Received: 20 October 2018 | Revised: 22 January 2019 | Accepted: 25 February 2019

DOI: 10.1002/ajmg.a.61126

AMERICAN JOURNAL OF medical genetics PART A WILEY

ORIGINAL ARTICLE

Clinical exome sequencing in 509 Middle Eastern families with suspected Mendelian diseases: The Qatari experience

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Background: Clinical exome sequencing (CES) is rapidly becoming the diagnostic test of choice in patients with suspected Mendelian diseases especially those that are heterogeneous in etiology and clinical presentation. Reporting large CES series can inform guidelines on best practices for test utilization, and improves accuracy of variant interpretation through clinically-oriented data sharing.

Methods: This is a retrospective series of 509 probands from Qatar who underwent singleton or trio CES either as a reflex or naïve (first-tier) test from April 2014 to December 2016 for various clinical indications.

Results: The CES diagnostic yield for the overall cohort was 48.3% (n = 246). Dual molecular diagnoses were observed in 2.1% of cases; nearly all of whom (91%) were consanguineous. We report compelling variants in 11 genes with no established Mendelian phenotypes. Unlike reflex-WES, naïve WES was associated with a significantly shorter diagnostic time (3 months vs. 18 months, $p < 0.0001$).

Conclusion: Middle Eastern patients tend to have a higher yield from CES than outbred populations, which has important implications in test choice especially early in the diagnostic process. The relatively high diagnostic rate is likely related to the predominance of recessive diagnoses (60%) since consanguinity and positive family history were strong predictors of a positive CES.

KEYWORDS

Arab, clinical exome sequencing, consanguinity, Mendelian diseases, Middle East, Qatar

1 | INTRODUCTION

Genetic heterogeneity, phenotypic variability, and disease rarity (with consequent lack of familiarity) are all factors that make the traditional diagnostic approach to genetic disorders, whereby a specific gene is selected for sequencing based on the clinical phenotype, very challenging. Whole exome sequencing, with its ability to interrogate many genes in both hypothesis-driven and hypothesis-free approaches, has revolutionized the diagnostic process, and clinical exome sequencing (CES) is now a widely adopted diagnostic test. For the purposes of manuscript terminology, CES can be performed as a first-tier test without performing any prior diagnostic workup, which is commonly, referred to as a "naïve" CES. It can also be performed as a "reflex" CES following prior inconclusive diagnostic workup consisting of imaging

studies and/or laboratory studies and/or other genetic tests (whole genome array CGH, single gene testing or NGS multigene panels).

The diagnostic yield of CES, commonly estimated at ~25%, is high compared to other diagnostic tools, including molecular karyotyping, which is officially endorsed by professional societies as a first-tier test in patients with developmental delay and those with congenital anomalies. Although CES has not yet been endorsed by professional societies as a first-tier test for patients with suspected Mendelian disorders, recent studies support such an indication for CES and suggest that earlier CES is associated with more favorable cost/benefit ratio (Stark et al., 2018).

In 2015, we reported a high diagnostic yield of CES based on our local experience with the first 149 patients undergoing this testing (Yavarna et al., 2015). The relatively high diagnostic yield appeared to correlate with the frequency of homozygous recessive etiologies, which accounted for the majority of cases. This is not surprising since the Middle Eastern population is characterized by a high level of consanguinity, which was found to strongly predict a positive CES result in that study. Since that study, several others have confirmed a relatively high molecular diagnostic yield of CES in Middle-Eastern families (Alfares et al., 2017; Al-Shamsi, Hertecant, Souid, & Al-Jasmi, 2016; Anazi et al., 2017; Charng et al., 2016; Fattahi et al., 2016; Monies et al., 2017). In this follow-up study, we expand the reporting of CES from more than 500 previously unpublished cases. In addition to confirming the trends observed in the smaller cohort, the large size of this cohort allowed us to report substantially more novel variants, including those in genes that we propose as novel candidate genes. Our expanded cohort also allowed us to observe the time-saving advantage of "naïve" versus "reflex" CES.

