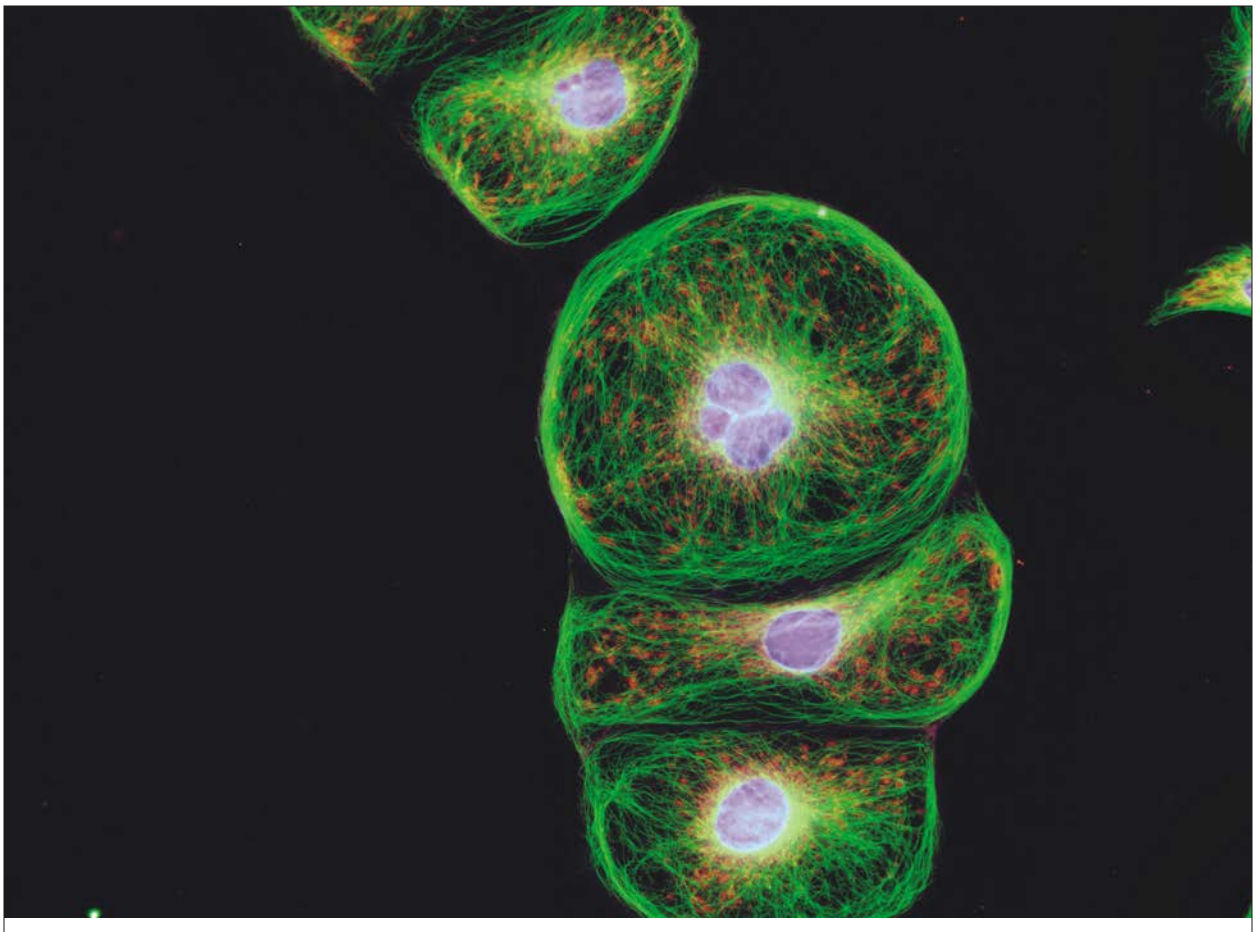

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Use of Exome Sequencing in Inborn Error of Metabolism: A Systematic Review

