pathology@viapath

Inside this issue

Message from the editor

Some of you may remember the "Record Breakers", a long running children's television programme. It was themed around breaking world records and the lyrics to its theme tune include "If you want to be the best…..dedication's what you need". I was reminded of this song as I read Norman's article outlining how steroid testing has changed over the years. It was his dedication that impressed me. He has spent 46 years studying steroids, a fantastic achievement.

Other articles within this edition of "pathology@viapath" also show how scientists are dedicated to improving the lives of patients. These include the development of a new screening service for Candida auris, the utilisation of therapeutic drug monitoring to help optimise treatment in individuals with certain infections caused by gram-positive bacteria and the use of next generation sequencing to diagnose inherited platelet disorders. Each project has required a great deal of dedication, and all will make a significant difference to patients' lives.

Improved Screening services for *Candida auris*

A novel screening method for Candida auris?

In Issue 4 of "pathology@viapath", an article entitled "*Candida auris*: A newly discovered multi-resistant pathogen" was published. It explained that *Candida auris* (*C. auris*) is an emerging multi-drug resistant fungal pathogen, dating back to 1996[2] in South Korea and that there is a high mortality rate of 50-60%, associated with the fungal pathogen.

C. auris has a very high outbreak potential, especially in high risk populations, and appears to be difficult to eradicate from the environment. Hence, strict levels of infection

control precautions are required, including isolation of infected patients. Screening of contacts is advised to detect early colonisation and help prevent the spread of infection.

Inaccurate identification of *C. auris* using classical screening methods (such as Chromagar and Sabouraud's Agar plate culture & Matrix-assisted laser desorption ionisation (MALDI) - Vitek mass spectrometry (VITEK® MS) with positives sent to the reference laboratory for MALDI-Bruker confirmation) can lead to a delay in recognising the infection or an outbreak. This can severely impact the patient outcome and also has cost implications. Thus, novel, specific diagnostic methods have been designed to overcome these limitations and improve patient care.

A new, rapid and precise *C. auris* polymerase chain reaction (PCR) was developed and validated to address the diagnostic screening issue. This improved the prevention measures at King's College Hospital by allowing patients suspected of having *C. auris* to be de-escalated when *C. auris* PCR was negative and by being informative for the infection control team to know when to employ increased vigilance if patients are *C. auris* PCR positive, through isolation and further screening. The *C. auris* PCR was validated on 320 specimens using both swab sets (groin, axilla, nose, throat) and urine samples. The *C. auris* PCR was estimated to detect as little as [100 organisms in 1 ml] = 1 organism in 10 µl of sample (less than 0.5 Mc Farland suspension), compared to a 2 Mc Farland suspension required for the Biomerieux, Vitek®2. The preliminary sensitivity and specificity data for the novel *C. auris* specific PCR was determined to be greater than 95% compared with classical methods. With high positive and negative predictive values, the *C. auris* PCR is suitable for the current diagnostic demand.

A second, different PCR method is used as a reference PCR to check the integrity of the specimen collected. It also has an 18S pan-fungal component, which can be used to confirm the first positive *C. auris* detected in a patient's specimen. The innovation of *C. auris* rapid detection PCR has enabled diagnostic screening methods that provide a more effective service, with a short turnaround time (24-48 hours) for identification (See figure 1 below).