2 | PATIENTS AND METHODS

2.1 | Patients

This study includes patients referred to the Clinical and Metabolic Genetics, Pediatrics Department at Hamad Medical Corporation (HMC) from April 2014 to December 2016. Patients were referred for a variety of reasons, including neurocognitive/neurodevelopmental or neuromuscular disorders, multiple congenital anomalies or disorders of other organ systems, such as immunologic, endocrine, or cardiac disorders (described below and in Table 2). Among the 509 patients, 359 underwent CES as a first-tier test (naïve CES), while 150 patients had a prior diagnostic workup with imaging and/or laboratory studies, including other genetic tests such as whole genome array CGH, single gene testing or NGS multigene panels, which did not establish a diagnosis (reflex CES).

Patients received pre- and post-test counseling about the scope of CES and its potential to reveal information unrelated to their original disease; informed consent was obtained from all patients or guardians by local clinicians. Peripheral blood samples from patients and their parents were obtained for CES as available. Detailed clinical data including medical history, family pedigree, thorough physical and dysmorphology examination, and any clinically indicated tests, such as MRI or genetic/metabolic were collected. The CES was performed as a part of the diagnostic work up and standard of care. In the case of an inconclusive CES, reanalysis of CES was done at least 1 year after the date of completion of the original CES. This study was approved by the local IRB (Study protocol no: MRC-01-18-273). The "solved" cases in our study were determined to be diagnostic based on molecular results of CES and reanalysis of CES and clinical correlation by a group of expert clinical geneticists, genetic counselors and clinical laboratory scientists.

2.2 | CES and variant calling

DNA extracted from peripheral blood samples was sent to a molecular diagnostic laboratory and CES, bioinformatics analysis and variant confirmation and interpretation were performed as reported earlier; when ordered based on clinical indication, mitochondrial testing was performed via a separate mitochondrial genome sequencing and deletion testing assay, with final CES and mitochondrial results returned as a single, combined report (Yavarna et al., 2015). Briefly, all variants were classified according to the ACMG guidelines (Richards et al., 2015). To score variants for the allele frequency criteria in the guidelines, gnomAD and the GME databases were used. A proprietary method for copy number variant (CNV) analysis was used (Retterer et al., 2015). All reported CNVs, as well as other clinically reported variants, were orthogonally confirmed using an appropriate secondary method, such as exon-level array CGH microarray.

2.3 | Statistical analysis

The significance of the diagnostic rate of trio-CES versus singleton-CES, p values was calculated by 1-tailed Fisher exact test. A p value of 0.05 was used as a significance threshold. Odds ratio and 95% confidence interval (CI) for the significance of difference in diagnostic rate were also calculated using SPSS.

3 | RESULTS

3.1 | Patient demographics

A total of 509 patients who presented with rare and diverse genetic disorders underwent CES. The male to female ratio was 1:1 (Female: N = 248, 49%; Male: N = 261, 51%). Patient ages ranged from prenatal to 53 years. Out of the 509 patients, 467 patients (92%) were children (18 years or younger); 265 (52%) were children younger than 5 years at the time of testing.

Trio (child-parents) or quad (child, sibling, parents) analysis was used for CES in 34% of cases (159/467) in the child-proband group, more than in the adult group (8/42 patients; 19%) (Odds ratio: 2.2 [95% CI, 0.99–4.8], $p < 0.03$), reflecting limited parental availability for adult patients. Males were slightly overrepresented in the childhood group (male: 252 of 467 patients, 54%; female: 215 of 467 patients, 46%; Odds ratio: 1.37 [95% CI, 1.0–1.7], $p < 0.01$) while the opposite was observed in the adult group (men: 9 of 42 patients, 21%; women: 33 of 42 patients, 79%; Odds ratio: 3.86 [95% CI, 1.887–7.901, $p < 0.01$]). Consanguinity or a positive family history was observed or reported in 65.0% and 32.0% of cases, respectively. The backgrounds of the probands included nationals from Qatar

TABLE 1 Patient demographics for 509 cases

Group	Sub-group	Number (%)
Gender	Male	261 (51%)
	Female	248 (49%)
Age	0 ≤ 5 years	265 (52%)
	5 ≤ 18 years	202 (40%)
	>18 years	42 (8%)
Nationality	Qatari	294 (58%)
	Other Arab countries	139 (27%)
	Indian subcontinent	76 (15%)
Parental consanguinity	Yes	332 (65%)
	No	177 (35%)
Family history	Positive	162 (32%)
	Negative	347 (68%)

(58.0%), other Arab countries (27.0%) and the Indian subcontinent (15.0%; Table 1).

