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Message from the editor

Incredibly, the first known mention of the symptoms of diabetes was in 1552 BCE, when an Egyptian physician documented frequent urination as a symptom of a mysterious disease that also caused emaciation. Centuries later, people known as "water tasters" diagnosed diabetes by tasting urine. If it tasted sweet, diabetes was diagnosed. Thankfully some advances have been made since then. Not only can we distinguish between Type I and Type II diabetes, but new markers are being developed which are increasing our understanding of the disease. One of these, being introduced by Viapath's Clinical Immunology department, is ZnT8. This increases diagnostic sensitivity by detecting a proportion of otherwise seronegative patients.

Personalised medicine is also changing the face of pathology. By taking into account variations in a patient's genes, environment and lifestyle, clinicians are able to target treatments more successfully and the aim is to improve outcomes for patients. In this edition, the revolutionising effect that next generation sequencing is having on molecular diagnostics and treatment strategies in cancer is reviewed.

New methodology for diagnosing Huntington Disease

What is Huntington disease?

Huntington disease (HD) is an inherited neurodegenerative disorder. Typically it is late onset with symptoms first appearing between 35-50 years. There is a more severe form of the disease, known as juvenile HD, which has a much earlier onset. The disease is progressive and symptoms vary but can include involuntary muscle movements, emotional problems, speech loss and dementia. HD is usually fatal about 15 to 20 years after symptoms start.

Huntington disease is caused by mutations in the *HTT* gene, which provides instructions for the production of a protein called huntingtin. The function of this protein is still unknown but it appears to play an important role in the neurons of the brain. The *HTT* mutation involves a region of DNA that consists of repeated cytosine, adenine, and guanine (CAG) nucleotides. This is known as a trinucleotide repeat. Normally, the CAG trinucleotide is repeated 6 to 35 times within the gene. However, in people with Huntington disease, the CAG segment is repeated more than 35 times. This leads to the production of an abnormally long version of the huntingtin protein which eventually leads to the death of neurons in certain areas of the brain. The number of CAG repeats relates to the severity of the disease; cases of juvenile HD are associated with 60+ repeats.[1,2]

Current diagnostic testing for Huntington disease

Current testing for Huntington disease involves amplification of the *HTT* CAG trinucleotide repeat region

using a fluorescent polymerase chain reaction (PCR) assay. The fluorescent PCR products are accurately sized on a capillary sequencer with an internal size standard that allows the determination of the CAG repeat count for each of the two copies (alleles) of the *HTT* gene in an individual. However an issue with this approach is that PCR preferentially amplifies smaller fragments over larger fragments. This can result in 'drop out' of very large alleles (e.g. some of those responsible for juvenile HD) meaning they are not detected (Figure 1).[3]

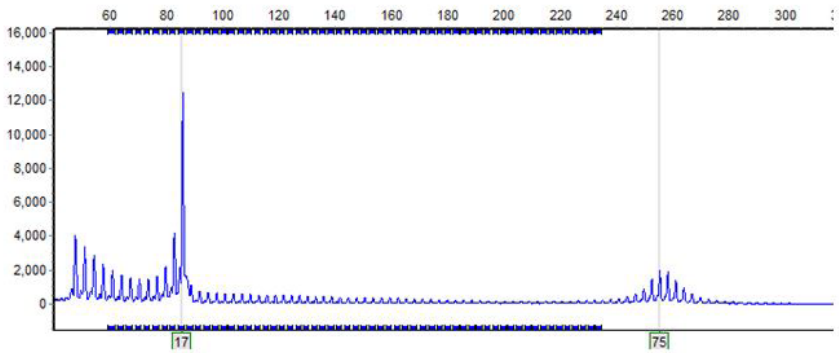

Figure 1 – Example of PCR bias towards shorter alleles. The peak for the allele with 17 CAG repeats (left) is much higher than the peak for the allele with 75 repeats (right).

Can diagnostic testing for Huntington disease be improved?

Viapath has recently introduced Oxford Nanopore long read sequencing technology into its Genetics Laboratory. This technology allows us to rapidly sequence long stretches of DNA using a small palm-sized device. Unlike short read 'next generation' sequencing; long read sequencing provides the ability to sequence the entire *HTT* CAG repeat region in a single read. It was therefore decided to investigate whether this technology could match the performance of traditional methods for diagnosing HD, and improve the ability to detect larger alleles.

