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Rapid exome sequencing in critically ill children impacts acute and long-term management of patients and their families: a retrospective regional evaluation

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ABSTRACT

Introduction

Genetic disorders are a significant cause of paediatric morbidity and mortality. Rapid exome sequencing was introduced by the National Health Service (NHS) in England on 1st October 2019 for acutely unwell children with a likely monogenic disorder, or to inform current pregnancy management where there was a previously affected child or fetus. We present results of a 12-month patient cohort from one large clinical genetics centre in England.

Methods

Patients were identified through local genetics laboratory records. We included all cases which underwent rapid exome sequencing between 1st October 2020 and 30th September 2021. DNA was extracted, quality checked and exported to the Exeter Genomic laboratory where library preparation, exome sequencing of all known human genes, gene-agnostic bioinformatic analysis, variant interpretation, MDT discussions and reporting were performed.

Results

Ninety-five probands were included. Trio analysis was performed in 90% (85), duo in 8% (8), singleton in 2% (2). The median turnaround time for preliminary reports was 11 days. The overall diagnostic yield was 40% (38 patients); 36% (34 patients) made solely on exome with a further 4% on concomitant exome and microarray analysis. Highest diagnostic rates were seen in patients with neuro-regression, skeletal dysplasia, neuromuscular and neurometabolic conditions.

Where the diagnosis was made solely through exome sequencing, management was altered for the proband or family in 97% (33/34). For the proband, this was most commonly that the diagnosis was able to inform current management and prognosis (20 patients, 59%), as well as direct specialist referrals (10 patients, 29%). For families, the exome sequencing results provided accurate recurrence risk counselling in 88% (30/34) with cascade testing offered if indicated in some families.

Conclusions

In the majority of cases, the genetic diagnoses influenced acute and long-term management for critically ill children and their families. Paediatric and neonatal clinicians in the NHS now have direct access to exome sequencing for their patients. The rapid turnaround time was particularly helpful to alter the management in acute clinical settings and is a powerful tool for diagnosing monogenic conditions. This study is an example of a highly successful integration of a national rapid exome sequencing service with diagnostic rates comparable to previously reported literature.

INTRODUCTION

Genetic disorders are a leading cause of infant mortality. An estimated 13% - 15% of children admitted to intensive care unit have an underlying genetic diagnosis.[1,2] Progression of disease can be extremely fast in this cohort of children and hence early genetic diagnosis is often helpful to influence acute management, which can subsequently reduce suffering, morbidity and mortality.[2-4]

Critically unwell children without a diagnosis often embark upon a diagnostic odyssey comprising multiple specialist consultations, invasive and non-invasive investigations including multiple genetic tests, sometimes continuing after the patient is deceased.[5]

In the past 6 years, there have been multiple studies demonstrating the utility of rapid (7-21days) and ultra-rapid (24 hours-7 days) whole exome sequencing (WES) and whole genome sequencing (WGS) in neonatal intensive care unit (NICU) and paediatric intensive care unit (PICU) settings. The reported diagnostic rates vary from 17-70% and the percentages of genetic diagnosis influencing management from 30%-100%.[1-3, 6-20] Diagnostic results for the patient also has implications for their wider family and can affect management of planned or ongoing pregnancies.

In the UK, the National Health Service (NHS) is a publically funded and government led healthcare system. The NHS provides services to all its population free of cost at the point of access. The genetics services in the UK are formed of 17 genetics centres which are recently reformed into Genomic Medicine Service Alliances (GMSA). These cover regions containing all tiers of healthcare including neonatal and paediatric intensive care units. As part of integrating next generation sequencing into mainstream medicine, NHS England published the National Genomic Test Directory[21] and launched the 'Rapid Exome Sequencing Service for acutely unwell children with a likely monogenic disorder' on 1st October 2019. This service is categorised with the code 'R14' in the requesting National Genomic Test Directory and is available in all regions of England with equity of access as one of the main aims. The R14 service is available predominantly for babies and children admitted to NICU and PICU who