3.2 | Patients and their clinical presentations

Patients presented with diverse clinical phenotypes: 229 (45.0%) had neurocognitive (NC), 45 (9.0%) neuromuscular (NM) disorders, 116 (23.0%) multiple congenital anomalies (MCAs), and 119 (23.0%) had other system manifestations (OSMs), such as endocrine, GI, or immunologic features (Table 2). The clinical presentation in several patients showed atypical phenotype meaning that the clinical presentation deviates from the established gene-linked phenotype as summarized in (Supporting Information Table S6).

3.3 | Diagnostic yield of CES

The overall diagnostic yield by the CES in this study was 48.3% (n = 246), (naive 48.0%, n = 173/359 vs. reflex CES 48.6%, n = 73/150). The molecular diagnostic rate for each of the phenotypic groups described above is shown in Table 2.

The solved cases (diagnostic yield) were stratified according to the major clinical phenotypes, NC: 106 (46.0%); NM: 29 (64.4%); MCAs: 45 (38.8%); OSMs: 66 (55.5%; Table 2).

The overall diagnostic yield for children younger than 5 years was 132/265 (49.0%). Of the children younger than 5 years at the time of testing (n = 265), trio-CES was performed for 32% (n = 87) of cases, and singleton-CES for 68% (n = 178) cases, respectively. The diagnostic yield of trio-CES was 53% (46/87), which was higher than for singleton-CES (85/177; 48%). The parental consanguinity in this group was 96/132 cases (72%).

The overall diagnostic yield for children aged 5–18 years was 47% (96/202). Out of the total number children aged 5–18 years (n = 202), trio-CES and singleton-CES were performed for 72 (35.0%) and 130 (65%) of cases, respectively. The diagnostic rate for trio-CES (43/72 cases; 59.0%) was significantly higher compared to singleton-CES (53/130 cases; 40.0%) (Odds ratio: 2.15 [95% CI, 1.2–3.9], p < 0.01) (Table 3). The parental consanguinity in this group was 62/97 cases (65%). The presence of consanguinity and positive family history predicted a higher clinical sensitivity (56.3%) as compared with those who lacked both (37.0%; p = 0.02). (Table 4).

3.4 | Characterization of CES molecular findings

Using CES, we identified 176 novel and 62 previously published pathogenic or likely pathogenic single nucleotide variants in known genes linked to the clinical phenotypes (Table 5, Supporting Information Tables S1–S3). More than half of the reported PV were homozygous variants in AR genes (60.0%) consistent with the high rate of consanguinity in the study population. Nevertheless, a considerable number of patients had PVs in AD (33%) or X-linked genes (5%), or de novo CNVs (2%) (Figure 1).

Out of AR cases, (60%) had homozygous variants for AR traits, 12 (2.3%) patients were compound heterozygous (Table 6) and three (0.5%) had two compound homozygous variants (a complex allele) in the same gene, which was consistent with their original clinical presentations (Table 7).

Two patients were homozygous for PV in genes currently only known to be associated with autosomal dominant disorders (Table 8).

Eleven (2.1%) patients had two molecularly-identified genetic disorders consistent with their original clinical presentation (Table 9). In addition, 210 variants of uncertain clinical significance (VUS) were reported. These variants were not included in the diagnostic yield calculations.

3.5 | Novel disease gene discovery

Eleven novel candidate genes were reported as potential contributors to the clinical presentation in the respective patients. These were considered as part of the overall diagnostic yield, and results were managed and returned clinically, with appropriate counseling.