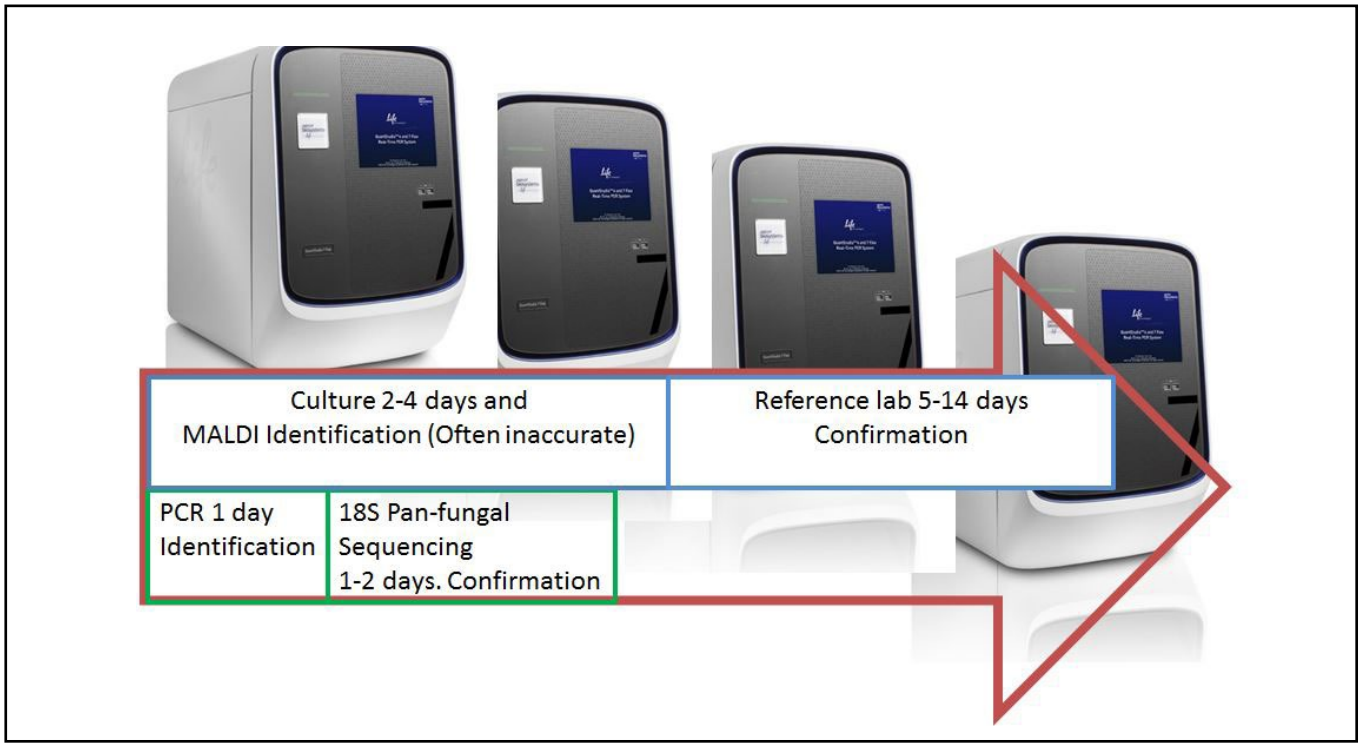

Figure 1: A comparison between the classical screening method and the molecular PCR screening method.

Improving Infection Control
Viapath is offering a *C. auris* service that can achieve high-throughput and deliver a clinically meaningful result, in a timely manner. This service will help to better manage patients suspected of being infected with *C. auris* using more appropriate treatments, reduce the burden on the availability of infection control isolation rooms and help to reduce the spread of infection. Thus greatly improving infection control.

Tests run Monday to Friday. With samples received in the laboratory before 12 pm, processing is performed on the following day.

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Developing a urinary steroid profiling service within the NHS: a personal journey recorded by Dr Norman Taylor

Nothing prepared me for 46 years (so far) of analysing steroids, a period which is more than half of the time since the first steroid was crystallised from human urine in 1929.

My early years
My birth year of 1947 was just months before the start of the NHS. I remember my parents' passionate support of its principles and with gratitude the local GPs, who would consent to visit for every childhood illness. Aged nine, I spent a week in hospital with appendicitis, returning home with a proud scar and a determination to become a doctor. Soon after, my best friend developed a sharp pain and after checking him and asking questions, I pronounced that he had appendicitis, which he did, but my scar was neater than his! I didn't make it to medical school, so turned to applied biology. After an HND and 2 years of voluntary work in Africa, I returned to find jobs suddenly scarce but landed one at St Thomas' Hospital as a technician tasked with analysing a single steroid. One year on, I transferred to the newly opened Clinical Research Centre where Cedric Shackleton had just returned from a post-doc and had started a lab for multicomponent steroid analysis. He coined the term 'urine steroid profiling'. I have been practising it ever since.

