Rapidly Evolving Applications of ‘Omics to the Diagnosis and Treatment of Genetic Disorders: a Time of Great Opportunity Fast Approaching- A Personal Appraisal

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Many Genetic Disorders have an Open Window of Opportunity

Andrew Wyeth: “Wind from the Sea”
Wherever possible, examples will be given from the speaker’s direct experience. Presentation not intended to be a comprehensive review. Will focus on outcomes rather than methodologies per se.

**AIM IS TO PROVIDE A PERSONAL PERSPECTIVE**
‘OMES AND ‘OMICS
The main ‘Omes and corresponding ‘Omics are linked ways of thinking about, studying, and organizing data and insights about biology:

1. **Proteome** and **Proteomics**
2. **Metabolome** and the **Metabolomics**
3. **Metallome** and the **Metallomics**
4. **Methods**: share a common dependence on high resolution (liquid or gas) chromatography, mass spectrometry (various formats), and new applications of statistics and mathematics (for example: Bayesian Mixed Effects Modelling) designed to bring order to, interpret and interlink very large data sets required to understand structure and process.

**THE ‘OMES AND THE ‘OMICS**
“Proteomics is the systematic study of the proteome: the complete set of proteins expressed by an organism, tissue, or cell. It includes the study of changes in protein expression patterns as related to diseases, environmental conditions, and therapeutic interventions”
Illustration of proteomic experimental output: Experiment resulting in the identification of new therapeutic targets by proteomic analysis – in this case of the cardiac left ventricle

**Research Article**

**Reversal of diabetes-evoked changes in mitochondrial protein expression of cardiac left ventricle by treatment with a copper(II)-selective chelator**

Mia Jüllig¹*, Xiuyin Chen¹*, Anthony J. Hickey¹, David J. Crossman¹, Aimin Xu¹,², Yu Wang¹,², David R. Greenwood¹,³, Yee Soon Choong¹, Sarah J. Schönberger¹, Martin J. Middleditch¹, Anthony R. J. Phillips¹ and Garth J. S. Cooper¹,⁴

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Figure 2. Scanned images of LV homogenates separated by 2-DE. Numbers 1–20 correspond to proteins as listed in Tables 1 and 2. (A) Representative control gel with numbers to the left of the gel indicating molecular weights (kDa) and numbers below indicate pIs. In (B), gel spots from the control gel (C) are compared with representative equivalent spots from untreated diabetic (D) and TETA-treated diabetic (T) groups in (B).
“The systematic study of the metabolome, the complete set of metabolites (small molecules; <1500 Da) expressed by an organism, tissue, or cell. It includes the study of changes in protein expression patterns as related to diseases, environmental conditions, and therapeutic interventions”

METABOLOMICS AND THE METABOLOME
“Metallomics is the systematic study of metallomes and the interactions and functional connections of metal ions and their species with genes, proteins, metabolites and other biomolecules within organisms and ecosystems, and includes their changes in disease and responses to therapeutic interventions”

METALLOMICS AND THE METALLOME
LESSONS OF THE WAR

To-day we have naming of parts. Yesterday,
We had daily cleaning. And to-morrow morning,
We shall have what to do after firing. But to-day,
To-day we have naming of parts. Japonica
Glistens like coral in all of the neighbouring gardens,
And to-day we have naming of parts...

Henry Reed "Naming of Parts." New Statesman and Nation 24, no. 598 (8 August 1942)

TO-DAY WE HAVE NAMING OF PARTS
Architecture of a Global Integrated ‘Omic Initiative: Where the Pieces Fit

Chromosome-based Human Proteome Project (C-HPP)
• Genetics provides **well-characterized patient populations**: ideal for application of the ‘Omics approaches, because of diagnostic certainty which is a huge advantage
• Many genetic disorders have a **Window of Opportunity** that is open for a time before phenotypic change ensures: **serves as target** for application of ‘Omics
• This speaker believes that the ‘Omics approaches, when linked to in-depth genetic understanding, will enable many new and effective ways of treating and ameliorating genetic disorders to be identified and implemented

**THE POTENTIAL RELEVANCE OF ‘OMICS TO GENETIC DISORDERS**
Second important theme: applications of ‘omics are identifying mechanistic overlaps between genetic disorders and non-genetic diseases (e.g. newly discovered links between Wilson’s disease (genetic disorder of the copper transporter ATP7B that leads to defective copper metabolism) and diabetes-evoked organ damage have provided important therapeutic insights into the latter that have advanced to phase 2B trials under the USFDA.