1. *CLCN3* (p.G327A heterozygous, de novo): Two-year-old female who presented with optic atrophy and overweight status.
2. *HSPB11* (p.L62AfsX14 homozygous, biparental): Two-year-old female with lung hypoplasia, polycystic kidneys, and cardiac hypertrophy.

TABLE 2 Distribution of diagnostic cases according to clinical presentations

Categories	Total number (%)		Total number of positive cases (%)	Singleton CES	Trio and Quad CES
Neurocognitive (NC) disorders	**229**	**45.0%**	**106 (46.0%)**	**63**	**43**
Neuromuscular (NM) disorders	45	9.0%	29 (64.4%)	15	14
Multiple congenital anomalies (MCAs)	116	23.0%	45 (38.8%)	32	13
Other system manifestations (OSMs)[a]	119	23.0%	66[a] (55.5%)	44	22
Total	509		246 (48.3%)	154	92

[a]Ten cases of each Endocrine and GI, and four cases of each nephrology, ophthalmology, Immunology, metabolic, pulmonology, rheumatology, dermatology, neurology.

TABLE 3 Molecular diagnosis rate of phenotypic subgroups by age group

	Age groups								
	≤5 years			5 ≤ 18 years			>18 years		
Categories	Total no	Singleton	Trio	Total no	Singleton	Trio and	Total no	Singleton	Trio and
Neurocognitive (NC) only	59	37	22	45	24	21	2	2	0
Neuromuscular (NM) only	12	6	6	16	9	7	2	1	1
Multiple congenital anomalies (MCA)	29	22	7	12	6	6	4	4	0
Other system manifestations	32	20	12	23	14	9	10	9	1
Total	132	85	47	96	53	43	18	16	2

3. *MACF1* (p.A3721T homozygous, biparental): Deceased female infant with multiple brain abnormalities (severe hydrocephalus and cerebral atrophy).
4. *XPOT* (p.C877X heterozygous, inheritance unknown): Forty-nine-year-old male with schizophrenia and a family history of dilated cardiomyopathy, psychiatric illness, and early Alzheimer disease. The reported variant was not detected in mother nor in brother with cardiomyopathy.
5. *GRM5* (AD) (p.S266T heterozygous, de novo): Eleven-year-old female with complex vocal and motor tics, learning disability, and obsessive–compulsive disorder.
6. *SCHIP1* (p.R452* homozygous): 4-year-old male of consanguineous parents with a history of hypotonia, macrocephaly, developmental delay, and abnormal MRI findings. This homozygous nonsense variant segregated in the proband's two similarly affected sisters.
7. *TUBA3E* (p.A150V and p.R215C compound heterozygous): Male with bilateral limb reduction, horseshoe kidney, undescended testes, and neuronal migration defect on brain MRI
8. *UNC13A* (p.V1619G homozygous, biparental): Male child with epileptic encephalopathy with intractable seizures and global developmental delay. The family history is significant for parental consanguinity.
9. *LAMTOR4* (p.Q90GfsX42 homozygous, biparental): Female child with Microcephaly, developmental delay, hypertonia, cortical hypomyelination, abnormal white matter, and dysmorphic features.
10. *MAP4K4* (p.S604T homozygous, biparental): Male child with microcephaly, speech delay, facial dysmorphism, developmental delay, and aggressive behavior. Family history is significant for a similarly affected sister who is also homozygous for this variant. The proband's two unaffected brothers were heterozygous for this variant.
11. *GALNT11* (p.R384X Homozygous biparental) Male child with disproportionate limb shortening, facial dysmorphism, short stature, failure to thrive

3.6 | ACMG secondary findings

Fifteen percent of patients/families opted to receive secondary findings. Secondary findings were reported in three probands (3.9% of those who elected to receive these findings) in our cohort, and was performed per to the American College of Medical Genetics and Genomics (ACMG) recommendations (Green et al., 2013). One patient had an autosomal dominant heterozygous pathogenic variant (p.E22K) in the *MYL2* gene associated with hypertrophic cardiomyopathy requiring medical action; one patient had an autosomal dominant heterozygous pathogenic variant (p.R248Q) in the *TP53* gene for Li–Fraumeni syndrome; and one female patient had an autosomal dominant heterozygous pathogenic variant (c.4136_4137delCT) in the *BRCA1* gene. The latter two patients were referred to a cancer center for further management.

