Horizon Scanning Series
The Future of Precision Medicine in Australia

Omics

This input paper was prepared by Professor David James and A/Professor Samantha Hocking (The University of Sydney)

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1. Current metabolomic and proteomic initiatives in Australia

Precision medicine is beginning to look beyond genomics for complex diseases. Proteomics and metabolomics are providing new paths to discovery and have clear application in the understanding, diagnosis and treatment of disease. Whilst next-generation sequencing allows for the analysis of genomes, including those embodying disease states, a major challenge is that most disorders are multifactorial. Systems approaches, including the analysis of proteins and metabolites, are required for a more in-depth understanding of these complex disease states. Unlike genomics, which is interpreted in a single dimension, proteomics in particular is extremely multifaceted, generating information representing protein modifications, which are amplified by their interconnectivity and activity in signaling networks, which can be highly divergent in space and time. Proteomics and metabolomics rely heavily on mass spectrometry (MS). Technological developments in MS instrumentation, sample preparation and computational analysis have recently matured to deliver discoveries across multiple areas of medical research. Moreover, there are many more technological developments on the horizon in MS and so there is huge potential in the proteomics and metabolomics arena.

Whilst genomics has potential in predictive and diagnostic medicine, influencing screening and therapeutic strategies, for many complex diseases, including but not limited to type 2 diabetes, cardiovascular disease and many types of cancer, the clinical value of the polymorphisms identified by large scale genome wide association studies (GWAS) and deep sequencing have been somewhat disappointing. In fact, for T2D it has been shown that incorporating SNP data adds no further value over and above traditional risk factors such as family history, fasting blood sugar and BMI[1]. In contrast, protein expression profiles, as measured from biopsied tissue and easily obtainable body fluids (such as blood plasma, blood exosomes, saliva and urine), yield a clear snapshot of the current health status of the individual, likely because this provides an integrated output of both genetic background and response to the environment. Applications of this technology exist across a broad range of disease types within and across disciplines, including oncology, neurology, cardiology, nephrology and infectious disease to name a few.

Thus, the future of precision medicine lies in <u>integration</u> of genomic analysis with proteomics and metabolomics, that <u>combined</u> will yield a much more accurate understanding of the links between genotype and phenotype. These systems approaches will provide better markers, effectors, and predictors of complex disease that are currently out of reach of any single analysis strategy used in isolation.

1.1. Metabolomics

Metabolomics is the study of small molecules, commonly known as metabolites, with cells, biofluids, tissues or organisms. All cells are constantly metabolising nutrients and other biosynthetic products to maintain homeostasis and support growth. The metabolome is conservatively thought to represent >100,000 organic molecules including lipids, sugars, amino acids and fatty acids. Through recent developments in chemistry and MS it is now feasible to quantitatively measure up to 4000 different products in a single drop of human blood and this technology is being developed at major centres throughout the world.

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In terms of metabolomics infrastructure, Australia is already well advanced. Investigators such as Prof Malcolm MacConville and Prof Peter Miekle in Melbourne and Dr John O'Sullivan at the Charles Perkins Centre in Sydney are well experienced in the application of metabolomics to clinical disease[2-6]. Prof Miekle has a wealth of experience performing lipidomic profiling in large human cohorts such as the FIELD study, and Dr O'Sullivan has recently returned from Harvard where he performed metabolomic profiling in numerous cohorts including the Framingham Heart Study, the Malmo Diet and Cancer Study, the Jackson Heart Study, and the Diabetes Prevention Program. Recently, the Western Australia Phenome Centre extends an international phenome centre network that links together metabolomic approaches to refining disease phenotypes and re-stratifying disease by incorporating metabolite data. Work is underway to link this phenome centre with other centres in Australia. There are now numerous examples of metabolomics informing disease phenotype (see below), which provide a blue-print for further interrogation of disease.