Newly discovered linkages between Huntington’s disease and diabetes that may lead on to insights concerning Alzheimer’s disease and new experimental therapeutic approaches to both.
Many disorders where there is clearly a genetic component have remained refractory to genetic analysis. Applications of ‘omics to these have provided unexpected insights that can lead to new experimental diagnostic and therapeutic approaches. Examples are type-2 diabetes and Alzheimer disease.
What and How?

- Identify novel disease mechanisms by ‘omics and molecular pathobiology (especially metabolomics; proteomics)
- Mechanistic modelling for development of novel, first-in-class therapeutics
- Use new clues from the relevant ‘omes e.g. those of diabetes, dementia (for example, Huntington’s), and age-related macular degeneration
• Discovery and evaluation of new drug therapies
• Biomarker Discovery
• New approaches for molecular pathology
• Bayesian mixed-effects modelling for data analysis
Centre for Advanced Discovery and Experimental Therapeutics (CADET)

Joint venture between the University of Manchester and the Central Manchester NHS Hospitals Foundation Trust

Co-Director – Prof Paul Bishop

Proteomics Section
Lead - Dr Richard Unwin

Metabolomics Section
Lead – Prof Garth Cooper

Biostatistics/bioinformatics
Lead – Dr Andy Dowsey
Where we are: interfaced between University of Manchester and CMFT
OUR MAIN TARGETS

- Diabetes (prevention/reversal of complications + prevention of diabetes itself)
- Nephropathy, cardiomyopathy, arteriopathy, retinopathy
- Other metabolic diseases
- Age-related macular degeneration
- Cardiovascular (heart failure, stroke, vascular disease)
- Dementia
- Ageing/frailty
Mechanisms in genetic disorders can provide crucial insights into similar processes that occur in common diseases
Application of proteomics to the pancreas in type-2 diabetes revealed a new protein hormone, amylin, that served as a basis for a new therapeutic molecule, Symlin, currently being used by ~200,000 patients in the USA for the treatment of diabetes.

Insights gained led to two further registered medicines for diabetes.

A WORKED EXAMPLE
Looking back to the beginning of the microscopic study of the pancreas in diabetes...

THE RELATION OF DIABETES MELLITUS TO LESIONS OF THE PANCREAS. HYALINE DEGENERATION OF THE ISLANDS OF LANGERHANS.

BY EUGENE L. OPIE, M. D.
Instructor in Pathology, Johns Hopkins University.
(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

J Exp Med 1900
Clues: islet amyloid in type-2 diabetes

Johns Hopkins University USA

Oxford University UK

Opie EL *J Exp Med* 1900

Clark A et al *Lancet* 1987
Islet amyloid under the polarizing microscope: route to the target

Cooper GJ et al *Lancet* 1987
Amylin: monomer of islet amyloid from pancreases of type-2 diabetic patients

Cooper GJS et al *PNAS* 1987
Discovery of a new protein hormone by proteomics

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8628–8632, December 1987
Medical Sciences

Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients

(amino acid sequence/calcitonin gene-related peptide/insulin A chain/Alzheimer disease/pancreatic islet)

G. J. S. Cooper* ‡‡, A. C. Willis*, A. Clark†, R. C. Turner†, R. B. Sim*, and K. B. M. Reid*

*Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, Parks Road, Oxford, OX1 3QU, United Kingdom; and Diabetes Research Laboratories, Radcliffe Infirmary, Woodstock Road, Oxford, OX2 6HE, United Kingdom

Communicated by David Phillips, August 4, 1987
First steps towards a new classes of medicine: what were the messages?