3.7 | CES reanalysis

CES was negative (nondiagnostic) for 27% of patients (136/509), with no reportable variants and/or where the reported variant did not explain the proband's phenotype. Nevertheless, reanalysis by CES revealed diagnostic results in 34 patients, corresponding to 25% of reanalysis cases. Supporting Information Table S4.

3.8 | CES shortens time to diagnosis

The average time to a definitive diagnosis from the patient's first visit to a genetic clinic was significantly shorter ($p \leq 0.0001$) for patients who had CES as a first-tier test (3 months; 173/246 positive cases) compared to those who had other diagnostic workups first, and who then later reflexed to CES (18 months; 73/246 positive cases). An early diagnosis can inform medical management and potentially improve patient quality of life. Supporting Information Table S5 summarizes treatable diagnoses identified by CES. It is also important for defining patient prognoses for families and may enable a patient to participate in clinical trials earlier. Finally, earlier diagnoses can also lead to earlier "cascade" or familial testing. Supporting Information Table S5.

TABLE 4 Distribution of cases for parental consanguinity and family history

Group	Parental consanguinity (PC)		Family history (FH)		Parental consanguinity and family history	
Classification	Yes	No	Yes	No	Yes/positive	No/negative
Total no	332	177	162	347	119	135
Solved	174 (52.4%)	70 (39.5%)	88 (54.3%)	156 (44.9%)	67 (56.3%)	50 (37.0%)
	Odds ratio: 1.68 (95% CI, 1.2–2.4) $p = 0.003$		Odds ratio: 1.45 (95% CI, 1.0–2.1) $p = 0.03$		Odds ratio: 2.19 (95% CI, 1.3–3.6) $p = 0.01$	

TABLE 5 Mutation analysis in diagnostic cases

Group	Gene			
Novel, likely pathogenic variants in known disease genes	Autosomal recessive (AR)	Autosomal dominant (AD)	Semi dominant	X-linked
	PIEZO1x3	*ACTA1*	*OPA1*	*COL4A5*
	LRP5	*SLC4A1*		*ATRX*
	PTH	*PLA2G7*		*DDX3X*
	GJC2	*KIF1A*		*USP35*
	ELOVL4	*EVC*		*HUWE1x2*
	EDNRB	*DES*		*KDM5C*
	COL7A1	*COL6A1*		*KIAA2022*
	CHRNAx2	*ABCB4*		*PDHA1x2*
	ANK3	*BSCL2*		*TAF1*
	CUL7	*ANKRD11*		
	AIPL1	*ASXL3*		
	ALDH7A1	*BRAF*		
	DAG1	*CAMTA1*		
	ACY1	*CHD2*		
	CNTNAP1	*ARID1Bx2*		
	B3GALNT2 x2	*COL11A1*		
	OCLN	*TBX18*		
	CENPF x2	*RAD21*		
	LONP1	*DSG2*		
	VPS13B	*DYNC1H1x2 COL5A2*		
	LRBA	*COL3A1*		
	GFPT1	*SLC1A3*		
	GLE1	*LDLR*		
	CWF19L1x3	*FGFR2*		
	XYLT1	*FOXP2*		
	ECEL1	*GATAD2B*		
	ATP6V0A4	*GRIN2Ax2*		
	SLC6A3x2	*HNRNPU*		
	DYM	*KMT2D*		
	EIF2B2	*KAT6Bx2*		
	SLC2A2	*KCNT1x2*		
	FKTN	*LDB3*		
	FBP1	*EFTUD2*		
	GCLC	*MED13Lx2*		
	PHKB	*MEF2Cx2*		
	NBEAL2	*AHDC1*		
	RAB27A	*NF1*		
	SLC46A1	*NOTCH1*		
	HPS1x2	*COL1A1*		
	HPS5	*PIEZO1*		
	SLC25A15	*PIK3CD*		
	IGHMBP2	*PIK3R1*		
	ITPA	*POGZ*		
	CC2D2Ax2	*PURA*		
	KCTD7	*RUNX2*		
	LIPH	*SALL4*		
	MED17	*SCN2A*		
	MICU1x7	*SETD2*		
	ATPAF2	*SHANK3*		
	RRM2B	*SLC9A9*		
	GNPTG	*ATL1*		
	MYBPC3	*SPECC1L*		
	KLHL41	*DNMT3A*		
	NEBx3	*TP63*		
	NHEJ1	*TCOF1*		
	NPC1			
	PDE6C			
	PEX2			
	PGAP3			
	PLEC			
	POMT1			
	LAMA1			
	SLC10A2x2			
	DNAH5			
	DNAH11			
	ASPM			
	RYR1			
	LIFR			
	TCTN3			
	TMPRSS4x2			
	CYP2R1			
	CYP2R1			