are acutely unwell and have features suggesting that a genetic cause is likely to be the underlying pathology, although it is also offered in some casesof neuroregression and for a couple who are currently pregnant and have had an affected child or fetus with a potential monogenic disorder. In these clinical situations, a rapid result is required, to assist with acute interventions or clinical decisions. In collaboration with a clinical geneticist, paediatric and neonatal clinicians can request testing for their patients and the testing is funded centrally by NHS England without direct cost to the patient or hospital in the free-at-the-point-of-delivery NHS system. Eligible patients are discussed with clinical genetics colleagues and approval is obtained after multidisciplinary team discussion with the Exeter Genomics laboratory (testing site). Following parental consent, trio blood samples are sent to the local genetics laboratory for DNA extraction, quantification and quality checks and subsequent sample export to the testing site. The library preparation, exome sequencing, bioinformatics analysis, variant interpretation and reporting is performed by the Exeter Genomics Laboratory[22]. This consists of trio (affected child and both unaffected parents) exome sequence analysis of the coding region and conserved splice sites of 23,244 genes. The DNA is prepared for sequencing as per the manufacturer's protocol (Twist Biosciences) and sequenced on a NextSeq 500 or NovaSeq sequencer (Illumina, San Diego, CA, USA). The data is processed following GATK (v3.4) best practice guidelines. An in-house bioinformatics pipeline is applied to identify *de novo*, compound heterozygous, homozygous or X-linked variants. Copy number variants are identified using read depth analysis with an-in house tool and comparing the test sample against reference samples. The variants identified are classified according to the ACMG-AMP guidelines for variant interpretation.[22,23]

Other genetic testing is completed at the relevant local genomic laboratories in parallel to the rapid exome sequencing such as microarray analysis and testing for relevant genetic conditions where exome sequencing has reduced sensitivity or is unable to detect the mechanism of disease. These include triplet repeat expansion disorders, methylation abnormalities seen in imprinting disorders and copy number variant mediated disorders. If a mitochondrial disorder is suspected, then mitochondrial DNA testing is performed through specialist genetics laboratories in England.

AIMS

We evaluated the cases which were referred to rapid exome sequencing from the West Midlands region between 1st Oct 2020 and 30th September 2021. Our aim was to evaluate the turnaround time to results, diagnostic rate and impact of a genetic diagnosis on patient and family management. We also aimed to evaluate demographics and locations of the referred patients and the spectrum of genetic diagnoses identified.

METHODS

All patients referred through the West Midlands region who had rapid exome sequencing testing under the R14 category activated between 1st October 2020 and 30th September 2021 were eligible and identified from the local genomic laboratory database. Exclusion criteria were where the test activation was outside of this time-frame and/or any patient where testing was declined or became ineligible by the R14 criteria before testing was activated. All testing was undertaken following informed parental consent. A standardised Microsoft Excel proforma was used to retrospectively collect data from patient case notes. Data were anonymised and analysed using Microsoft Excel.

Primary outcomes were the turn-around time to results and the diagnostic rate.

Secondary outcomes were the impacts on management of a genetic diagnosis for probands and families, demographics and the spectrum of molecular diagnoses made.

RESULTS

Over the course of the defined time period, a total of 102 patients were identified, of which 7 were excluded. Therefore, 95 patients were included, of which 79 (83%) were tested for the indication of an acutely unwell baby or child and the remaining 16 (17%) to inform management of a current

pregnancy in a family who had a previously affected fetus or child. Demographics and diagnostic rates for the 95 cases are detailed in table 1.

The majority of the cases were enrolled from either NICU or PICU (40% and 34% respectively). The remaining patients (25%) were at home or other ward environments; these were cases where testing was occurring due to a new pregnancy with a history of a sibling or fetus with a likely genetic condition. Some cases of neuroregression were also enrolled where the genetic diagnosis would impact management if they became ill such as withdrawal of care although the number of such cases was restricted nationally.

A likely genetic cause for the features was identified in 34 cases (diagnostic rate of 36%) including 3 partial diagnoses. An additional 4 diagnoses (4%) were made on concurrent microarray analysis with subsequent exome confirmation. The total diagnostic rate was therefore 40% (38 cases), with no genetic cause identified in 60% (57 cases). Table 2 provides details of all diagnoses made exclusively on exome analysis.

Patients presenting with developmental regression, neuromuscular disorders, suspected neuro-metabolic/mitochondrial disorders, hypotonia or skeletal phenotypes had higher diagnostic rates (between 50-75%).

A variant of uncertain significance (VUS) was reported in 3 patients (3%). In all VUS cases, the result was discussed with the referring clinical team on multidisciplinary team meeting and only those thought to be relevant (e.g. where additional information/testing now or in the future may lead to variant re-classification) were included in the final report[3].

