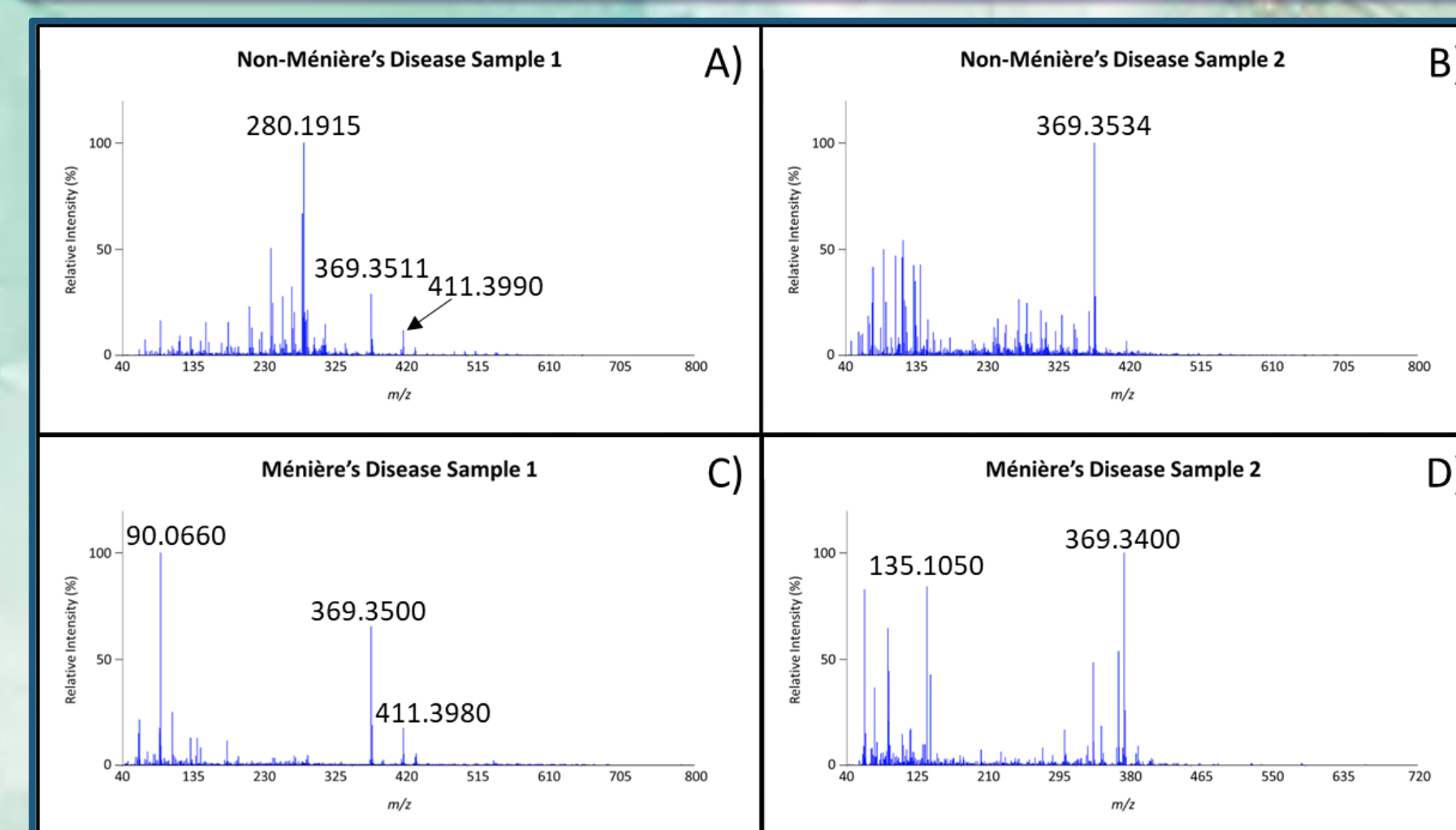


### DART-HRMS

- Soft ionization technique
- Open-air, ambient ionization method
- Direct analysis of samples
- Millimass unit accuracy
- Nanogram detection limit
- Rapid analysis
- Positive-ion mode
- 350 °C