(Continues)

TABLE 5 (Continued)

Group	Gene			
	EIF2AK3 *WWOX* *POLH* *TBCD* *PEX13* *RPIA x2* *FANCA* *FBXL4* *SPTA1*			
Variants in novel candidate genes	*HSPB11* *CDK9 x2* *KIAA0195* *UNC13A* *TRAF3IP2* *LAMTOR4* *SCHIP1* and *IQCJ-SCHIP1* *MAP4K4*	*TUBA3E* *CLCN3* *GRM5* *XPOT* *GALNT11*		
Previously reported P/ LP variants in known disease genes	*SIX6* *MYL2* *COL7A1* *CC2D2Ax2* *TMIE* *VPS13B* *SRD5A3* *MCEE* *LAMA2 x2* *PCNT* *DDCx2* *PLA2G6* *ISCA2* *UQCRQ* *C12orf57* *RSPH9* *SNX14* *SEPN1* *CLCN1* *MKKS* *ABCB4* *SEPSECS* *XYLT1* *SGCA* *OTOF* *RNASEH2C* *BCS1L* *SLC24A5* *TMPRSS3* *PEX1* *PEX5* *GALC* *TRAPPC9*	*TCOF1* *SMARCB1* *SYNGAP1* *MBD5* *MYH3* *PTPN11x3* *TP53* *RYR1* *LDB3* *TPM2* *MAP2K1* *CSNK2A1* *FLG* *SATB2* *TRPV4* *NSD1* *FGFR2* *TNNI2* *MYL2* *TBCD* *PPP2R5C* *CDK13*	*LDLR*	*FOXP3* *MECP2* *MECP2* *MECP2* *CLCN4*
Novel copy number variants	• *KPTN* deletion (exons 9–12) • 2.9 Mb deletion in 15q15.3–15q21.1 • 1.3 kb deletion in 5q12.1 • 283 kb duplication in 16p11.2 • De novo 361 Mb duplication of 17p13.3 • Partial gene duplication in *DNAH5* • Partial gene duplication involving *MICU1*			
Mitochondrial pathogenic findings	• Heteroplasmic 5 kb deletion of the mitochondrial genome encompassing the following genes: *MT-ATP8*, *MT-ATP6*, *MT-CO3*, *MT-TG*, *MT-ND3*, *MT-TR*, *MT-ND4L*, *MT-ND4*, *MT0TH*, *MT-TS2*, *MT-TL* and *MT-ND5* • *MT-CYB* p.Gly167Asp (G167D)			

4 | DISCUSSION

We previously reported that the diagnostic yield of CES was 60% in 149 patients from Qatar. In this larger study, as we continually enrolled a new cohort of 509 CES with rare and diverse disorders, the overall diagnostic yield of CES was 48.3% (n = 246). The lower diagnostic rate in this current study may be related to the strict use of ACMG guidelines for variant classification, which was not used in the first cohort, but may also involve other factors. Within our cohort, the highest diagnostic rate (64%) was observed for patients with neuromuscular disorders.