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ABSTRACT

Over the last few years, the use of Exome Sequencing (ES) has significantly improved our understanding of many complex diseases. Inborn Errors of Metabolism (IEM) are a genetically heterogeneous group of diseases caused by a defect in a metabolic pathway, leading to the production and/or accumulation of toxic metabolites in the body. In this review, we aimed to analyze all the available publications and highlight the advantage issues in the conduct and interpretation of these studies and also to establish, if, the existing data supports any polymorphism to be conclusively associated with IEM. We systematically searched PubMed using MeSH terms include, "Metabolism, Inborn Errors" and "Exome" and all other possible combination from 1/1/1980-29/6/2014. The search returned 54 unique articles of which 19 articles met the established inclusion criteria and were included in the analysis. Overall, 19 studies were selected, include Leigh syndrome (LS, n = 4), Brown-Vialetto-Van-Laere syndrome (BVVL, n = 4), 3-Methylglutaconic aciduria (3-MGCA, n = 3), Niemann-Pick disease type C (NPC, n = 1), Inborn errors of vitamin B12 (n = 2), Inborn error of folate metabolism (n = 2), Pentosuria (n = 1) and Combined Malonic and Methylmalonic Aciduria (CMAMMA, n = 2). Considering the complex etiology, it is enormously doubtful that any single SNP contributes significantly to the development of IEM. Consequently, conducting future studies that focus on other low penetrance polymorphisms using more comprehensive techniques such as ES for identification of potential genetic variations.

Key words: Exome sequencing, inborn errors of metabolism, low penetrance polymorphisms, genetic variations

INTRODUCTION

Exome Sequencing (ES) is an effective approach to selectively sequence the genomic coding regions as a cheaper, but still effective alternative to whole genome sequencing. Over the last few years, the use of ES has significantly improved our understanding of many complex diseases (Rabbani *et al*., 2014). ES is an innovative technology has brought a paradigm-shift in medical research and clinical practice, which the cost reduction of this way enables personalized medicine to come to maturity (Kaname *et al*., 2014).

Inborn Errors of Metabolism (IEM) are a genetically heterogeneous group of diseases caused by a defect in a metabolic pathway, leading to the production and/or accumulation of toxic

metabolites in the body. Thus far, over 1000 different IEM have been introduced, while individually rare, the incidence has been shown to be around 1 in 800 (Mak *et al*., 2013). The last decade has witnessed significant progress in newborn screening as a mandatory public health strategy in most developed and developing countries, due to the advent of tandem mass spectrometry. The IEMs are present in all ethnic groups and across every age. Some IEMs are responsive to treatment with hopeful outcomes. ES allows inexpensive simultaneous detection of more than 30 different IEMs in one single blood spot specimen at a reasonable cost, with worthy analytical accuracy and precision. Furthermore, it is more than a test and it warrants systematic healthcare service delivery across the pre-analytical, analytical and post-analytical phases.

In this review, we aimed to analyze all the available publications and highlight the advantage issues in the conduct and interpretation of these studies and also to establish if the existing data supports any polymorphism to be conclusively associated with IEM.

MATERIALS AND METHODS

We conducted the first systematic review to collect evidence from the literature and clinical expertise of all IEMs with a focus on those that are detected using exome sequencing. We aim to raise awareness of formulate a diagnostic protocol using exome sequencing to support clinicians in the effective identification of these IEMs. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (http://www.prisma-statement.org/) (Liberati *et al*., 2009a-c).

Information sources: A systematic search was performed to identify all studies of IEMs from the PubMed database, checking and retrieving relevant studies from reference lists and consulting with experts. We considered only articles that were published in English language and conducted in humans.

Search strategy: We systematically searched PubMed using MeSH terms include, ("Metabolism, Inborn Errors"[Mesh]), "Exome"[Mesh] and all other possible combination from 1/1/1980-29/6/2014. The public MEDLINE database was searched according to a standard four-step protocol, as described in the following sections and summarized in Fig. 1.