1.2. Proteomics

Proteomics refers to the use of MS to measure quantitatively numerous proteins or their modifications in biological specimens. In light of very recent technology developments this approach has undergone striking improvements and each year major vendors such as Thermo Scientific[TM] release instruments with vast gains in sensitivity and speed of acquiring biological data. This technology is evolving so rapidly that only major centres that are willing to commit to regular upgrades of their existing infrastructure are capable of staying at the forefront. At present, these gains in instrument performance have not yet reached a plateau and so we can expect even greater gains over the next decade. Thus, in terms of future expectations and promise of revolutionary breakthroughs, this technology is likely to be at the cutting edge.

In Australia, most universities have a mass spectrometry core facility. Centres of excellence in proteomic research include the Australian Proteomics Analysis Facility (APAF) which has received recurrent NCRIS funding to support the further development of proteomic analysis under the guidance of Professor Mark Molloy and ProCan led by Professors Phil Robinson and Roger Reddel. At APAF, proteomic analysis of body fluids, tissues or cells is well-established and proteomic analysis of human plasma is under development[7]. Professor Molloy is a member of the Global Human Proteome Project and has a particular research interest in cancer proteomics including the discovery of cancer biomarkers. ProCan has received funding from the Australian Cancer Research Foundation and aims to determine the proteome of biobanked human cancer tissues in order to better match treatments to disease. As yet, there has been no concentrated effort in developing proteomic methodologies for diseases other than cancer and no coordinated effort in establishing clinical human plasma proteomics. Globally, two centres of excellence lead the field in human plasma proteomics, Professor Ruedi Aebersold in Zurich and Professor Mattias Mann in Munich. Both centres have recently published landmark studies utilising novel methodologies to characterize the human plasma proteome [8,9]. The Mass Spectrometry Core Facility, Charles Perkins Centre, The University of Sydney is currently spearheading a project in human plasma proteomics, under the leadership of Drs Ben Parker, Mark Larence and Sean Humphrey, exploring novel methodologies in sample preparation and separation that can compensate for the dynamic range of proteins in human blood allowing Australian research centres access to non-targeted clinical plasma proteomics. Since 2015, The Proteomics research lab at the Walter and Eliza Hall Institute in Melbourne has made considerable progress in developing the capabilities for translational proteomics (now one of three core aims of the group – collaboration, development and clinical translation). Over the past two years the lab has initiated and undertaken a growing number of clinical translational projects, including childhood leukaemia's, ovarian/colorectal/breast Cancer, acute rheumatic fever, Parkinson's disease and renal transplant monitoring. In 2013, with funding from the ACRF and institutional co-investments, the University of Melbourne and the Victorian Comprehensive Cancer Centre (VCCC) established the first dedicated Translational Proteomic Facility in Australia for the

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customized quantification of cancer-associated protein expression and posttranslational modifications in individual patient tumour samples. This information is being used to better match specific treatments to each patient, and to determine how likely a patient's tumour is to respond to new generation targeted chemotherapies. In non-oncological disease, Bio21, University of Melbourne, in collaboration with Diasorin Inc, is developing targeted MS methods to accurately quantify parathyroid hormone (PTH) in clinical plasma samples of patients with chronic renal failure undergoing dialysis in whom traditional PTH immunoassays have limited specificity.

2. How Metabolomics and Proteomics might develop in Australia over the coming decade

2.1. Proteomics

In the same way, the Human Genome Project facilitated the discovery of the genomic contribution to disease pathogenesis, the recently published human proteome[10] will enable the identification of proteome changes associated with a particular disease. However, unlike the genome, the proteome is dynamic – that is, the proteins expressed by tissues during a disease are not the same as those expressed prior to the disease, after the disease, or in an individual unaffected by the disease. This means that an individual's proteome can be mapped serially over time, enabling a comparison of the temporal proteome with the individual's personal archived proteome, rather than comparison with a bio-banked library average. As technology and bioinformatics advance in the next 10 years, the analysis of personalised patient proteomes will become a reality in the clinic. This will revolutionalise the current approach to diagnostics and prognostics with clinical proteomics becoming the preferred methodology for identifying biomarkers of rare diseases of unknown aetiology, diagnosing communicable and non-communicable disease, monitoring response to therapeutic regimens, identifying mechanisms underlying adverse reactions and determining disease progression.