Methodology

Forty-eight samples previously tested by PCR with CAG repeat counts ranging from 15 to 101 were selected. Two kilobase long-range PCR products covering the *HTT* CAG repeat region were generated. Libraries were constructed and sequenced on an Oxford Nanopore MinION. The error rate of Oxford Nanopore sequencing made data analysis challenging. We therefore ran all data through an algorithm called repeatHMM (https://github.com/WGLab/RepeatHMM) which applies a Hidden Markov statistical model to estimate the repeat count.[4]

Results

All of the alleles (15 – 101 repeats) were successfully detected by repeatHMM. A high correlation between the repeatHMM repeat counts and the PCR repeat counts was observed (Figure 2). Results from repeatHMM and PCR generally fell into the same HD classification categories. Best practice guidelines state that acceptable error limits

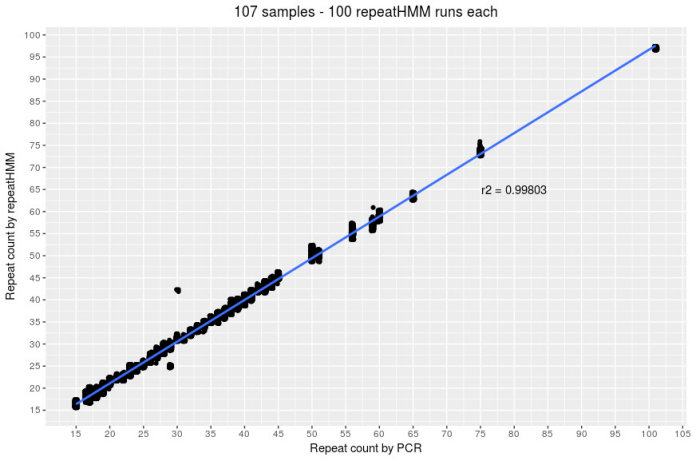

Figure 2 – Plot showing correlation between CAG repeat counts reported by repeatHMM results and PCR

are +/-1 repeat for alleles that are <42 repeats in length. Repeat counts in the intermediate and reduced penetrance ranges (where accuracy is most critical) were generally within +/-1 repeat of the PCR result. It was observed that repeatHMM slightly overestimated results at the lower limits and underestimated results at the upper limits of the repeat sizes. It was also observed that some outliers required further investigation. However the initial results were very promising.

Is this test the way forward for Huntington disease?

In the first instance we are implementing Oxford Nanopore sequencing as a reflex test in juvenile HD cases where we have failed to detect a pathogenic allele. Based on the promising initial results, we are hopeful that, with a little more optimisation, this test will be used more widely. We are working with Oxford Nanopore Technologies and other laboratories to share expertise and resources, which is allowing the development of this and other exciting new Nanopore tests. We are enthusiastic about the breadth of opportunities this new cutting edge technology presents us with and are grateful to the Viapath Innovation Fund for funding this project.

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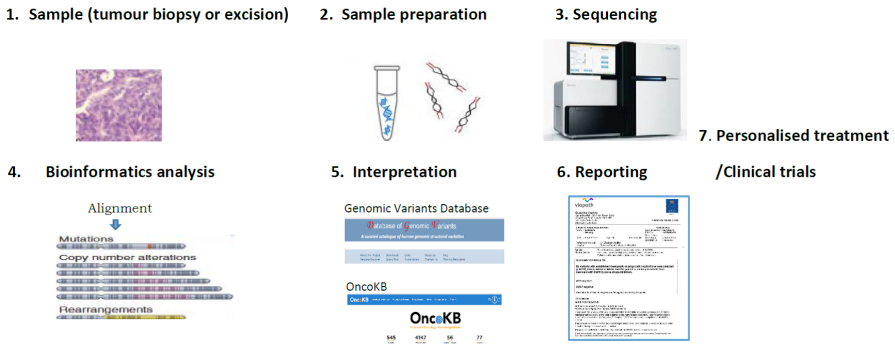
Precision Medicine is changing the face of pathology

What is precision medicine?