The early years of steroid testing
Steroids first came to the fore in the modern era during the study of soluble physiologically-active agents that could be extracted from the adrenals, ovaries and testes. The

thirties and forties saw crystallisation of individual steroids, testing of hormonal activity and structural determination. Steroid synthesis in quantities sufficient to be useful therapeutically began in the early fifties, revolutionising several areas of medicine, including moderation of the immune system, assisting fertility and in contraception. The first inborn error of steroid metabolism was fully characterised at this time. This needed methods for the analysis of natural steroids in body fluids. Although steroid hormones in blood were the major focus, urinary metabolites proved to be very informative in providing a composite picture of steroid metabolism. This remains true to this day, with all significant new findings on steroid metabolism still originating with observations in urine. The continuing challenge is to separate and detect individual steroids from within a 'soup' containing an uncountable number of related compounds.

'Steroids first came to fore in the modern era during the study of soluble physiologically- active agents that could be extracted from adrenals, ovaries and testes '

During a time of rapid development of new techniques, Cedric Shackleton went through chromatography on paper, thin layer plates, and finally gas chromatography-mass spectrometry (GC-MS) within a mere 4 years (1966-70). He was the first in the UK to use capillary column gas chromatography for steroid analysis: it provided enormously improved resolution. By the time I joined him in 1972, these techniques were all in place and have not changed in principle since. Over the next few years, along with my co-worker, John Honour (who went on to run the other Supraregional Assay Service for this within England), disorder after disorder came under our scrutiny. We soon realised that each had its own 'fingerprint' and were able to identify all the major components, very much helped by the resources of the Medical Research Council Steroid Reference Collection, which kept hundreds of crystalline pure steroids and provided small amounts for identification purposes. We found many previously undescribed steroids. In 1980, we jointly published an atlas of steroid profiles to aid steroid disorder identification by others.

An evolving service

A service to provide steroid profiling on patients was never systematically planned, but simply grew from a creative interaction with clinicians and clinical biochemists. The organisation and ethos of the NHS has been critical: we continue to learn together in a free, non-hierarchical exchange. Few, if any, countries in the world can match the UK for this. Among the great benefits of GC-MS is that it can be used non-selectively: whatever is there in greatest amounts will be seen. All the data are electronically stored and can always be interrogated, so steroids that were not at first considered significant can be looked for if new information emerges. The combination of 'fingerprint' and searchability has also meant that, with careful record-keeping, we can use previous findings on a given disorder to aid identification of new cases. A highly developed database helps in finding parallels, such as locating other patients who are being treated with the same drug. It also enables us to identify previous encounters with a patient and so provide continuity: trends in single patients can be monitored and earlier findings made known to a clinician who may not be aware of them.

Using GC allied with tandem mass spectrometry (GC-MS/MS) increases sensitivity and specificity and has an incredible and still largely unrealised potential to delve further into steroid metabolism. A doctoral project by Sofia Christakoudi in our laboratory revealed around 450 different steroid metabolites in newborns with 21-hydroxylase deficiency, of which only 15 or so had been previously described in the literature[1-6].

Serum steroid analysis does, of course, remain the mainstay of clinical investigation, for which immunoassay reigns supreme.

Some of its limitations of specificity are well known, such as when quantifying testosterone in women, whilst others are less familiar. For instance, steroids at pathologically high levels may unexpectedly interfere, an example being when serum from newborns with severe 21-hydroxylase deficiency shows a 'normal' serum cortisol concentration when in fact the true value is very low.

