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Research Article

Next Generation Sequencing in Metabolic Myopathies: Experience from a Tertiary UK Adult Neurometabolic Unit

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Abstract

We investigated the diagnostic yield of a 30 gene sequencing panel for metabolic myopathy (MM) in 54 patients presenting to our tertiary neuro-metabolic clinic. A diagnosis was achieved in 11% of patients however there were many variants of unknown significance. Targeted genetic testing is preferable if a clear phenotype is encountered, but distinguishing features are not always present in patients presenting with a potential MM. In such cases, use of an NGS panel can facilitate diagnosis.

Keywords: *Metabolic; Myopathy; Rhabdomyolysis; Sequencing; Storage; Exercise*

Introduction

Hereditary metabolic myopathies in adults are characterised by defects in muscle energy production typically manifesting as exercise intolerance, muscle cramps, weakness, and rhabdomyolysis. Most disorders are inherited in an autosomal recessive fashion, with some X-linked and rare autosomal dominant gain-of-function mutations also described. Diagnosis is challenging as symptoms are often vague and may be disregarded by both the patient and clinician [1]. Diagnosis allows implementation of measures to prevent rhabdomyolysis (e.g. dietary changes, supplements, exercise programmes, and specific treatments).

Traditionally, these disorders have been diagnosed by biochemical tests and muscle biopsy with genetic confirmation by candidate gene testing performed depending upon the results, however this is invasive and often very slow. Genetic testing using next generation sequencing (NGS) systematically screens for pathogenic variants in multiple genes in parallel and has the potential to improve the diagnostic process in such patients, facilitating diagnosis in cases where clinical features are not classical. NGS is a relatively new technology and the identification of variants of unknown significance (VUS) are common, representing a challenge for clinicians [2]. Over time many apparent VUS are being re-categorised as either pathogenic or benign as evidence accumulates, however follow-up of patients with VUS varies amongst institutions [3]. We analysed results of an NGS approach to the diagnosis of metabolic myopathies in adult patients referred to a tertiary metabolic/neuroscience centre in the UK.

Methods

An NGS panel of 30 genes (Supplementary material) associated with metabolic myopathies has been used in the neurology and metabolic departments of Salford Royal NHS Foundation Trust since 2015. We performed a retrospective case note review of patients identified from a local database of adults presenting to our service with symptoms suggestive of a metabolic myopathy who had been investigated using this NGS panel.

We collected data regarding age at presentation, maximum serum total creatine kinase level, episodes of rhabdomyolysis and other presenting symptoms. Results of any previous genetic testing were also obtained. Variants from the NGS panel were assessed for pathogenicity using the American College of Medical Genetics (ACMG) criteria [4].

NGS panel

The NGS panel used in this study was provided by Sheffield Regional Genetics Centre on the Illumina MiSeq using the MiSeq Reagent Kit v2 performing 2 x 150 bp end paired reads. Reads were aligned to human genome build hg19 and annotated using dsSNP and COSMIC. Variants were also filtered against in-house polymorphism lists prior to being reported back to the referring clinicians. All clinically significant sequence variants were confirmed by Sanger sequencing.

Results

We identified 54 patients from our database with an average age of 32.9 years, 16 of whom were female. 37/54 (69%) patients received NGS as a first-line genetic investigation. Other targeted genetic tests undertaken prior to NGS included: *PYGM* (14/54), *CPT2* (3/54) and a mitochondrial DNA panel (2/54).

Overall, 6/54 (11%) patients referred for NGS panel sequencing had pathogenic variants achieving a definitive diagnosis without the need for further investigation (Table 1). Most of these patients (5/6, 83%) had at least one episode of rhabdomyolysis, compared to 26/44 (61%) of those where a definitive diagnosis was not reached without further investigation, however this difference did not reach statistical significance ($p = 0.387$). None of these patients required muscle biopsy for diagnosis.

We identified 4 patients who were carriers of known pathogenic mutations in metabolic myopathy genes however did not carry any other variants *in trans* to allow a conventional diagnosis (Table 1). There remains no consensus on how to classify a patient as a manifesting heterozygote and, as such, a diagnosis made on such evidence may be controversial. For the patients with mutations in *GBE1* [5] and *PGAM2* [6] there is evidence for manifesting heterozygotes in the literature so a contribution of these variants to the patient's phenotype could be considered a possibility.

One patient was found to be compound heterozygous for VUS in *PHKG1*. This 25 year old male presented with dizziness and reduced exercise tolerance. He had bilateral ptosis with myopathic facies. Biochemical testing for Pompe disease and common *PYGM* mutations was negative. His NGS panel returned two variants (p.E195K and p.V218I) in trans in *PHKG1* which has been suggested as a candidate gene for Glycogen Storage disorder type IX (GSD IX) as the protein product is known to interact with other important genes for the condition (*PHKA1, PHKA2, PHKB* and *PHKG2*) [7]. Both of these variants are rare (< 0.001 MAF), predicted to be damaging to protein function and occur within residues showing evolutionary conservation. This is coupled with a muscle biopsy which has shown excessive glycogen storage in our proband. Functional analysis was undertaken by measuring plasma phosphorylase kinase levels and glycolytic enzyme analysis. The white blood cell total phosphorylase A and B was normal (42 units) although the ratio of phosphorylase A to total was slightly low at 0.32 (reference range 0.4 - 0.83), suggestive of GSD IX, although we do acknowledge much lower values in classical cases.