There were two cases of glucose-6-phosphate dehydrogenase (G6PD) deficiency identified as incidental findings. A *de novo TGFB2* variant was also found incidentally, it was not thought to be contributing to the presenting phenotype but was discussed and reported as likely pathogenic. This patient was referred to the cardiology team for ongoing monitoring.

Requestors

The largest number of exome analysis (33, 35%) were requested by neonatal teams caring for babies on NICU, this was followed by clinical genetics who requested 24 (25%). Paediatric intensivists on PICU and paediatric neurologists also requested a large proportion of exomes, 19 (20%) and 10 (11%) respectively. The remaining 7 exomes (7%) were requested by other mainstream paediatric specialities.

Turn-Around Times

The median turnaround time for reports was 11 days. Final reports were delivered within 14 days in 74 (78%) and 10 (11%) were issued between 15 and 21 days. The longest turnaround time to final report was 101 days.

Management

The genetic or partial diagnosis influenced the overall management in 97% of cases (33 cases). In 88% (30 cases) the genetic diagnosis was primarily of utility for supporting genetic counselling by providing an accurate recurrence risk and provision of a prenatal diagnosis where indicated. There were multiple management implications in a proportion of cases (Table 3).

DISCUSSION

This study illustrates the successful integration of rapid exome sequencing into routine clinical practice allowing mainstream clinicians such as those working in neonatal and paediatric intensive care settings to access exome sequencing for their patients. This success is demonstrated by the high requesting rates in mainstream specialities.

Turn-Around-Time

The rapid turn-around time (TAT) of exome results (14 days in 78%) is an important advantage of this type of testing in the context of NICU/PICU cases and current pregnancies.

Where final reports required more extensive timelines, this was mainly due to the complexity of the findings which required multidisciplinary team discussions and/or arranging further testing to aid the result interpretation.

Alternatives in literature include ultrarapid exome sequencing, which give a faster result but require significantly more resources. Balancing the need for a rapid response with the ability to provide a service to a wide number of patients with the resources available is important within health systems, while also considering that consenting for a genetic test can be a difficult decision for many parents with the many potential outcomes explained to them. Providing a result for a child who is critically unwell within 24 hours, while potentially clinically informative, might not allow parents adequate time to absorb all the information and prepare themselves for a diagnosis at a stressful time, or

when palliative care routes are to be considered with a lethal diagnosis. It may be recommended with larger amounts of data, to further analyse how many diagnoses would change management and outcomes for a child if made within 24-48 hours rather than 7-10 days to understand the scale of any benefits in shortening turnaround times further. This would also need to be explored with studies looking at the psychological impact on families of these two approaches.

One of the delays identified in a local pathway audit was in time between consenting, time for samples to be taken and sent to the regional genetics laboratory from hospitals. Postulated and reported issues contributing to this include difficulty in availability of both parents for trio samples, local phlebotomy organisation particularly during the COVID pandemic, communication between laboratory and clinical staff, particularly when samples need to be sent from one hospital to another for DNA extraction. Misunderstandings in local pathology laboratories, logistics of sample transport, and potential lack of training of the clinical staff to understand the need for rapid dispatch to the genetics laboratory were likely contributing factors to delay, adding multiple days to the turn-around-time between consent and result, rather than time from commencement of testing to result.

Diagnostic Rate

The total diagnostic rate of 40% in this cohort is comparable with other similar methodologies such as reported from China and Australia (31-52%)[18-19] within their healthcare systems. Diagnostic rates in other studies appear to range from 17-70%; the largest cohort to date reported in Jama Paediatrics in 2017 had a diagnostic rate of 37% in trio rapid exome sequencing and a similar TAT of 13 days. [1-3, 6-20] Two other studies in China and Australia[18-19] have reported a similar sample size with diagnostic rates of 31% and 51% respectively, they were more selective in recruiting cases and some used both exome and genome sequencing, which increases diagnostic yield.

The majority (89%) of exomes performed in our study were trios. This allows a gene-agnostic approach in analysis and provides immediate inheritance information, which can be used both for

variant classification and for counselling patients and families. Singleton analysis was performed in two cases where trio samples were not available, but this was after individual case discussion with clinical scientists to use gene panel testing. On a larger scale this approach would not be possible due to the intensity of time and interpretation process required.

Diagnostic rates vary with phenotype assessed prior to testing, with developmental regression achieving the highest diagnostic rate. However, as the sample number is small in these categories it is difficult to draw firm conclusions as to whether these findings would be replicated across a larger population.