From urine steroid profiling to serum steroid panelling

Liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is far more specific than immunoassay, is now coming into use, but is not yet available on automatic analysers. It has mostly been used to directly replace single steroid assays, but it is perfectly possible to quantify many steroids simultaneously. We now have a method, developed by David Taylor and Lea Ghataore[7], that targets all the major intermediates in the steroid hormone synthesis pathways. This will be launched as a service soon. Since the technique requires prior setting up for the particular steroid targets, it should be called serum steroid *panelling* and not *profiling*. Because many steroids have the same general structure and so have identical molecular weights, differentiating the critical ones is a challenge. Comparing findings in urine and serum on single patients has already revealed errors in results obtained by LC-MS/MS by other services[8]. The learning continues…

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For further information on Viapath's Steroid Analysis, please contact:
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New test: Teicoplanin Assay

New test: Teicoplanin Assay
Viapath has added teicoplanin to the repertoire of assays offered by the Therapeutic Drug Monitoring (TDM) laboratory based at King's College Hospital.

What is teicoplanin?
Teicoplanin (Targocid, Sanofi-Aventis) is a glycopeptide antibiotic used in the prophylaxis and treatment of serious infections caused by Gram-positive bacteria, including multi-resistant staphylococci. However, there are reports of *Staphylococcus aureus* with reduced susceptibility to glycopeptides and increasing reports of glycopeptide-resistant enterococci. Teicoplanin is similar to vancomycin, but has a significantly longer duration of action, allowing once daily administration after the loading dose. Teicoplanin is available in oral, intravenous, or intramuscular preparations.

Why is teicoplanin measured?
Plasma/serum teicoplanin concentrations are not measured routinely because a relationship between plasma/serum concentration and toxicity has not been established. However, monitoring the teicoplanin concentration can be used to optimise treatment in individuals with severe sepsis or burns, deep-seated staphylococcal infection (including bone and joint infection), or endocarditis. Pre-dose ('trough') concentrations should be greater than 15 mg/L (greater than 20 mg/L in endocarditis or deep-seated infection such as bone and joint infection), but less than 60 mg/L.

How is teicoplanin measured?
Teicoplanin is measured on an automated analyser, the Indiko Plus (ThermoFisher Scientific). The teicoplanin assay is a homogeneous particle-enhanced turbidimetric immunoassay and is based on competition between the drug in the sample and the drug coated onto a microparticle for antibody binding sites of the teicoplanin antibody reagent. The teicoplanin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-teicoplanin antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically. When a sample containing teicoplanin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with maximum rate of agglutination at the lowest teicoplanin concentration and the lowest agglutination rate at the highest teicoplanin concentration (Figure 1).

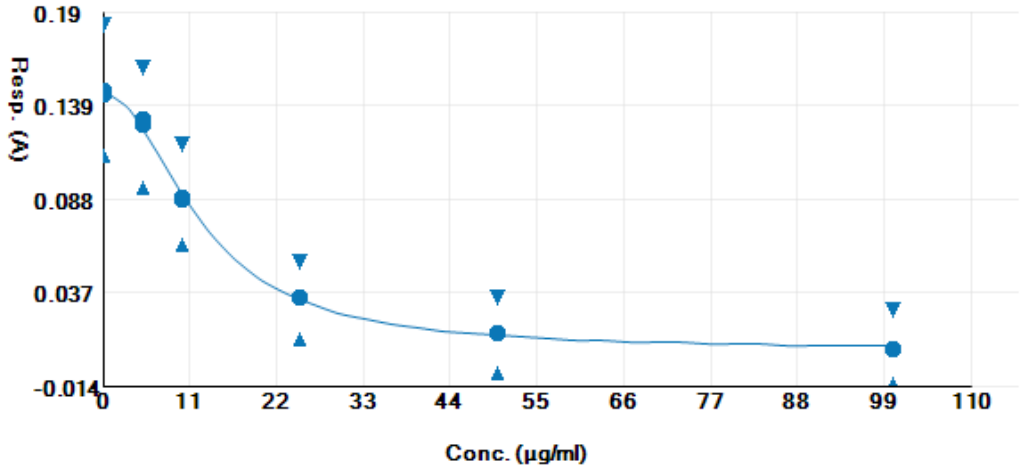

Figure 1 – Typical teicoplanin calibration curve

Sample requirements

2 mL EDTA whole blood or 1 mL plasma or serum is required for analysis. Ideally samples should be taken pre-dose (a 'trough' sample).