2.2. Proteomics for biomarker identification and monitoring

Although still in its infancy, proteomics for the detection of biomarkers has focused on two main areas: biomarkers for diagnosis of disease and biomarkers that indicate disease severity or disease stratification. In contrast to current clinical markers, for example prostate specific antigen (PSA) as a biomarker for prostate cancer, biomarkers identified by MS can be multiple and analysed simultaneously from a single sample. This has the potential to reduce costs and significantly improve time to diagnosis, reducing time to initiation of therapy and thereby improving patient outcome. Furthermore, as MS analysis can use a non-targeted approach, there is the potential to discover novel biomarkers, even in rare diseases where little or nothing is known about the underlying pathophysiology. Candidate biomarkers for a number of diseases have been discovered using proteomics analysis of body fluids including saliva for oral squamous cell carcinoma[11], cerebrospinal fluid for amyotrophic lateral sclerosis[12], urine for urological malignancies[13] and blood for inflammatory bowel disease[14], to name a few. One can envisage that proteomic analysis of an easily attainable body fluid, such a blood, urine or saliva will replace the need for invasive diagnostics tests such as endoscopy or angiography for diagnosis and monitoring of disease within the next decade.

2.3. Proteomics for diagnostics

Clinical proteomics has applications in the rapid diagnosis of infectious disease. At present, proteomic analysis can identify *E. coli, Salmonella, Campylobacter, Clostridium* (including *C. difficile), L. monocytogenes, Mycobacterium, Staphylococci, H. pylori* and enterobacteriaceae from biological samples[15-34] and has been reported to be faster and less costly than conventional methodologies relying on isolation and culture [35-37]. Moreover, proteomic methodologies can determine antibiotic resistance in addition to bacterial strain in a single test, if the antibiotic resistance mechanisms are reliant upon alterations in protein expression[38-40]. Host proteomic signatures that can differentiate

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bacterial from viral infection are an active area of research with the potential to rapidly distinguish a patient with a viral infection from a bacterial infection, limiting the inappropriate use of antibiotics which is contributing to the global emergence of antibiotic resistance[41].

2.4. Proteomics for therapeutic monitoring

In the same way that proteomics can be utilized to detect a biomarker indicative of the presence of disease, serial monitoring of the proteome could be used to assess disease response, or lack thereof, to a therapeutic strategy. Proteomic-based pharmacokinetic measurements of biotherapeutics may replace traditional assays within the next decade[42].

2.5. Proteomics for ongoing health maintenance

The premise underlying Precision Medicine is that individuals have a unique response to disease, drugs, diet and their physical environment, based on a combination of their personal genetic blueprint and their pre-birth and early life exposure to environmental factors. This unique combination, programs individuals for distinct health outcomes and determines how they will interact or respond to their particular environment or certain drugs. By utilizing a combination of detailed phenotypic analysis and personalized omics data (genomic, proteomic and metabolomics) better assessment of disease risk, enhanced understanding of disease mechanisms and optimal prevention and treatment strategies can be realised. By following individuals longitudinally over the next decade and collecting large amounts of biomedical information, including molecular, genomic, cellular, clinical, behavioural, physiological and environmental parameters, we will develop a set of biomarkers that not only enables the stratification of individuals into various risk groups but more importantly accurately predicts the optimal dietary, therapeutic and behavioural environments to promote and prolong health.

2.6. Metabolomics

Metabolomics offers many benefits in terms of providing novel insight into complex disease. As the terminal products of the genome and most proximal to phenotype, metabolomics can be a powerful source of novel markers, effectors, and predictors of disease. There are numerous examples of how metabolomics has provided clinically relevant findings in the past, including the discovery of: circulating plasma branched chain and aromatic amino acids as potent predictors of future diabetes[43]; a novel endogenous endocannabinoid marker of fatty liver disease[5]; a novel metabolite conferring the beneficial effects of exercise on fat browning and hepatic beta-oxidation[6]; markers of new onset atrial fibrillation[44]; markers of myocardial infarction[45,46]; markers of acute kidney injury[47]; tryptophan markers of pulmonary hypertension[48]; metabolite markers of longevity and wellbeing[49]; an amino acid signature of human pancreatic adenocarcinoma[50]; metabolite markers of cardiac work and stress[51]; acylcarnitines as predictors of cardiovascular mortality in dialysis patients[52]; a lysine metabolite as a predictor of future diabetes over a decade in advance[53,54]; an amino acid signature of acute cardioembolic stroke[54]; a triacylglycerol signature of insulin resistance[55]; metabolite markers of cardiac chemotoxicity[56]; metabolite signatures of exercise[57]; metabolite markers of uremia[58], amongst others.