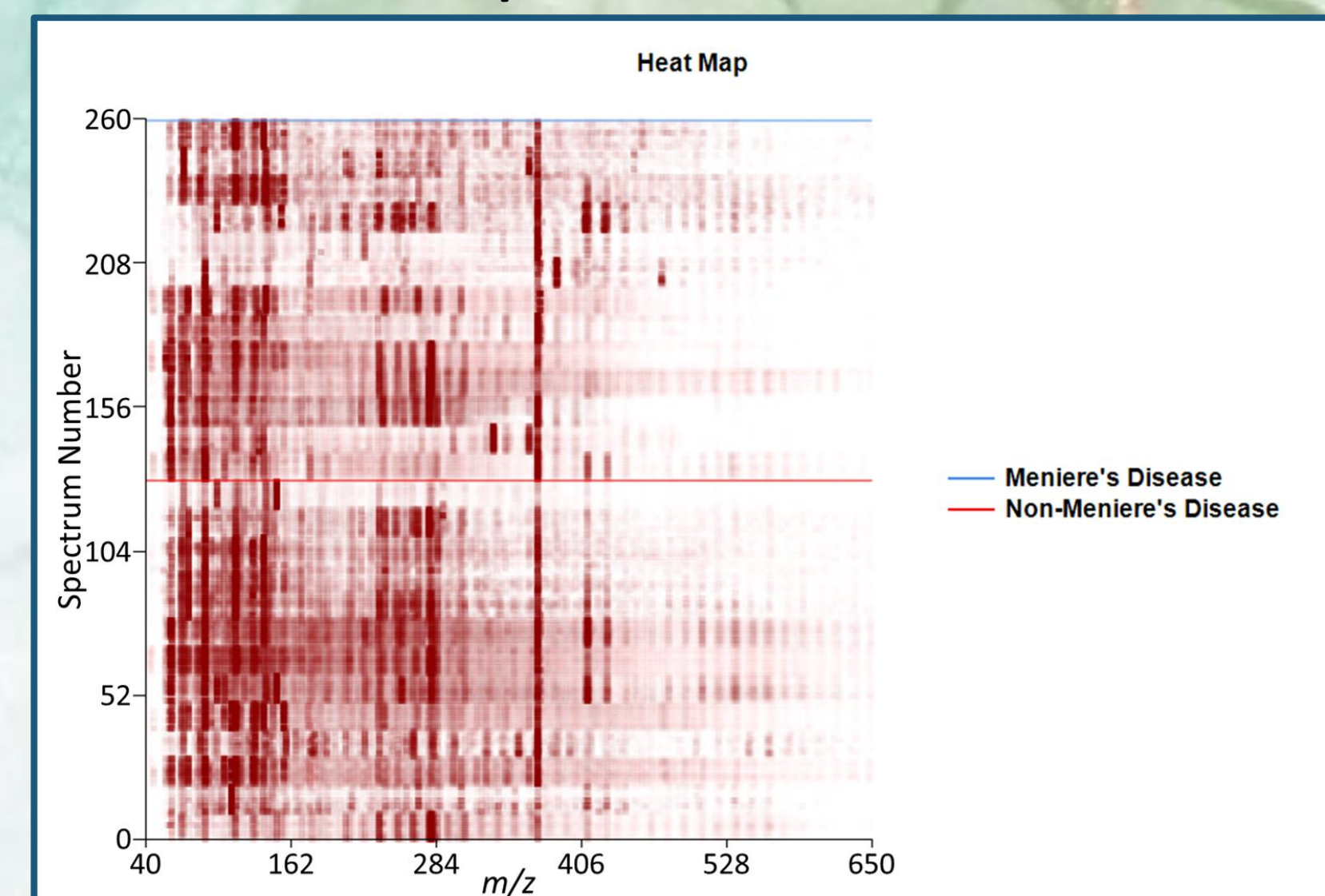
### RESULTS: DART-HRMS



Representative DART mass spectra of earwax from donors without (Panels A&B) and with Ménière's disease (Panels C&D). Even with the observed intra-class variation in mass spectral patterns, a general trend was that Ménière's disease samples exhibited lower levels of detected molecules.