Turn-around time is 5 working days.

Further information on requesting a teicoplanin assay, including a downloadable request form, can be found on the Viapath website:
www.viapath.co.uk/our-tests/teicoplanin

For additional details on teicoplanin, please email:
kch-tr.toxicology@nhs.net

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Changes in HbA1c reporting

Rationale for change

Viapath performs HbA1c testing at several of its sites, including St Thomas' Hospital, King's College Hospital and the Princess Royal University Hospital. To meet with national guidance, from 1st February 2018, Viapath standardised its reference ranges and interpretative guidance for HbA1c: the standardised non-diabetic reference range on all samples being 20 – 41 mmol/mol in IFCC units or 4.0 – 5.9% in DCCT units. Dual unit reporting will be continued.

Interpretative guidance

HbA1c result interpretation depends on whether the test is being used for the diagnosis of type 2 diabetes mellitus (T2DM) or for monitoring diabetic glycaemic control. Our reports are now aligned to latest guidance and contain interpretative information for both possible scenarios. The exact report layout will depend on the Viapath site generating the result, due to I.T. differences across our sites.

	IFCC units (mmol/mol)	**DCCT units (%)**
HbA1c for diabetes mellitus diagnosis		
Normal	20–41	4.0–5.9
Pre-diabetes	42–47	6.0–6.4
Diabetes Mellitus	≥48	≥6.5
HbA1c in monitoring established diabetes		
Target	48–58	6.5–7.5

Please note that in accordance with NICE guideline PH38 2012 (updated 2017), diagnosis of T2DM in asymptomatic individuals requires two results ≥48 mmol/mol (≥6.5%). If the second sample is <48 mmol/mol (<6.5%) the person should be treated as at high risk of diabetes.

Other considerations

The diagnosis and monitoring of diabetes mellitus using HbA1c is dependent on normal erythropoiesis, no genetic or chemically altered haemoglobins, normal glycation and normal erythrocyte destruction rates. These should be considered as part of routine patient management, and complementary glucose measurements used in situations where HbA1c results may not be reliable.

Advice/ Feedback

We would like to take this opportunity to apologise to our users for any previous confusion resulting from non-standardised HbA1c reporting practices.

Please do not hesitate to contact us if you have any questions or feedback:

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References:
NICE PH38 2012 (updated 2017):
https://www.nice.org.uk/guidance/PH38

New vitamin status testing service

Viapath is introducing a new service, Viapath Nutris. This service will allow patients to go online, select a test panel and, in some cases, have results within 24 hours of having their blood drawn.

Which vitamin test panels will be launched?

Viapath Nutris will initially focus on vitamin B_{12} and related tests. In Issue 9, of "pathology@viapath", the relevance of Vitamin B_{12} deficiency and the need to offer more sophisticated testing, particularly to patients with pernicious anaemia, was discussed. This article can be viewed at *www.viapath.co.uk/news-and-press/are-you-suffering-from-a-vitamin-b12-deficiency-and-need-additional-help article*

Many of those who suffer from a vitamin B_{12} deficiency also suffer from iron, folate and vitamin D deficiencies. In fact, 30% of people who have a vitamin B_{12} deficiency also manage a deficiency for one or all of these.

Initially, four test panels will be offered.

B12 Express	My B12	My B12+	Nutristasis Vitality
Next day turnaround*	7-day turnaround**	7-day turnaround**	7-day turnaround**
Measures Total B_{12} and Active B_{12}, both of which are needed for healthy nerves, brain function and red blood cells.	A comprehensive B_{12} panel including Total B_{12}, Active B_{12}, and methylmalonic acid (MMA). MMA is considered the closest to a gold standard available for detecting B_{12} insufficiency.	This extended panel supports investigation of not only Total B_{12}, Active B_{12} and methylmalonic acid (MMA), but also folate (B_9) and homocysteine.	This extended panel tests for key markers that are linked with fatigue and anaemia.
Active B_{12}	Active B_{12}	Active B_{12}	Active B_{12}
Total B_{12}	Total B_{12}	Total B_{12}	Total B_{12}
-	Methylmalonic Acid (MMA)	Methylmalonic Acid (MMA)	Methylmalonic Acid (MMA)
-	-	Folate (B_9)	Folate (B_9)
-	-	Homocysteine	Homocysteine
-	-	-	Ferritin
-	-	-	Vitamin D