Precision medicine (sometimes known as personalised or stratified medicine) is an emerging approach to the treatment and diagnosis of disease that takes into account variations in a patient's genes, environment and lifestyle. It allows clinicians to better target treatments to an individual's circumstances and aims to improve outcomes for patients. It is of particular relevance to patients with cancer and was discussed recently at the "European Association for Cancer Research/ Organisation of European Cancer Institutes Joint Course: Molecular Pathology Approach to cancer".

Molecular pathology is one of the linchpins of precision medicine with respect to the treatment of cancer patients; therapeutic decisions can no longer be made solely on the basis of histopathological diagnosis, as genomic analysis of human cancers has resulted in the identification of genetic determinants of tumour development, cancer progression and therapy response.



Schematic representation of molecular analysis workflow

The use of precision medicine in cancer diagnosis and treatment

Massively-parallel sequencing or next-generation sequencing (NGS) has enabled the genetic characterisation of many cancers and identified driver genetic alterations which can be used for targeted therapies and predict treatment response.

Lung and colorectal cancer (CRC) are two of the most common cancer types, which are often diagnosed at late stages leading to limited therapeutic options. Major advances in molecular characterisation of these tumours has identified specific gene alterations such as *ALK* and *ROS1* rearrangements and *EGFR*, *NRAS*, *KRAS* and *BRAF* mutations which are targets for specific treatment, thus increasing therapeutic options for many patients and enabling a more personalised approach to patient management.

Viapath's Cancer Genetics service has implemented a targeted NGS panel using the Swift Biosciences Accel-Amplicon EGFR Pathway panel as part of the routine diagnostic pathway of lung and colorectal cancer, which is UKAS accredited to ISO15189 standard. The assay allows simultaneous identification of the majority of the therapeutically and prognostically relevant *EGFR, KRAS, NRAS* and *BRAF* gene variants, and is highly reproducible, giving high success rates (>95%) even for small biopsies yielding low DNA mass and significantly improving the management of patients and expanding their treatment options. For example, lung cancer patients with sensitising *EGFR* variants (~19%) receive treatment with EGFR tyrosine-kinase inhibitors (TKIs), and CRC patients who show no evidence of *KRAS* or *NRAS* mutations are treated with anti-EGFR monoclonal antibody therapy.

The panoply of genes with variants of known therapeutic relevance is rapidly and constantly expanding, and as identification of such variants becomes clinically relevant more comprehensive gene panels are needed in the diagnostic pathway. To meet this need the department is validating a 57 gene NGS panel for translation into routine diagnostic use. This will not only allow for detection of a larger spectrum of variants in lung and colorectal cancers but will also provide a basis for molecular diagnosis/treatment of other solid tumours.

In summary, introduction of NGS and small inhibitory molecules is revolutionising molecular diagnostics and treatment strategies in cancer and has contributed to the ongoing development of personalised medicine to improve outcomes for patients with solid tumours.

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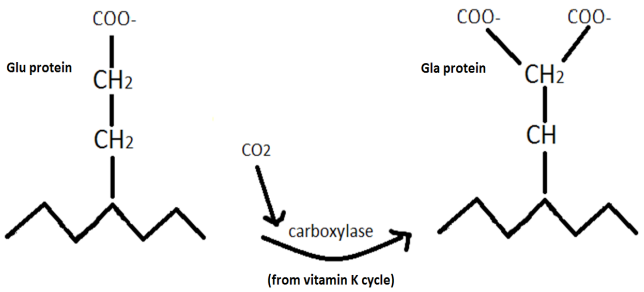
Early experience with the interpretation of dp-ucMGP: an indicator of vascular calcification

FOCUS 2018, the national meeting of the Association for Clinical Biochemistry and Laboratory Medicine, covered various topics on laboratory medicine and provided an opportunity for an enhanced, constructive dialogue on Viapath's new diagnostic test - Matrix Gla Protein (MGP).

The role of Matrix Gla Protein

Matrix Gla Protein (MGP) is a 84 amino acid extra hepatic vitamin K-dependent protein which is synthesized by chondrocytes and vascular smooth muscle cells. It plays an essential role in the regulation of bone and soft tissue calcification by directly inhibiting calcium precipitation and crystallization, and is the most potent inhibitor and reverser of arterial calcification currently known. Knock-out mice lacking MGP develop extensive and premature calcification in arteries and cartilages and die within six to eight weeks of birth as a result of blood-vessel rupture.