It is also important to highlight that due to the close proximity of metabolites to phenotype, their effect size is commonly far greater than that of genetic variants. For example, in a groundbreaking study that revealed the potent biomarker utility of branched chain and aromatic amino acids, the upper quartile levels of the metabolite leucine had an odds ratio (OR) of >4 for development of future diabetes over a decade in advance (after adjustment for age, sex, BMI, fasting glucose, and parental history of diabetes)[43], whereas SNPs are often associated with disease at ORs of 1-1.5[59]. Combining just three metabolites – phenylalanine, tyrosine, and isoleucine – increased the OR for future diabetes to 7.6 (after adjustment for age, sex, BMI, fasting glucose, and parental history of diabetes)[43]. This underscores their power as biomarkers as compared to genetic variants. Despite

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the shortcomings of genomics in this arena, it can be useful when combined with exploratory metabolomics as a means to aid identification of novel unknown metabolites[60,61].

2.7. Research methods in proteomics and metabolomics

In addition to sophisticated liquid chromatography and mass spectrometry (LC/MS) equipment, careful consideration must be given to study design and robust sample handling. The above metabolomics studies were performed in carefully annotated and phenotyped human cohorts, mostly community-based cohorts that are most representative of the larger human population, e.g. the Framingham Heart Study. Care was taken to address confounding variables, e.g. bloods were taken on fasting individuals at the same time of day, in the same centre, processed using the same tubes, manual handling errors eliminated, stored quickly at -80C according to strict protocols. This attention to detail is essential, particularly in exploratory and hypothesis-generating research approaches. These robust approaches are being replicated in some centres around the world, but not all cohorts are as robust.

In Australia, the dearth of such cohorts is a disadvantage, but also an opportunity. As metabolomics requires such stringent sample handling conditions, many of the clinical metabolomics expertise in Australia have received training in centres where this level of care and attention was *in situ*. Therefore, new Australian precision medicine approaches can harness this expertise and ensure that the relevant protocols are in place. In addition, these new Australian initiatives can learn from the mistakes of older, ossified institutions with restrictive political systems. Another advantage of these younger centres, is the ability to incorporate younger disciplines and novel approaches to integrating systems biology. For example, at the Charles Perkins Centre at the University of Sydney many disciplines cross-pollinate under one centre, and a core "omic" technology and next-generation bioinformatics capability synthesizes data integration across multiple disciplines. Here, proteomics and metabolomics are routinely performed in obese individuals who are "phenotyped" by multiple and related disciplines (before and after perturbation): diabetology, sleep medicine, cardiovascular disease, imaging, psychology, nutrition, hepatology, and so on. Bioinformaticians who have direct experience with this type of data perform quality control, help study design, and in addition to standard univariate and multivariate analyses, perform machine learning approaches that frequently uncover novel and unanticipated associations that are further probed in the wet labs. The clinic on site allows careful monitoring, follow up, and sample acquisition; the clinical trials centre facilitate validation of findings in larger cohorts and translation of findings and development of human intervention studies. Other centres, such as the Western Australia Phenome Centre at Murdoch University take similar approaches, and initiatives have already begun linking these centres.

Another unique opportunity in Australia is the availability of indigenous populations. The accelerated rates of obesity-driven disease in Aboriginal Australians[62] when exposed to a Western diet, compared to those of Australians of European origin, offers a unique opportunity to study gene-environment interaction not commonly available. Conserved disease pathways across the races and divergent pathways together may identify novel strategies for targeting these diseases.

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