Why methylmalonic acid & homocysteine make a difference

Vitamin B_{12} insufficiency/deficiency is common in mixed patient populations. However, there is no single marker which can reliably diagnose vitamin B_{12} status. The timely detection and correction of vitamin B_{12} deficiency prevents macrocytic anaemia, elevated homocysteine (thrombotic risk factor), potentially irreversible peripheral neuropathy, memory loss and other cognitive deficits.

Low concentrations of serum vitamin B_{12} are often used to assess vitamin B_{12} status but this approach generates a high rate of false negative results. Emerging evidence indicates that 'active B_{12}' may be a more reliable indicator of vitamin B_{12} status and so active vitamin B_{12} has been included, as standard, in all the panels.

For further investigation, metabolic markers of vitamin B_{12} status include circulatory levels of methylmalonic acid and homocysteine. Elevated concentrations of methylmalonic acid are considered the most representative marker of metabolic vitamin B_{12} insufficiency. However, poor availability of testing means that this test is often not available to patients.

Find out more about the new service

For further information about this service, please visit the Viapath Nutris Facebook page.

The Viapath Nutris website will go live towards at the end of February and will have details on how to order the test.

Https://nutris.viapath.co.uk

The application of next generation sequencing to the diagnosis of inherited platelet disorder

What are inherited platelet disorders?
Platelets are small anucleate cells, produced in the bone marrow, which play a major role in blood clotting. Normally there are between 150,000 - 450,000 platelets/uL of blood. Patients with a low platelet count, defined as less than 50,000 platelets/uL of blood, are described as having thrombocytopenia and this may be a sign that the patient has an inherited platelet disorder.

The inherited platelet disorders (IPDs) are a large group of individually rare diseases characterised by one or more of the following: thrombocytopenia, altered platelet shape, size, structure and/or function. The main symptom of the IPDs is mucocutaneous bleeding of varying severity, ranging from easy bruising to severe bleeding after injury or surgery.

'Genetic analysis offers the potential of a quick and definitive diagnosis and clinicians are increasingly requesting this as first line test if they suspect IPD'

Worldwide, the estimated frequency of the IPDs is around 1 in 10,000[1] but this is likely to be an underestimate as many affected individuals have very mild or even no bleeding symptoms and often only come to medical attention following an incidental finding of thrombocytopenia during routine blood testing. Although severe bleeding is not an issue for the vast majority of patients, a number of the IPDs are associated with serious extra-haematological symptoms such as pre-senile cataracts, early onset sensorineural deafness, renal failure and an increased risk of developing cancer, especially leukaemia, so identifying those at risk is important for their future management and quality of life.

How are the IPDs diagnosed?
Currently diagnosis is based on bleeding history, platelet count and platelet function tests. However, these are labour intensive and not very well standardised between laboratories. In fact studies have shown that they are unable to detect a platelet defect in around half of the patients' referred[2].

Genetic analysis offers the potential of a quick and definitive diagnosis and clinicians are increasingly requesting this as a first line test if they suspect an IPD. However, deciding which gene to analyse can be difficult - platelet development is a complex, multistage process and involves the expression of many genes, and mutations in any one of these could potentially give rise to an IPD (Table 1). So far mutations have been found in more than 50 genes in association with platelet disorders.

In Viapath's Molecular Haemostasis laboratory the targeted analysis of 15 genes by PCR and Sanger sequencing is currently offered. However as several genes may need to be analysed, diagnosis can be time consuming and costly. Next generation sequencing technologies offer a rapid method for determining the causative variant in affected individuals as they permit the analysis of multiple genes simultaneously. Thus a pilot study was carried out to investigate the validity of this approach.