MGP activation is expressed through two posttranslational processes: the phosphorylation of three serine residues in positions 3, 6, and 9 (although the role of this phosphorylation process is still not well understood) and the carboxylation of five glutamate residues. For this reason, different forms of MGP are circulating in plasma which may have different physiological roles and functions. Studies on the different species of MGP have shown that the dephosphorylated and uncarboxylated (dp-ucMGP) form is the only variant associated with a response to vitamin K supplementation. Increased levels of dp-ucMGP reflect an increased propensity for vascular calcification and decreased availability of vitamin K in the vessel wall. The measurement of dp-ucMGP has been suggested to be a functional indicator of vitamin K status in tissues that utilize MGP. Also, it is possible that the measurement of dp-ucMGP reflects vitamin K status.



undercarboxylated **γ-carboxylated MGP**

Measurement of MGP

In a recent study, we measured the desphosphorylated-uncarboxylated form of MGP (dp-ucMGP) using an automated IDS-iSYS InaKtif MGP assay based on chemiluminescent technology to begin to evaluate the clinical utility of this marker in patients with established vascular risk factors..

35 plasma samples were analysed from patients with: type 2 diabetes mellitus (T2DM), (n=4); chronic kidney disease (CKD), (n=4); rheumatic disease (RD), (n=3); hypertension (HTN), (n=6); raised total cholesterol (>4mmol/L), (n=11); and cardiovascular disease (CVD) (n=6). In addition, 80 plasma samples were analysed from postmenopausal osteoporotic women aged ≥55 y who were receiving oral bisphosphonate treatment and had low serum vitamin K_1 concentrations ranging from 0.05-0.35µg/L [fasting reference range; 0.17–0.68µg/L].

The MGP cut-off value <750pmol/L was adopted from manufacturer's reference range. It was calculated from 132 apparently healthy donors from 18 to 59 years of age, using the IDS-iSYS InaKtif MGP assay.

The incidence of elevated dp-ucMGP was: 33% in CVD (mean 641pmol/L); 33% in RD (mean 725pmol/L); 33% in HTN (mean 674pmol/L); 25% in T2DM (mean 716pmol/L); and 18% in raised cholesterol (mean 610pmol/L). In the osteoporotic women, 9% had a dp-ucMGP that exceeded the cut-off value (mean 534pmol/L). In addition, in keeping with previous published studies (Riphagen 2017, Theuwissen 2014), we found a significant association between age ($r=0.31$, $p < 0.05$) and BMI ($r=0.4$, $p=0.001$) with dp-ucMGP. Both increasing age and BMI are associated with the risk of CVD and the correlation observed here may suggest that the link between obesity and CVD could be modulated by MGP but further research is needed.

Conclusion

Our results show more >750 pmol/L values in patients at high risk of CVD, and a positive correlation with age and BMI: both CVD risk factors. Both increasing age and BMI are associated with the risk of CVD and the correlation observed here may suggest that the link between obesity and CVD could be modulated by MGP but further research is needed. When the serum vitamin K_1 levels were spilt into quartiles, there was no significant difference in dp-ucMGP concentrations. The vitamin K_1 concentrations were below the reference range median; thus evaluation with a larger range is needed.

Studies have shown that vitamin K supplementation reduces dp-ucMGP and improves vascular health. Monitoring dp-ucMGP levels may allow modulation of arterial calcification and CVD progression, particularly in those with increased age and BMI.

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Viapath Career Development Programme – A personal review

The Viapath Career Development Programme (VCDP) was reviewed in the last issue of "pathology@viapath". It is a 2 day training course, led by Viapath's Future Leaders in Innovation, aimed to guide junior employees at Viapath who want to learn more about career progression within healthcare science. Here Jadie Baxter, a Senior Assistant Technical Officer in the Red Cell Laboratory, discusses her reasons for attending the course and what she gained from it:

What are your aspirations for your future career?

I hope to do my IBMS registration portfolio, a master's degree and become a registered Biomedical Scientist.