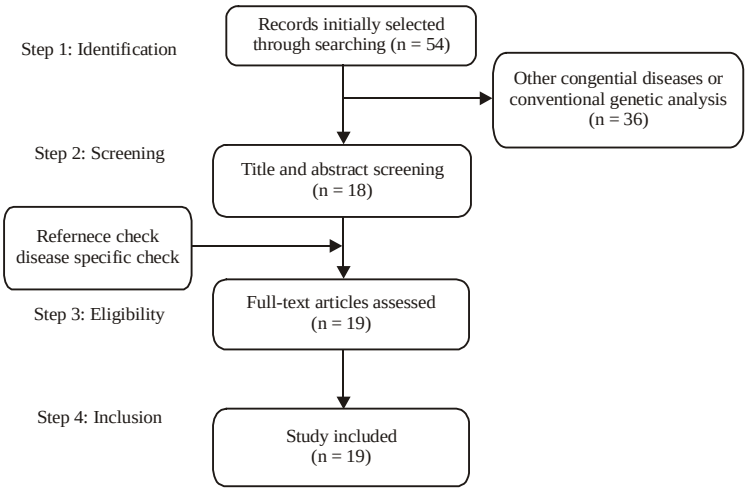

Fig. 1: Summary of a standard four-step protocol for literature review

Screening: Two authors (FR and HG) screened separately title and abstracts from relevant articles from the literature review. Genetic screening using exome sequencing containing relevant data were selected. Articles were excluded from full text analysis according to the following exclusion criteria: (1) The article mentioned IEM without data pertaining to any of the chosen diseases; (2) An article using conventional genetic and molecular diagnosis mentioning IEM in cases where the two authors did not agree, records were kept and included in the next step. Then we compared the results of ES to the phenotype findings from two websites, including Online Mendelian Inheritance in Man (OMIM) (Hamosh *et al*., 2005) and GeneCard (Hurrell *et al*., 2007).

Eligibility: The same two authors accessed the full texts of all remaining articles (n = 19) and checked them further for eligibility according to the same exclusion criteria used in the abstract screening stage.

Inclusion: Among the eligible records, information on key IEMs disease features as well as exome sequencing result was included.

Data extraction: From each study, information like: author, year of publication, country of origin, IEMs type, No. of cases, detected gene and genotyping method was extracted.

RESULTS

The PubMed search returned 54 unique articles, of which 19 articles met the established inclusion criteria and were included in the analysis. Overall, 19 studies were selected, include Leigh syndrome (LS, n = 4), Brown-Vialetto-Van-Laere syndrome (BVVL, n = 4), 3-Methylglutaconic aciduria (3-MGCA, n = 3), Niemann-Pick disease type C (NPC, n = 1), Inborn errors of vitamin B12 (n = 2), Inborn error of folate metabolism (n = 2), Pentosuria (n = 1) and Combined Malonic and Methylmalonic Aciduria (CMAMMA, n = 2) (Table 1). Exome sequencing

Table 1: Summary of included studies

Countries	Reported in same IEM	No. of cases	IEM	Candidate gene	Findings	References
Japan	No	3 probands	Leigh syndrome	GYG2	A possible link between GYG2 abnormality and Leigh syndrome	Imagawa *et al.* (2014)
Canada	Yes	2 patients	Brown-vialetto-Van-laere syndrome	SLC52A2	Exome sequencing helps in Early recognition of this disorder is critical, given its potential treat ability	Srour *et al.* (2014)
UK	Yes	18 patients	Brown-vialetto-Van-laere syndrome	SLC52A2	Riboflavin supplementation can ameliorate the progression of the disease, particularly when initiated soon after the onset of symptoms	Foley *et al.* (2014)
Israel	Yes	4 patients	3-Methylglutaconic aciduria (3-MGCA)	SERAC1	Exome sequencing has notable potentials for diagnosis of atypical cases	Sarig *et al.* (2013)
Iran	Yes	2 probands	Niemann-pick disease type C (NPC)	NPC2	Exome sequencing has notable potentials for diagnosis of atypical cases	Alavi *et al.* (2013)
Spain	Yes	1 proband	Leigh syndrome and 3-Methylglutaconic aciduria (3-MGA-uria)	SERAC1	Usefulness of exome sequencing to reveal the genetic bases of human rare diseases	Tort *et al.* (2013)