Development of a new testing regime for IPD
A targeted exome sequencing was used to analyse a panel of 60 genes in 8 of our patients who had been investigated previously and in whom no putative pathogenic variant had been identified and the results are presented in Table 1.

Patient	Diagnosis	NGS results
1	Familial macrothrombocytopenia	*MYH9* - p.Val1516Met; c.4546 G>A (Het)
2	Macrothrombocytopenia	*MYH9* - p.Asp1424Asn; c.4270 G>A
3	Macrothrombocytopenia	*GP1BA* - p.Asn150Ser; c.449 A>G
4	Familial thrombocytopenia	*GFI1B* - p.Cys168Phe; c.503G>T
5	Thrombocytopenia	*GFI1B* - p.Cys168Phe; c.503G>T
6	Thrombocytopenia	*TBXAS1* - p.Arg169His; c.506G>A
7	Familial thrombocytopenia	*FLI1* - p.Arg438His; c.1313G>A and *FERMT3* - p.Gly44Arg; c.130G>A
8	Familial thrombocytopenia	Causative variant not identified

Table 1: Variants identified in patients with IPD using next generation sequencing

Putative pathogenic variants were identified in "platelet genes" in 7 of our 8 patients, five of which were clearly causative.

Patients 1 and 2 had a variant in *MYH9*, both of which have been reported in association with thrombocytopenia[3]. The *MYH9* gene codes for the heavy chain of non-muscle myosin IIA, a cytoskeletal protein which is involved in several processes including cell motility and maintenance of cell shape. Some variants in *MYH9* have been associated with early onset deafness and/or cataracts but these were not detected in this study.

Patient 3 was found to have a heterozygous variant in *GP1BA*. This gene codes for part of the GP1b-IX-V complex which is required for megakaryocyte maturation and normal platelet morphogenesis and *GP1BA* variants have been reported in autosomal dominant Bernard-Soulier Syndrome[4].

Two unrelated patients, 4 and 5, were found to have a heterozygous variant in *GFI1B*, which has been reported previously in patients with mild to moderate thrombocytopenia[5]. Variants in *GFI1B*, which encodes a transcription factor, have been associated with an increased risk of developing leukaemia and other cancers[6] so the patients will be informed and monitored.

Putative pathogenic variants were also identified in platelet-specific genes in 2 other patients which are likely to be responsible for their thrombocytopenia. A novel heterozygous mutation in *TBXAS1* was identified in patient 6. Variants in this gene, which codes for thromboxane synthase, have been reported in autosomal dominant bleeding disorder, platelet type 14[7]. However affected individuals usually present with significant bleeding, defective platelet aggregation and normal platelet count, which suggests that there may be other moderating factors contributing to the milder phenotype in this patient.

In patient 7 heterozygous variants in 2 genes, *FLI1* and *FERMT3,* were identified. The transcription factor *FLI1* is involved in regulating gene expression during platelet production and variants have been reported in association with mild thrombocytopenia, reduced dense granule secretion and symptoms such as eczema and alopecia[8]. A recent study found that *FLI1* variants are common in patients with thrombocytopenia[9]. *FERMT3* encodes kindlin which has been shown to be involved in integrin activation and inside-out signalling. To date only a few

pathogenic variants have been reported in this gene, and all of them are associated with Leukocyte adhesion deficiency-3 which is characterised by immune deficiency and Glanzmann Thrombasthenia-like bleeding[10]. The phenotypic data on our patient was incomplete but they were reported to have only mild bleeding so analysis is ongoing to determine if these variants are truly causative.

Thus, using this new approach putative pathogenic variants were identified in all but 1 of our 8 patients which is a diagnostic hit rate of ~80%. These exciting results clearly demonstrate that next generation sequencing can provide a rapid molecular diagnosis in patients with IPDs even in the absence of detailed phenotypic data. This test is now being adopted as part of our diagnostic repertoire and it is anticipated that it will significantly improve patient treatment and prognosis.

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