### RESULTS: HEAT MAP

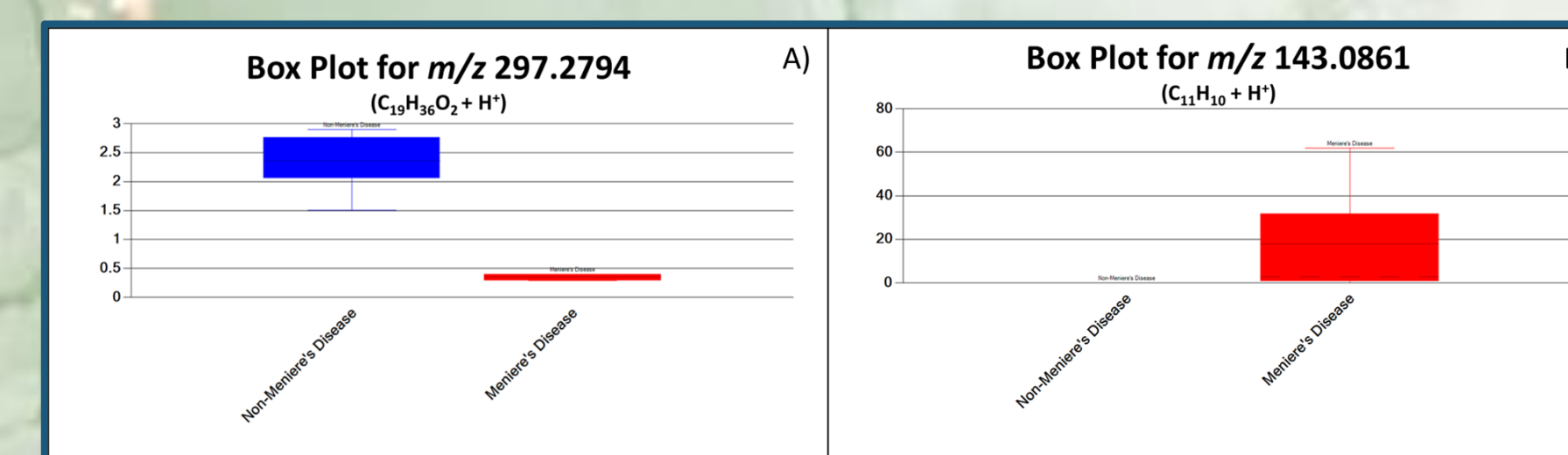
- 551 Potential compounds in earwax
  - Alkanes, alkenes, fatty acids, ester, triglycerides, cholesterol derivatives, amino acids, etc.
- Published or suspected to be in this matrix



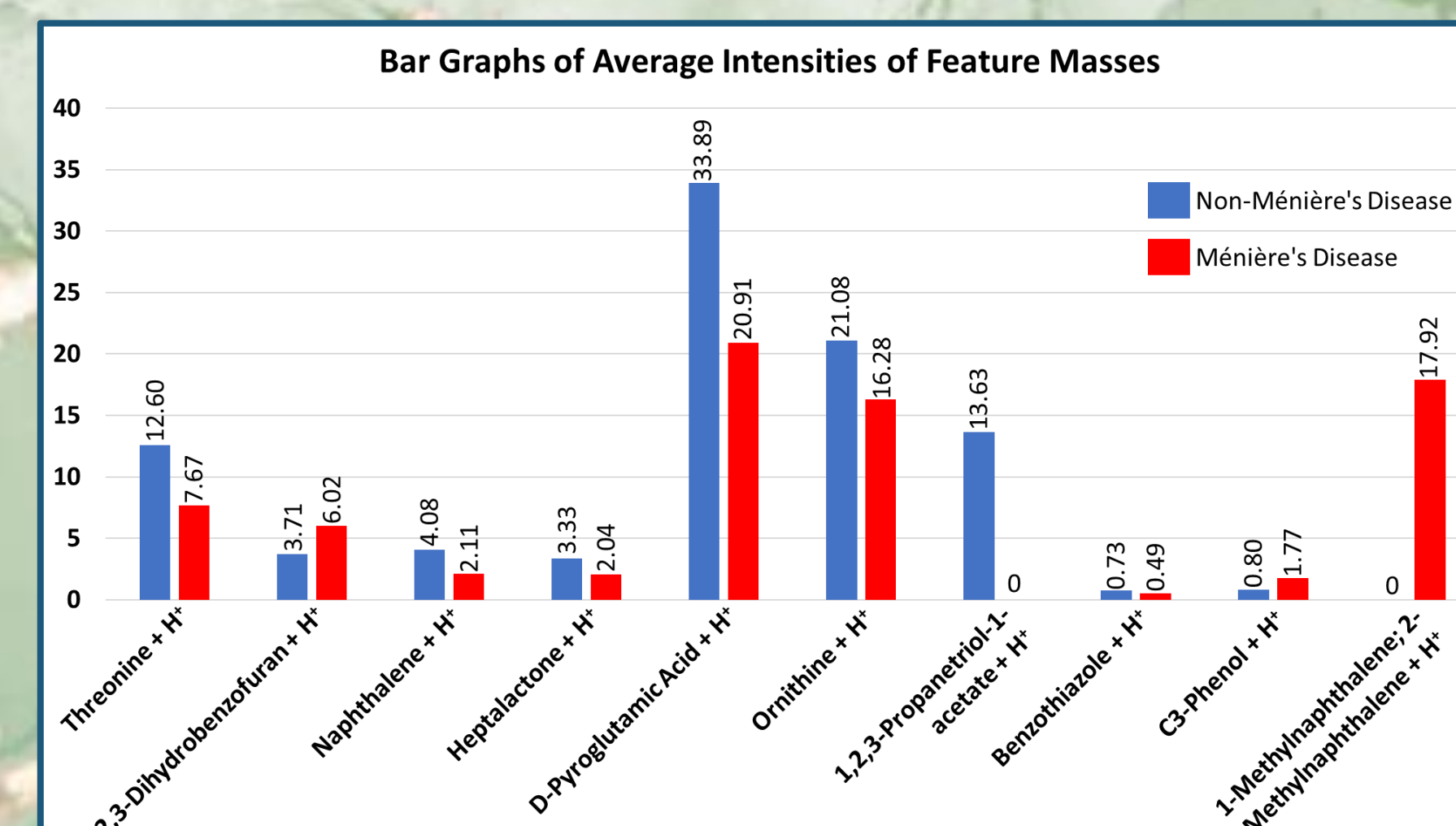
Heat map rendering of the DART-HRMS data. A dark red color indicates a high intensity for the specific  $m/z$  value.