Why did you attend the VCDP?

I saw it as an opportunity to obtain some information and guidance about the possible career pathways as well as to learn about some of the disciplines and opportunities available at Viapath. I hoped to improve my knowledge and skills which will help me to achieve my ambition of becoming a Biomedical Scientist.

What did you gain from the programme?

I feel that I gained a much better understanding of the opportunities available to me in healthcare science and also within Viapath. I also acquired knowledge on how to improve my job applications and interviews as well as more in depth information about what is required for the completion of the IBMS registration portfolio.

How will you use the skills that you learnt to develop your career?

I learnt about the Viapath Insight Programme which encourages Viapath employees to spend a day in a different department to see what their work entails. I'm scheduled to go to St Thomas' Newborn Screening laboratory which will be interesting and very valuable as my laboratory performs their second-line screening tests.

I personally found the Viapath Career Development Programme to be very useful and I'm grateful that I was able to attend.

Figure 1 - Jadie Baxter - Senior Assistant Technical Officer

Figure 2 - VCDP delegates

New test for diagnosing diabetes: Zinc transporter-8 autoantibodies

Why is the diagnosis of diabetes important?

Diabetes mellitus is a chronic disease present in an estimated 3.7 million members of the UK population. The condition is a consequence of either a deficiency in the production of insulin by the pancreas or by the ineffectiveness of the insulin produced. The outcome of this results in increased glucose concentrations in the blood, which can have damaging multi-organ effects, particularly on the blood vessels and nerves.

Type 1 diabetes (T1D) is caused by T-cell mediated destruction of the beta cells in the pancreas. The process of beta cell destruction is marked by the production of autoantibodies to the beta cell, which occurs over many years. Several different autoantibodies have been identified and they are useful clinically for:

I. Diagnostics: to distinguish between Type 1 and Type 2 diabetes

II. Predictors: to indicate subjects at increased risk of future T1D development

III. Monitoring: to monitor the success of clinical islet transplantation

Diabetes mellitus is a chronic disease present in an estimated 3.7 million members of the UK population.

Testing for diabetes

Whilst laboratories have traditionally offered a panel of glutamic acid decarboxylase 65 (GAD) and protein tyrosine phosphatase IA-2 (IA-2) testing, zinc transporter 8 (ZnT8) has been newly identified as a target of humoral immunity in T1D. This means that testing for ZnT8 is less readily available.

ZnT8 is a pancreatic β-cell secretory granule membrane protein that is a member of a large conserved family of cation efflux proteins. It is highly expressed on the membrane of pancreatic islet beta cell insulin secretory granules[1]. ZnT8 is essential for exporting zinc into the lumen of beta cell secretory granules, with cytoplasmic zinc uptake allowing insulin crystallisation for storage in a hexameric form[2]. ZnT8 autoantibodies are a promising marker as the target is expressed more specifically in the insulin□ containing secretory granules than both GAD and IA-2. Recent data from Viapath's Clinical Immunology laboratory demonstrates that the inclusion of an additional ZnT8 antibody for T1D leads to the detection of otherwise seronegative patients, thus allowing clinical trial enrolment and appropriate treatment regimens to be followed. Positive GAD, IA2 and ZnT8 antibodies in combination are associated with younger age of onset of autoimmune diabetes and more severe insulin deficiency. The combined measurement of GAD, IA2 and ZnT8 antibodies is highly specific for T1D and can increase the detection rate to 98% at disease onset.

For further information or any enquiries, please contact the Clinical Immunology Laboratory: kch-tr.immunology@nhs.net

There are two principle forms of diabetes:
Type 1: occurs when the pancreas fails to produce insulin.
- Develops most frequently in children and adolescents.
- Accounts for 10% of diabetes cases.

Type 2: results from the body's inability to respond properly to the action of insulin produced by the pancreas.
- Accounts for around 90% of all diabetes cases
- Occurs most frequently in adults but is being noted increasingly in adolescents.