Molecular and functional characterization of amylin, a peptide associated with type 2 diabetes mellitus

(genomic clone/carboxyl-terminal amidation/chromosome 12/glycogen synthesis/insulin resistance)

A. N. Roberts*, B. Leighton†, J. A. Todd‡, D. Cockburn§, P. N. Schofield¶, R. Sutton‡, S. Holt§ǁ, Y. Boys§ǁ, A. J. Day*, E. A. Foot†, A. C. Willis*, K. B. M. Reid*, and G. J. S. Cooper*,**

*Medical Research Council Immunochemistry Unit, Department of Biochemistry, †Department of Biochemistry, §Genetics Laboratory, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom; ¶Cancer Research Campaign Developmental Tumours Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom; ‡Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, United Kingdom; and ¶¶Nuffield Department of Surgery, John Radcliffe Hospital, University of Oxford, Headington, Oxford OX3 9DU, United Kingdom

Communicated by David C. Phillips, August 21, 1989 (received for review February 13, 1989)
Human amylin: two distinct SARs within one molecule: distinct structural underpinning for hormonal activity and cytotoxicity with signaling via two different receptors/pathways = isolate one to make the first medicine

The intramolecular disulphide bond and COOH-terminal amide are both required for amylin’s hormonal activity; however, neither are necessary for it to elicit β-cell apoptosis, which activity centres on amylin \textsubscript{20-29}

Chimaera...
Invention of a molecular chimaera: 

**Symlin®**

Fig. 1. A comparison of the amino acid sequences of human (H) [1], rat (R) [7] and cat (C) amylin [8], with those of human CGRP-1 (CGRP-1 H) [36] and CGRP-2 (CGRP-2 H) [42], and rat CGRP α (CGRP α R) [83] and rat CGRF β (CGRF β R) [84]. At the bottom the consensus sequence for this grouping of amylin and CGRP is shown. The shaded boxes indicate regions of sequence identity. The underlined sequence in human amylin indicates approximately the amyloidogenic region of the human amylin molecule [58]. The circled residues in the human amylin sequence indicate those residues not conserved between human and rat amylin, and the shaded circles those residues likely to be involved in the formation of islet amyloid by amylin. All molecules share a disulphide bond, linking Cys-2 with Cys-7 [1,36] and are carboxy terminally amidated.
Outcome: founded company to drive therapeutic development
Symlin, a new medicine for diabetes derived by proteomic means is registered for the treatment of diabetes (T1D and T2D) by the USFDA in 2005

OUTCOME
Applying this insight, the team at Amylin Pharmaceuticals between 1987 and 2012 made three new anti-diabetic medicines (first-in-class) - all registered by the US Food & Drug Administration for treatment of diabetes: Symlin (2005), Byetta (2005) and Bydureon (2012)
Proteomic Analysis of the Human Brain in Huntington’s Disease Indicates Pathogenesis by Molecular Processes Linked to other Neurodegenerative Diseases and to Type-2 Diabetes
SOME WORKED EXAMPLES FROM APPLICATIONS OF METABOLOMICS

Power to detect and measure unexpected phenotypic linkages in complex diseases
Robust Early Pregnancy Prediction of Later Preeclampsia Using Metabolomic Biomarkers
Louise C. Kenny, David I. Broadhurst, Warwick Dunn, Marie Brown, Robyn A. North, Lesley McCowan, Claire Roberts, Garth J.S. Cooper, Douglas B. Kell, Philip N. Baker and on behalf of the Screening for Pregnancy Endpoints Consortium
Hypertension 2010;56:741-749: originally published online Sep 13, 2010;
DOI: 10.1161/HYPERTENSIONAHA.110.157297
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association. All rights reserved. Print ISSN: 0194-911X. Online ISSN: 1524-4563
Identification of new first-in-class therapeutic molecules

USE OF METABOLOMICS FOR ELUCIDATION OF EXPERIMENTAL THERAPEUTICS FOR TYPE 2 DIABETES
Doubling of survival in diabetic transgenic mice by treatment with an orally-active oligomer suppressor
Phase 3 clinical trial of a new experimental amylin-amyloid suppressor in type-2 diabetes
The unexpected: tissue damage in diabetes closely related to that in Wilson’s disease

Selective Divalent Copper Chelation for the Treatment of Diabetes Mellitus

G.J.S. Cooper*1,2

1Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, and School of Biomedicine, The University of Manchester, and Manchester Academic Health Sciences Centre, Manchester, UK

2School of Biological Sciences and Maurice Wilkins Centre for Molecular Biodiscovery, Faculty of Science, University of Auckland, Auckland 1010, New Zealand