### RESULTS: FEATURES OF IMPORTANCE

- From heatmap, analysis of variance performed to reveal which masses were within a 95% confidence limit.
  - Data was normalized
  - Tolerance: 10 mmu
  - Threshold: 0.01%
- 100 Feature masses were found to differentiate Ménière's disease and non-Ménière's disease samples.



Representative box plot rendering for an individual  $m/z$  value. Panel A shows that  $m/z$  297 has a higher intensity in non-Ménière's disease samples compared to Ménière's disease samples. Panel B shows that  $m/z$  143 is present in Ménière's disease samples but absent in non-Ménière's disease samples.



Bar graph rendering comparing the intensities of a subset of the 100 features that were found to be characteristic for differentiating earwax from patients with and without Ménière's disease. Some intensities were comparable while others showed distinct intensity differences.

### RESULTS: CHEMICAL MARKERS

Protonated Monoisotopic Mass	Formula	Tentative Identity	Class Marker
135.0657	$C_{10}H_{20}O_4$	1,2,3-Propanetriol 1-acetate	Non-Ménière's Disease
143.0861	$C_{11}H_{20}$	1-Methylnaphthalene; 2-Methylnaphthalene	Ménière's Disease
185.2269	$C_{13}H_{28}$	Tridecane	Ménière's Disease
195.1134	$C_{16}H_{24}N_2O_2$	Dipyrrrolypyrazine dione octahydro; Dihydromethylphenanthrene; Methylphenylethyl benzene	Ménière's Disease
211.2426	$C_{15}H_{30}$	1-Pentadecene	Ménière's Disease
213.1855	$C_{17}H_{32}O_2$	6-Tridecenoic acid	Non-Ménière's Disease
214.2171	$C_{13}H_{27}NO$	N-Methyl-dodecanamide	Ménière's Disease
267.3052	$C_{19}H_{38}$	Nonadecane	Ménière's Disease
311.1800	$C_{21}H_{42}$	Methylphenyl ethylbenzene	Non-Ménière's Disease
361.3107	$C_{24}H_{46}O_2$	5 $\beta$ -Cholanic acid	Ménière's Disease
401.3783	$C_{23}H_{46}O$	Unknown Cholesterol derivative	Non-Ménière's Disease
479.5192	$C_{26}H_{48}O$	Tritricontanone	Ménière's Disease
653.6237	$C_{28}H_{48}O_2$	Cholesteryl stearate	Non-Ménière's Disease
757.6346	$C_{28}H_{48}O_2$	45:4 Triglyceride	Non-Ménière's Disease
775.6816	$C_{28}H_{48}O_2$	46:2 Triglyceride	Non-Ménière's Disease

Potential presence/absence markers derived from the bar graph renderings of the feature masses.

### RESULTS: PLS ANALYSIS

- Performed partial least squares (PLS) analysis the using 100 feature masses of importance
- Leave-one-out cross validation (LOOCV): 83.08%
- Screened external validation samples
  - 1 Tentative Ménière's disease sample
  - 1 Confirmed Ménière's disease sample
  - 5 Non-Ménière's disease samples
  - 85% External validation prediction accuracy

### CONCLUSIONS

- It was revealed 15 potential compounds could serve as present/absent chemical markers for Ménière's disease.
- This suggests that earwax can serve as a viable and readily accessible matrix that when analyzed by mass spectrometry could provide a rapid and accurate diagnostic approach for Ménière's disease.

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