As expected, autosomal recessive variants accounted for the majority of identified causative variants. Consistent with our prior published experience, we note that this pattern extends to genes that had only been linked to human diseases in the autosomal dominant mode of inheritance (Monies, Abouelhoda, et al., 2017). In addition,

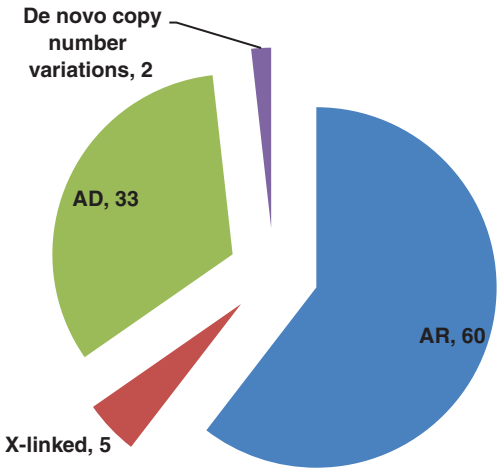

FIGURE 1 Distribution of cases based on mode of inheritance [Color figure can be viewed at wileyonlinelibrary.com]

enhanced autozygosity facilitated the co-inheritance of more than one homozygous disease-causing variant with resulting dual molecular diagnoses in several cases. The apparently lower incidence of these "multilocus" phenotypes compared to a previous estimate is likely related to our strict use of ACMG guidelines to call pathogenic variants. Indeed, our estimate is nearly identical to another study involving a comparable Middle Easter population that applied to the same criteria (Monies et al., 2017).

Eleven novel candidate genes were reported in this cohort of patients. These genes had not been reported in associated with human disease or the published data to support human disease association may not yet be definitive. While supporting data for the candidacy of these genes (e.g., model organism data, intolerance of the gene to sequence variation, tissue or developmental timing of expression, or knowledge of the gene function and pathway analysis) are suggestive, we emphasize that these remain candidates pending future confirmation through the reporting of similarly affected patients.

Others have noted the importance of reanalyzing CES to improve the diagnostic rate (Ewans et al., 2018; Shamseldin et al., 2017; Wright et al., 2018). However, it is likely that CES will never reach 100% diagnostic rate even in patients with a clearly genetic etiology because of technical limitations. Despite the encouraging early results

TABLE 6 Patients with compound heterozygous P/LP variants

Patient	Clinical indication	Gene	Disease	Variant	cDNA
1	Intrahepatic cholestasis, elevated liver enzymes, vitamin D deficiency, and short stature	*CYP2R1*	Vitamin D25-hydroxylasedeficiency	p.L257SfsX6 p.L89R	c.266T>G
3	Seizures, hypomyelination, lactic acidosis, cryptorchidism, and a history of intrauterine growth retardation and premature birth	*ALDH7A1*	Epilepsy, pyridoxine-dependent	p.V481E p.C154R	c.1442T>A c.460T>C
4	Possible Joubert syndrome	*CC2D2A*	Joubert syndrome	p.L457X p.E998V	c.1370T>A c.2993 A>T
5	Cerebellar ataxia, cerebellar atrophy, and intellectual disability. The family history is significant for two siblings with similar features.	*CWF19L1*	Spinocerebellar ataxia 17	IVS8-2A>G p.V229F	
6	Arthrogryposis, cerebral palsy, and developmental delay	*DAG1*	Alpha-dystroglycanopathy	p.T531N p.A98V	c.1592 C>A c.293 C>T
7	Elevated liver enzymes, elevated creatine kinase, and motor delay	*MICU1*	MICU1-related muscular dystrophy	p.Q185X Partial gene duplication	c.553 C>T NA
8	Congenital hydrocephalus and developmental delay	*B3GALNT2*	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies, Type A, 11)	IVS2-1G>A p.M75I	c.261-1G>A c.225G>A
9	Slow progressive congenital myopathy	*PLEC*	Muscular dystrophy, limb-girdle, Type 2Q	p.A2110V p.R307C	c.6329 C>T c.919 C>T
10	Cerebellar ataxia, cerebellar atrophy, and intellectual disability	*CWF19L1*	Spinocerebellar ataxia 17	IVS8-2A>G p.V229F	c.850-2A>G c.685G>T
11	Allergic colitis	*SLC10A2*	Primary bile acid	p.C106X p.P251L	c.318 C>A c.752 C>T
12	Autism spectrum disorder	*ANK3*	Autism spectrum disorder	p.P1489S p.S2366P	c.4465 C>T c.7096T>C