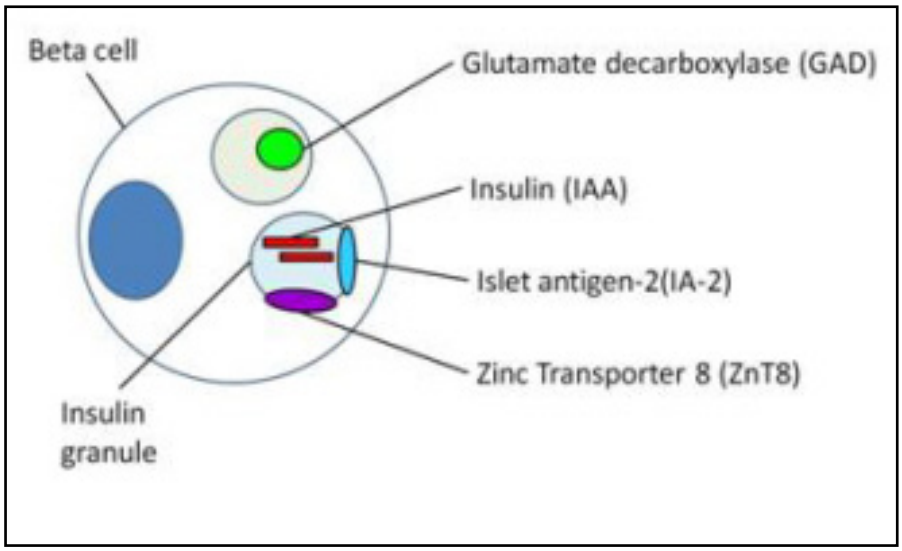

Figure 1- Antibody targets within the beta cells of the pancreas associated with Type 1 Diabetes4. GAD, IA2 and ZnT8 form our new triplicate testing panel

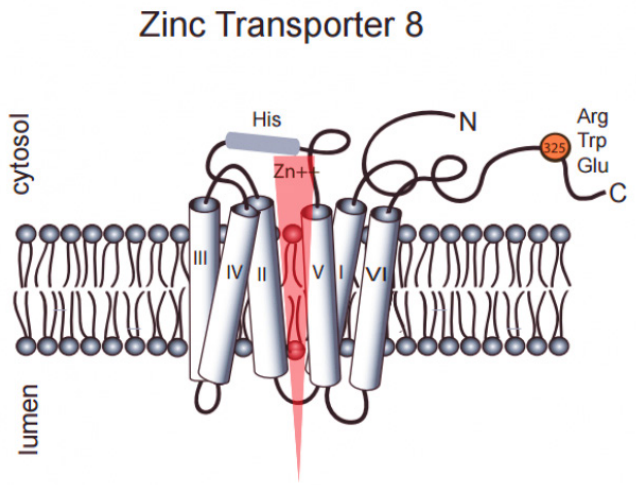

Figure 2 – Schematic diagram of ZnT8 showing the six transmembrane domains forming the membrane pore. This is flanked by N- and C-terminal domains and a histidine loop, which binds zinc ions3

Key facts:

- ZnT8 antibodies are a useful new marker of T1D, which are now included in the testing panel in Viapath's Clinical Immunology laboratory alongside GAD and IA-2.
- A consultant-led service is provided, with the tests routinely clinically interpreted by Professor Mark Peakman.
- Measuring this antibody increases diagnostic sensitivity, which will allow detection of a proportion of otherwise seronegative patients.
- ZnT8 provides additional diagnostic confirmation in >10% of samples tested, increasing confidence in borderline or low positive results that may have previously triggered repeat confirmatory testing or genotyping.
- Both the prevalence of ZnT8 positivity and the antibody titres are higher in younger patients.

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Viapath's Innovation Academy Symposium

Viapath's annual Innovation Academy Symposium attracts leading and up-and-coming members of the scientific community, and also provides networking opportunities. The symposium includes presentations on current trends and developments.

The theme of the 8th Symposium is 'Stretch & Hold Forth' and will feature exciting presentations covering a range of topics including, the development of a novel laboratory diagnostic for ***Parkinson's disease***, the application of ***nanopore technology, 'flying high' in London with Drones*** and and much more. There is also an opportunity to hear about the latest in scientific innovation from our developing scientists with the Excellence in Pathology award.

Join Viapath at Skinners' Hall on December 7

For more information on the event and to register your interest to attend please email:
InnovationAcademy@viapath.co.uk

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