While WES offers a good diagnostic rate it is important to be aware of the potential limitations and therefore what concurrent testing should be employed. While all four copy number variant diagnoses made on microarray in our cohort were confirmed on WES, WES is not as sensitive as microarray for this indication and can miss smaller changes, therefore microarray testing should be considered simultaneously for all patients. In a similar way, for patients presenting predominantly with hypotonia, multiplex ligation dependent probe amplification (MLPA) of *SMN1* should be considered to identify spinal muscular atrophy. Additionally, it should be noted that while some imprinting defects such as a deletion or uniparental disomy may be identified, methylation defects would remain undetected by WES. If an imprinting condition such as Prader-Willi is suspected, targeted testing should be undertaken in parallel to WES to prevent delays in identifying a genetic diagnosis.

Incidental and Uncertain Findings

Incidental findings are results of potential clinical significance which are unrelated to the primary purpose of the test. There is the additional possibility that trio testing can reveal non-maternity and non-paternity. Current ACMG guidelines recommend the reporting of incidental findings and suggest that these should be included in pre-test counselling[24]. Incidental findings may be a cause of

trepidation for clinicians, however we demonstrate an extremely low yield within our cohort, including non-paternity (1 patient only). The three incidental genetic diagnoses were significant and in themselves warranted further management. Uncertain findings, where genetic variants are identified but not thought to be related or where there is insufficient evidence can be a source of anxiety for clinician and patient/ parent. This report demonstrates a very low number of such reports, with only 3 being felt significant enough after multidisciplinary discussion to report, this reflects careful bioinformatics pipeline and the clinical scientist team input during variant analysis. We therefore encourage clinicians to follow the recommendations to include these possibilities in pre-test counselling as although these findings are rare, they can be significant. The national consent form for the R14 service specifies that these are discussed with the families.

Impact on Patient and Family Management

In the 34 patients where a diagnosis was made, nearly all experienced a change in management for the proband or their family, demonstrating the clinical utility of the testing. This is higher than in other literature and when considering the patient only, not the family, the proportion is around 60%. However, the impact of a diagnosis on management has not been consistently reported across most studies, with reports of an impact ranging widely from 30-100%.

In 24%, the diagnosis supported reorientation to palliative care which is a highly significant clinical decision and implication for the patient and family, where doubt of an underlying diagnosis and its outcome can lead to ongoing futile care and/or difficulty in decision making. In 3% a post-mortem examination was avoided and in another 3% a liver transplantation occurred after diagnosis was made. These are life-changing decisions and interventions supported by accurate and timely genetic information provided by the rapid exome sequencing. Patients were able to gain more information about their condition or prognosis or be referred to specialists for care as a result.

The majority of parents were able to receive accurate recurrence risk counselling and a small number had cascade screening through the family, allowing for better informed preconception and prenatal counselling and testing. As our study only covers a short period it is possible that over time more of these families will access cascade screening, prenatal counselling and possibly even preimplantation genetic diagnosis.

It is also important to note that several of the diagnoses made within the cohort were well recognised genetic conditions such as Kabuki syndrome, Robinow syndrome and CHARGE syndrome, other diagnoses were made of ultra-rare genetic conditions such as ATP6AP1-related liver condition and PAX1-related otofaciocervical syndrome type 2. Without exome sequencing the diagnostic delay for these patients may have been very protracted.

Future Directions

As mentioned the current R14 rapid exome service within England is open only to eligible patients with a likely monogenic disorder in the neonatal or paediatric intensive care setting, in some cases of neuroregression or where there is a current pregnancy with a previously affected child. However there are other indications with a potentially high diagnostic yield where patients may benefit from rapid diagnosis to inform management even outside of the intensive care setting. This is likely to form part of future indications for rapid exome or genome sequencing with fast track testing more readily available. However, NHS England has launched genome sequencing for all such indications and as a result testing is available with a faster turnaround time than before.

The Genomic Medicine Service in England has introduced prenatal rapid exome sequencing for fetal anomalies with a likely monogenic disorder using a large panel of genes where features may be evident prenatally. This is indicated for those where a molecular diagnosis may influence pregnancy or early neonatal management and follows from the PAGE and BOOST studies[25-26]. It is possible that as rapid exome sequencing becomes more available prenatally, that this may change the numbers of

children referred for rapid exome sequencing in the postnatal period or potentially affect the diagnostic rate for this service, as the neonatal age group have a slightly higher diagnostic rate than older children.