We also identified one patient with an *RYR1* VUS (pT3711M) which has not previously been described in the literature but was susceptible to malignant hyperthermia on functional biopsy testing. A further patient with a single *CPT2* VUS (pM342T) was also found to have

No	Age	Symptoms	Episode of Rhabdo	Highest CK	Free Carnitine	Variants	Diagnosis
Conventional Diagnosis							
1	29 F	Muscle aches after exercise	Yes	6000	Normal	*CPT2* p.S113L Homozygous *ACADVL* p.L299del Carrier	CPT2 deficiency
2	24 M	Muscle aches after exercise	Yes	13 000	Normal	*CPT2* p.S113L Homozygous	CPT2 deficiency
3	41 M	Muscle aches during concurrent illness	Yes	60 000	Normal	*CPT2* p.P50H *CPT2* p.P595fs *LPIN1* exon 18 deletion Carrier	CPT2 deficiency
4	16 M	Intermittent flu-like symptoms	Yes	19 900	Raised (40.1) Normal profile	*PYGM* p.R50* Homozygous	GSD5
5	35 M	Muscle aches after exercise, myoglobinuria, second wind phenomenon. Consanguineous parents.	No	3000	Normal	*PYGM* p.L5* Homozygous	GSD5 Previously tested for PYGM and negative
6	39 M	Muscle aches after exercise.	Yes	15 000	Not tested	*RYR1* p.S1728F Heterozygous	RYR1-related myopathy PYGM neg
Heterozygotes for pathogenic alleles							**Comment**
7	33 M	Muscle aches after exercise. Myopathic muscle biopsy.	Yes	17 000	Not tested	*GBE1* c.691+2T>C	GSD IV PYGEM neg
8	32 M	Muscle stiffness and dark urine.	Yes	80 000	Raised (44.6) Normal profile	*GAA* c.-32-13T>G	Pompe
9	23 F	Fatigue and muscle aches. Myopathic EMG.	Yes	11 000	Normal	*ETFDH* pL334P	MADD PYGM neg
10	28 M	Muscle cramps, episode of renal failure but not rhabdomyolysis.	No	2000	Not tested	*PGAM2* pW78*	GSD X

Table 1: *Six of 54 patients where a diagnosis was confirmed from the next generation sequencing panel without the need for further investigation. Four other patients were carriers for pathogenic mutations. CK, total serum creatinine kinase. MH: Malignant Hyperthermia; GSD: Glycogen Storage Disorder; CPT2: Carnitine Palmitoyltransferase 2 Deficiency; MADD: Multiple Acyl-CoA Dehydrogenase Deficiency.*

reduced enzyme activity on skin fibroblasts. 15 further VUS in 13 patients were identified, for which we had no biochemical or histological evidence of pathogenicity had been obtained during the study period. Four of these VUS occurred in *RYR1*.

Discussion

The overall diagnostic yield of the metabolic myopathy NGS panel in our cohort was 11%. It is noteworthy that Patient 5 was found to have GSD V due to homozygous p.L5* variants which was not picked up by common 'hot spot' *PYGM* mutation testing, whilst Patient 4 was found to have GSD V due to p.R50* variants which would have been picked up on targeted common *PYGM* mutation testing had it been employed.

Another important consideration for the NGS panel is the VUS burden. *RYR1* variants are common and, in this study, five patients underwent muscle biopsy for functional studies to establish the impact of an *RYR1* variant found on NGS. To date, confirmation of malignant hyperthermia susceptibility has only been found in one of these patients. Therefore, it is possible that the number of muscle biopsies avoided by use of an NGS panel is negated by the need to clarify the significance of *RYR1* variants.

There are few reports on the use of NGS in a metabolic myopathy cohort, however, Wu., *et al.* performed panel sequencing on 169 patients with muscle weakness, rhabdomyolysis and hyperCKaemia. This panel included 183 genes and was primarily aimed for the detection of muscular dystrophies however the diagnostic yields in the rhabdomyolysis and hyperCKaemia cohorts were 33% and 31% respectively [8]. These newer technologies have been praised in reports by Walters., *et al.* who describe the use of whole exome sequencing as paramount in the diagnosis of a 14 year old girl with McArdle disease despite initial negative metabolic testing and non-specific electromyography [9]. This reflects the potential benefits of NGS where traditional testing has failed to identify a diagnosis.

Although our results are of interest, this was a relatively small retrospective study and we also relied upon clinical documentation as a proxy for clinical suspicion of MM. There is the possibility that clinical suspicion of MM was actually low and hence referral for a non-invasive NGS test was thought preferable by the referring clinician.

Conclusion

The use of an NGS gene panel can help in the diagnosis of complex cases with variable phenotypes however its diagnostic yield is still low. Wherever possible, targeted genetic tests are preferable to avoid VUS and to streamline diagnosis. There does not appear to be enough evidence at present to suggest an NGS panel as a first-line investigation for MM.

Ethical Publication

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Author Contributions

AJ collected date, contributed to bioinformatic analysis and wrote the manuscript. JBL and RS provided expert opinion and reviewed the manuscript. RS conceived the idea for this study. All authors read and approved the final manuscript prior to submission.

Competing Interests

None declared.

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Ethics Approval

Not required.

Patient Consent Statement

Not applicable.

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