Table 1: Continue

Countries	Reported in same IEM	No. of cases	IEM	Candidate gene	Findings	References
USA	Yes	4 probands	Leigh syndrome mitochondrial complex I deficiency coenzyme Q10 deficiency	MT-ATP6 NDUFV1 COQ2	Exome sequencing has notable potentials when conventional diagnostic testing failed	Dinwiddie *et al.* (2013)
Netherland	Yes	3 probands	Leigh syndrome	SLC19A3	High doses of biotin or thiamine maybe is beneficial in leigh syndrome	Gerards *et al.* (2013)
Canada	Yes	1 proband	Inborn errors of vitamin B12 (cobalamin)	ABCD4	Successful application of exome sequencing for diagnosis of a rare case	Kim *et al.* (2012)
Switzerland	No	-	Inborn errors of vitamin B12	ABCD4	Identified causal mutations and new disease	Coelho *et al.* (2012)
USA	Yes	44 patients	Brown-vialetto-Van laere syndrome	SLC52A2	Excellent candidate therapy for the mutation-positive patients	Johnson *et al.* (2012)
Netherlands	Yes	1 patient	3-Methylglutaconic aciduria (3-MGCA)	SERAC1	Mutations in the phospholipid remodeling gene	Wortmann *et al.* (2012)
Canada	Yes	5 inborn	Inborn error of folate metabolism	FOLR1 MTHFR FTCD	Successful application of exome sequencing for diagnosis of a rare case	Watkins and Rosenblatt (2012)
USA	Yes	44 cases	Brown-vialetto-Van laere syndrome	SLC52A2	Exome sequencing leads to excellent candidate therapy	Johnson *et al.* (2012)
USA	No	15 families	Pentosuria	DCXR	Illustrates the power of modern genomics to elucidate the mechanism of mutational action	Pierce *et al.* (2011)
Canada	Yes	1 infant	Inborn error of folate metabolism	MTHFD1	Reinforces the power of exome sequencing for the discovery of novel genes, even when only a single proband is available	Watkins *et al.* (2011)
USA	No	9 patients	Combined Malonic and Methylmalonic Aciduria (CMAMMA)	ACSF3	Value of exome sequencing of a limited number of patients for the identification of novel disease genes	Sloan *et al.* (2011)
Canada	No	2 probands	Combined Malonic and Methylmalonic Aciduria (CMAMMA)	ACSF3	Value of exome sequencing of a limited number of patients for the identification of novel disease genes	Alfares *et al.* (2011)
UK	Yes	9 probands	Brown-vialetto-Van laere syndrome	SLC52A3 (C20orf54)	Identifying genes in rare recessive disorders	Green *et al.* (2010)

findings compared to the phenotypic findings reveals that the ES results are more specific (Table 2). The use of ES leads to early diagnosis of disorders critically and consequently gives potential treat ability. Overall, 19 articles investigated 116 candidate genes and 173 different polymorphisms in association with IEM.

DISCUSSION

Even though there have been several narrative reviews on such topic, to the best of our knowledge, this is the first systematic review analyzing the use of ES in IEM. In summary, we reviewed the available literature on ES studies of IEM and compared the presentation of GYG2, MT-ATP6, SLC19A3, SLC2A2, SLC52A2, SERAC1, NPC2, ABCD4, FOLR1, MTHFR, FTCD, MTHFD1, DXCR, ACSF2 and C20orf54 polymorphisms with previous reported diseases.

Leigh syndrome globally affects 1/40,000 newborns and is characterized by the presence of developmental delay and lactic acidosis, which has a mean life expectancy variously estimated at

Table 2: Comparison of the exome sequencing findings to reported syndromes

IEMs syndromes	Genes	OMIM ID	Reported disease for gene*
Leigh syndrome	GYG2	300198	Glycogen Storage Disease (GSD)
	MT-ATP6	516060	NARP, LHON, MIBSN, MC5DM1 syndromes
	SLC19A3	606152	THMD2 syndrome
Brown-vialetto-van-laere syndrome	SLC2A2	138160	FBS, Non-insulin dependent diabetes mellitus
	SLC52A2	607882	BVVLS2
	SLC52A3	613350	BVVLS1, FALOND
3-Methylglutaconic aciduria	SERAC1	614725	Leigh-like syndrome and 3-methylglutaconic aciduria
Vitamin B12 metabolism	ABCD4	603214	MAHCJ
Folate metabolism	FOLR1	136430	NCFTD
	MTHFR	607093	MTHFRD, NCFTD, FS-NTD, schizophrenia
	FTCD	606806	FIGLU-URIA
	MTHFD1	172460	FS-NTD, CRC, spina bifida
Pentosuria	DCXR	608347	Pentosuria
Combined malonic and methylmalonic acid uria	ACSF3	614245	CMAMMA