The introduction of whole genome sequencing (WGS) is envisaged to ultimately replace whole exome sequencing within NHSE. A transition to WGS which allows the analysis of a wider genomic region and at a higher level of resolution, is likely to be allied to an increase in the diagnostic yield. WGS also has the advantage of identifying a wider range of genetic variants eliminating the need to request multiple tests (e.g WES with microarray analysis). The careful delineation of the applicability of this technology is needed to limit the uncertainty of the results and the identification of variants unrelated to the reason for testing.

Gathering feedback from families who have utilised this service, after the acute clinical time period is another area where further research should be conducted to identify areas of benefit to families and areas of the service that can be enhanced to improve patient experience.

Limitations

This is a study in a single region of England and is beholden to the inherent limitations of using retrospective patient documentation from a single specialty for information. It may be that this has underestimated the potential impacts on management as they may not be documented outside of paediatric services notes or apparent yet, at the child's current age in the time-frame included. However, the results of testing and turnaround times are taken directly from the laboratory reports to maximise accuracy in these results. While the results may be informative in terms of the national service, the involvement of mainstream clinicians and the patient population in our area may not be directly comparable to all other regions of the UK or further afield. However, it is reflective of the clinical application and practice that is occurring outside of a research setting and so does provide insight into some of the successes and pitfalls of the programme.

Conclusion

This report demonstrates the success for our centre of rapid exome sequencing being embedded into routine clinical practice for patients with equity of access for all eligible patients. The service is a successful model for allowing mainstream clinicians access to testing with supervision of clinical geneticists. There is clinical utility of rapid exome sequencing in identifying the genetic diagnosis and impacting immediate and long-term management in acutely unwell children and their families with the information being used to inform current pregnancy management as well as future family planning. The unique challenges of mainstreaming a complex specialised diagnostic test can be surmounted by multidisciplinary team working and this should be encouraged widely. Whilst advances in the technology with the implementation of more advanced methodologies will inevitably solve some of the unsolved cases and lead to an increase in diagnostic rates, the re-analysis of already available WES data as the patients phenotype evolves and additional new genetic data becomes available in the literature, needs to be considered as an invaluable option to improve the diagnostic rate.

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Contributions

H.McDermott designed the proforma for data collection and drafted sections of the original manuscript. C.Sherlaw-Sturrock collected data from patient records and also drafted sections of the original manuscript. J.Baptista carried out variant interpretation analysis and reviewed and revised the manuscript. L. Hartles-Spencer coordinated sample processing and reviewed and revised the manuscript. S.Naik conceptualised the evaluation, co-designed the proforma for data collection and reviewed and revised the manuscript. All authors meet the following criteria: (1) substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of the data, (2) drafting the work or revising it critically for important intellectual content, (3) final approval of the completed version, (4) accountability for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration

No conflicts of interest and no funding to declare.

All reported genetic variants are uploaded to the NHS consortium project within the DECIPHER database to be available to the NHS scientific community.

Ethical Considerations

In accordance with the Health Regulation Authority (HRA) tool, this local service evaluation did not require ethical approval but was registered with trust governance.

Due to the presence of ultra-rare conditions, no phenotypic data are available in conjunction with individual results to preserve patient anonymity.

Table 1: Demographics, referral information and diagnostic rate for R14 Referrals

	Number of Patients N (%)	Diagnostic Rate N (%)
Test Indication		
Acutely Unwell Children	79 (83%)	30 (38%)
Current Pregnancy with Previous Affected Child	16 (17%)	4 (25%)
Sex of Proband		
Male	56 (59%)	21 (38%)
Female	39 (41%)	13 (33%)
Ethnicity of Proband		
Asian (Pakistani/Indian/Other)	29 (31%)	12 (41%)
White (British/Irish/European)	25 (26%)	10 (40%)
Other	5 (5%)	2 (40%)
Mixed Ethnicity	3 (3%)	0 (0%)
Black African/Afro-Caribbean	2 (2%)	0 (0%)
Unknown/ Unavailable	31 (32%)	10 (32%)
Age of Proband at Referral		
Neonates (<1 month)	40 (42%)	14 (35%)
Infants (1 month-12 months)	31 (32%)	10 (32%)
Children (1-16 years)	25 (26%)	7 (28%)
Proband deceased	10 (10%)	3 (30%)
Location During Referral to R14		
Neonatal Intensive Care (NICU)	38 (40%)	16 (42%)
Paediatric Intensive Care (PICU)	32 (34%)	9 (27%)
Other (home, High Dependency, ward)**	24 (25%)	9 (38%)
Unknown	1 (1%)	0 (0%)
Type of Testing		
Trio	85 (90%)	31 (36%)
Duo	8 (8%)	2 (25%)
Singleton	2 (2%)	1 (50%)
Phenotype		
Multiple congenital anomalies and/or dysmorphism syndromes	32 (34%)	9 (28%)
Neurological (including neurodevelopmental/ seizures/ encephalopathy/ structural brain anomalies)	25 (26%)	11 (44%)
Single system disorder with possible monogenic cause	7 (7%)	0 (0%)
Arthrogryposis/neuromuscular	6 (6%)	3 (50%)
Neurometabolic/mitochondrial	6 (6%)	3 (50%)
Skeletal abnormalities	5 (5%)	3 (60%)
Developmental regression	4 (4%)	3 (75%)
Hypotonia	4 (4%)	2 (50%)
Cardiomyopathy	3 (3%)	0 (0%)
Hydrops fetalis	3 (3%)	0 (0%)