Abstract: Oxidative stress and mitochondrial dysfunction have been identified by many workers as key pathogenic mechanisms in ageing-related metabolic, cardiovascular and neurodegenerative diseases (for example diabetes mellitus, heart failure and Alzheimer’s disease). However, although numerous molecular mechanisms have been advanced to account for those processes, their precise nature remains obscure. This author has previously suggested that, in such diseases, these two mechanisms are likely to occur as manifestations of a single underlying disturbance of copper regulation. Copper is an essential but highly-toxic trace metal that is closely regulated in biological systems. Several rare genetic disorders of copper homeostasis are known in humans: these primarily affect various proteins that mediate intracellular copper transport processes, and can lead either to tissue copper deficiency or overload states. These examples illustrate how impaired regulation of copper transport pathways can cause organ damage and provide important insights into the impact of defects in specific molecular processes, including those catalyzed by the copper-transporting ATPases, ATP7A (mutated in Menkes disease), ATP7B (Wilson’s disease), and the copper chaperones such as those for cytochrome c oxidase, SCO1 and SCO2. In diabetes, impaired copper regulation manifests as elevations in urinary CuII excretion, systemic chelatable-CuII and full copper balance, in increased pro-oxidant stress and defective antioxidant defenses, and in progressive damage to the blood vessels, heart, kidneys, retina and
Outcomes of insight: multiple lines of evidence for structural and functional regeneration

REGENERATION OF THE HEART
Copper(II) selective chelation Protects & Restores Contractile Filaments in Heart of Diabetic Animals

Non-Diabetic (glucose 5 mM)  Untreated Diabetic (glucose 30 mM)  Treated Diabetic (glucose 30 mM)

Laser Confocal Microscopy

Cooper GJS et al *Diabetes* 2004
Reversal of heart failure in diabetic rats

Lu J et al Cardiovasc Diabetol 2013
Completed phase 2 trial: amelioration of LVH in diabetic patients after 12 months’ treatment with a copper(II)-selective chelator

Multivariate regression model predicting change in LVM_{bas} from indicated variables

<table>
<thead>
<tr>
<th></th>
<th>β coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
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<tbody>
<tr>
<td>Intercept</td>
<td>26.60</td>
<td>3.31</td>
<td>0.004</td>
<td></td>
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<tr>
<td>Serum albumin at baseline</td>
<td>-0.60</td>
<td>0.58</td>
<td>-5.04</td>
<td>&lt;0.001</td>
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<tr>
<td>Age</td>
<td>+0.43</td>
<td>0.18</td>
<td>3.50</td>
<td>0.003</td>
</tr>
<tr>
<td>Urinary copper excretion*</td>
<td>-0.42</td>
<td>0.07</td>
<td>-3.43</td>
<td>0.003</td>
</tr>
</tbody>
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*. Total urinary copper excretion over 12-months’ treatment derived from area-under-curve.
Expected to progress to phase 3 trials under FDA 2Q2015
Lines of structural and functional evidence

REVERSAL OF DIABETIC NEPHROPATHY IN THE RAT
Restoration of the filtration apparatus by Cu(II)-selective chelation

Kureishy N et al *In Preparation* 2014
New experimental diagnostic and therapeutic approaches for genetic disorders: understand the phenotype
A major advantage is certainty of diagnosis in well-defined populations
‘Omics: ability to detect and harness the unexpected
Many genetic disorders have a long window of opportunity that should be explored if no genetic mechanism-based therapy is available
Case-control ‘omic studies using non-affected family members a key strategy
Challenge of scale: large numbers of genetic disorders and where to start?

SUMMARY: APPLICATION OF ‘OMICS METHODS TO DERIVE NEW DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR GENETIC DISORDERS
Support

New Zealand
Ministry for Business, Innovation and Employment
Health Research Council
University of Auckland
Paykel Trust
Auckland Medical Research Foundation
Department of Education through a grant to the Maurice Wilkins Centre
Endocore Research Associates

UK
University of Manchester
National Institute of Health Research
North-western Development Agency
Central Manchester Hospitals (NHS) Foundation Trust
Manchester Area Health Sciences Centre
MRC
University of Oxford
Window of Opportunity

Andrew Wyeth: “Wind from the Sea”