Rapid Identification of New Biomarkers for the Classification of GM1 and GM2 Gangliosidoses: A Coupled ^{1}H NMR and LC/MS-Linked Metabolomics Strategy

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LAY SUMMARY

Both GM1 and GM2 gangliosidoses are debilitating diseases without any available cures or specific treatments. However, the use of some aggressive clinical management approaches has succeeded in enhancing the lifespan and quality of life for patients afflicted by these disorders. Unfortunately, to date the diagnoses of these conditions is very complicated, since they rely on the time-consuming evaluation of a whole series of comprehensive observations, many of which require laboratory tests to be performed on a range of samples collected from these patients. Moreover, such diagnoses may be compromised by a partial absence or lack of specimens, which may result in a significant number of cases remaining undiagnosed. Therefore, there is a major requirement for a more disease-specific and rapid strategies for the diagnosis and severity monitoring of these debilitating conditions.

In this pilot investigation we employed 'state-of-the-art' analytical techniques with the ability to simultaneously detect and monitor hundreds of molecules present in body fluid specimens collected from humans, in order to seek and identify those which serve as reliable indicators of disease activity and progression in patients with gangliosidoses, specifically GM1 (class II) and GM2 (late-onset Tay-Sachs) diseases. These molecules are known as 'biomarkers', and one of the major techniques employed in this study, nuclear magnetic resonance (NMR) analysis, takes advantage of the differing magnetic properties and locations of particular atoms in molecules when such body fluids are placed within a very powerful magnetic field. From the analytical results acquired, we then used sophisticated computer-based statistical methodologies in order to identify and recognize biomarkers present in such body fluids by comparing the amounts of these present in GM1 and GM2 diseases to those in healthy, age-matched human controls (this is known as *metabolomics analysis*). To the best of our knowledge, this is the very first NMR-based metabolomics investigation of these gangliosidosis conditions. These studies were supported by the application of another multi-analytical strategy known as liquid chromatography-mass spectrometry (LC/MS), a technique which involves the prior separation of a large number of biological molecules.

This research project analysed urine, blood and cerebrospinal fluid (CSF) samples collected from the above groups of gangliosidosis patients, and also from age-matched healthy control participants. In total, more than 120 body fluid samples were analysed, and the unique analytical ability of the NMR technique utilised allowed us to detect and monitor up to 110 molecules simultaneously in each body fluid collected. Indeed, the exact quantities of each of 100, 50 and 30 molecules in urine, blood plasma and CSF samples, respectively, were measured, i.e. a grand total of almost 8,000 biochemical analysis measurements were made. Moreover, each blood plasma sample was analysed under two different experimental conditions, so this increased the number of measurements performed by approximately another 2,000. Additionally, we are currently exploring the blood plasma compositions of a further 37 GM2 patients and 30 age-matched healthy controls, so that will boost this investigation with a further 3,350 measurements.

The first phase of the investigation was focused on an exploration of the molecular profiles of blood plasma molecules in patients with GM1 (class II) disease, and was this successful in detecting unusual 'patterns' of these in such patients, specifically higher levels of key amino acids. Indeed, distinctions between these two classes of body fluids were very clear and highly statistically significant. The provision of valuable diagnostic information for this condition is rapid, with the analysis performed taking approximately 15 minutes per sample. This information has also allowed us to detect faults in the sequence of some chemical conversion processes occurring in the human body, and which are manifested in patients with this disease, but not in healthy humans. These observations will help us to detect suitable 'targets' for future drug development programmes.

However, for the study focused on late onset Tay-Sachs GM2 patient urine samples, this distinction was less clear, although we did find some significant changes in the amounts of a small number of biological molecules when compared to those of control samples collected from healthy individuals.

Overall, these results will be valuable for the clinical management and monitoring of patients with these disorders, and will also allow us to explore their responses to drug treatments. In the near future, this information will be used to enhance our understanding of these diseases, and will also help us to design new treatments (including combination therapies) for these devastating

conditions. Further experiments are currently underway, and our novel results will be submitted for publication in reputable scientific/clinical journals soon.