Table 2 Table of single gene diagnoses made on exome sequencing analysis.

Genetic Diagnosis	Gene
ABCA12-related harlequin ichthyosis (AR)	*ABCA12*
ABCD5-related retinal dystrophy with leukodystrophy (AR)	*ABCD5*
ACTA1-related nemaline myopathy (AD)(DN)	*ACTA1*
ANKRD11-related disorder (AD)(DN)	*ANKRD11*
ARX-related lissencephaly (XL)	*ARX*
ATP6AP1-related liver condition (XL)(DN)	*ATP6AP1*
BRAT1-related neurodevelopmental disorder (AR)	*BRAT1*
CHARGE syndrome (AD)(DN)	*CHD7*
Osteogenesis imperfecta type 2 (AD)(DN)	*COL1A2*
CSPP1-related Joubert syndrome (AR)	*CSPP1*
DNM1-related developmental and epileptic encephalopathy (AR)	*DNM1*
FOXF1-related congenital alveolar capillary dysplasia (AD)(DN)	*FOXF1*
FOXF1-related congenital alveolar capillary dysplasia with misalignment of pulmonary veins (AD)(DN)	*FOXF1*
G6PC-related glycogen storage disease (AR)	*G6PC*
Gaucher disease type 2 (AR)	*GBA*
GLDC-related non-ketotic hyperglycinaemia (AR)	*GLDC*
KCNQ2-related epileptic encephalopathy (AD)	*KCNQ2*
KMT2D-related Kabuki syndrome (AD)(DN)	*KMT2D*
KCTD7-related neurodegenerative disorder (AR)	*KCTD7*
LAMB2-related Pierson syndrome (AR)	*LAMB2*
MFSD8-related neuronal ceroid lipofuscinosis (AR)	*MFSD8*
MYRF-related cardiac urogenital syndrome (AD)	*MYRF*
PAX1-related otofaciocervical syndrome type 2 (AR)	*PAX1*
Multiple congenital anomalies-hypotonia-seizures syndrome type 1 (AR)	*PIGN*
Pelizaeus-Merzbacher disease (XL)	*PLP1*
Robinow syndrome (AR)	*ROR2*
RYR1-related congenital myopathy (AR)	*RYR1*
SCN2A-related seizures (AD) (DN)	*SCN2A*
SLC6A5-related hyperekplexia (AR)	*SLC6A5*
STXBP1-related developmental and epileptic encephalopathy (AD)(DN)	*STXBP1*
TCIRG1-related osteopetrosis (AR)	*TCIRG1*
SCN2A related disorder (AD) (DN) *	*SCN2A*
UPB1-related beta-ureidopropionase deficiency (AR) * (this diagnosis was made twice)	*UPB1*

(AD=Autosomal dominant inheritance, AR=Autosomal recessive inheritance, XL=X-linked inheritance, DN=confirmed de novo,*=partial diagnosis)

Table 3 Overall implications of management in the 34 patients with a genetic diagnosis through exome sequencing R14 testing

Management Implication	N	%
Proband Management		
Referral to Specialist(s)	10	29%
Informs current management & prognosis	20	59%
Re-Orientation of Care/ Palliation	8	24%
Modification of Treatment (pharmacology, transplant)	1	3%
Surveillance Recommendations	4	12%
Avoided Invasive Testing or Post-Mortem	1	3%
Relevance to parents and or other family members		
Accurate recurrence risk and Prenatal diagnosis/PGD	30	88%
Cascade screening	3	9%