*Data has retrieved from GeneCards and OMIM, NARP: Neuropathy, ataxia and retinitis pigmentosa syndrome [MIM: 551500], LHON: Leber hereditary optic neuropathy [MIM: 535000], MIBSN: Mitochondrial infantile bilateral striatal necrosis [MIM: 500003], MC5DM1: Mitochondrial complex v deficiency, mitochondrial 1 [MIM: 516060], THMD2: Thiamine metabolism dysfunction syndrome 2 [MIM:607483], FBS: Fanconi-bickel syndrome [MIM:227810], BVVLS2: Brown-vialetto-van laere syndrome 2 [MIM:614707], MAHCJ: Methylmalonic acid uria and homocystinuria type cblJ [MIM:614857], NCFTD: Neurodegeneration due to cerebral folate transport deficiency [MIM:613068], MTHFRD: Methylenetetrahydrofolatereductase deficiency [MIM:236250], ISCHSTR: Schemic stroke [MIM:601367], FS-NTD: Folate-sensitive neural tube defects [MIM:601634], FIGLU-URIA: Glutamate formiminotransferase deficiency [MIM:229100], CRC: Colorectal cancer [MIM:114500], Pentosuria [MIM: 260800], CMAMMA: Combined malonic and methylmalonicaciduria [MIM:614265], BVVLS1: Brown-vialetto-van laere syndrome 1 [MIM:211530] and FALOND: Fazio-londe disease [MIM:211500]

3-5 years. Many studies using conventional genotyping methods, such as PCR-RFLP, Massively Parallel Sequencing (MSP), single specific primer-PCR and direct sequencing reported mtND2 to mtND6 as the most frequent mutation associated with Leigh syndrome. These techniques also helped to identify other mutations such as SLC19A3 (Vernau *et al*., 2013), C19orf79 (Lim *et al*., 2014), NDUFS1 (Tuppen *et al*., 2010), SURF1 (Hurrell *et al*., 2007), SUCLA2 (Vernau *et al*., 2013) and MT-ATP6 (Manfredi *et al*., 2002) hence, sometimes failed in the detection of functional mutations (Lee *et al*., 2001). Moreover, mutation in GYG2, MT-ATP6 and SLC19A3 genes has been reported in association with ES. The other IEMs included in this study also followed the same pattern. Significant validation of ES for clinical applications includes diagnosis of developmental delay, metabolic disorders, skeletal abnormalities and multiple congenital anomalies (Linderman *et al*., 2014). Besides, this approach is a useful tool for confirming the possible link between a gene and a specific syndrome, early detection that gives potential treatability, notable potentials diagnosis of atypical and rare cases especially when conventional diagnostic testing failed and discovery of novel genes, even when only a single proband is available. Recently, many researchers claimed the usefulness of ES in detection of mutations in gene responsible for some IEM, especially on SLC19A3 mutations in Leigh-like syndrome, as well as provided important information confirming the role of the ES in Leigh syndrome and the beneficial effect of biotin and/or thiamine treatment for patients harboring mutations in the gene encoding hTHTR2, SLC19A3 (Gerards *et al*., 2014, 2013; Kevelam *et al*., 2013; Van Der Knaap and Kevelam, 2014).

Though ES has generated high-quality data, it require more bioinformatics and statistical sophisticated tools, the increasing number of ES studies shows the power of this approach in mapping genes involved in complex disorders. However, there are still a large number of Mendelian diseases with unknown genetic causes. Unquestionably, the data generated in ES technologies will continue to grow, the role of bioinformatics such as the development of tools for variant analysis in the process of quality control, alignment, variant identification and downstream association, becomes more and more crucial in the analysis and interpretation of sequencing data.

ES is a promising method in the differential diagnosis of human hereditary diseases, because the majorities of pathogenic mutations are localized in exons and splice sites. Many researchers discuss the clinical application of ES with special emphasis on the diagnosis of the diseases of interest. Choi *et al*. (2015) applied ES to diagnose the first reported Korean patient with Congenital Disorder of Glycosylation Ia (CDG-Ia), which was misdiagnosed as Glycogen Storage Disease (GSD) (Choi *et al*., 2015).

CONCLUSION

Although some genes show promise, the conventional genetic studies in IEM susceptibility have so far been insufficient to confirm any association. The existence of small number of studies decreases the possible generality of results and the statistical power as well. Considering the complex etiology, it is enormously doubtful that any single SNP contributes significantly to the development of IEM. Consequently, conducting future studies that focus on other low penetrance polymorphisms using more comprehensive techniques such as ES for identification of potential genetic variations. ES might also help to identify new mutations that may contribute in a significant way to IEM pathogenesis. Moreover, ES studies using multistage design might be more fruitful to investigate the role of genetic components in complex diseases such as IEM. Understanding the intricate mechanisms of genetic pathways involved in IEM etiology and pathogenesis would be helpful to better identify subjects at high risk.

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