TABLE 7 Patients with two homozygous variants in the same gene

SN	Clinical indication	Gene	Disease	Variant	cDNA
1	Skeletal dysplasia with spine and femur with Swedish key/monkey wrench appearance. Parental consanguinity	*XYLT1*	Desbuquois dysplasia Type 2	p.A21GfsX173 IVS7-3C>T	c.62delC c.1588-3C>T
2	Nonimmune hydrops	*PIEZO1*	Lymphatic dysplasia with nonimmune hydrops fetalis	p.E679X p.A1496V	c.2035 G>T c.4487 C>T
3	Pericardial effusion, cardiomegaly, ascites, hepatomegaly, short limbs, hydrops, and echogenic bowel and kidney. Parental consanguinity	*SPTA1*	Hereditary spherocytosis type 3	p.W279X p.N1934S	c.836 G>A c.5801 A>G

TABLE 8 Patients with homozygous variants in genes typically associated with AD traits

Patient	Clinical indication	Gene	Disease	Variant	cDNA
1	Epilepsy, hemiparesis, and migrational anomalies	*SLC1A3*	Episodic ataxia type 6	p.K60Q	c.178 A>C
2	Failure to thrive, lung fibrosis, vasculitis, rash, recurrent angiodema, alopecia, and intermittent limb edema	*TMEM173*	STING-associated vasculopathy with onset in infancy	p.R281W	c.841 C>T

from WGS in these "negative" cases, the full potential of WGS in patients who could not be diagnosed by CES remains to be seen (Alfares et al., 2018). Testing other tissue sources as well as additional modalities at the RNA, epigenetic, and multilocus levels will likely be necessary to resolve an even higher proportion of cases.

The value of an early diagnosis for most patients is to improve management and quality of life. It is also of critical importance to the patient's family as defining a diagnosis allows specific prognostic predictions, connecting to other families and patient support groups. Although there are limited treatments available for many patients, an earlier diagnosis may allow these patients to participate in available clinical trials.

A limitation of interpretation of variants found by CES in ME patients has been the lack of reliable control cohort data, such as estimating the minor allele frequencies of variants in healthy individuals from the Middle East. However, this obstacle can be overcome by establishing databases of normal variants and disease-causing variants in Arab population. Examples of current efforts in Qatar and the Arabian gulf region include establishing the Qatar Genome Program (QGP) and the Saudi Human Genome Program (SHGP).

TABLE 9 Patients with dual molecular diagnoses

SN	Inheritance	Gene	Disease
1	AR AR	*NBEAL2* *DNAH5*	Gray platelet syndrome Primary Ciliary dyskinesia
2	AD AD	*EFTUD2* *NOTCH1*	Mandibulofacial dysostosis Adams–Oliver syndrome 5
3	AD AR	*RYR1* *PIEZO1*	RYR1 related disorder (neuromuscular) Lymphatic dysplasia with nonimmune hydrops fetalis
4	AR AD	*LRP5* *PLA2G7*	Van Buchem disease Type 2 Platelet-activating factor acetylhydrolase deficiency
5	AD Mitochondrial	*TPM2* *MT-CYB*	Arthrogryposis Leber hereditary optic neuropathy
6	AD AR	*SLC1A3* *MCEE*	Episodic ataxia Type 6 Methylmalonic aciduria
7	AR AR	*GLE1* *ATM*	Lethal congenital contracture Syndrome-1 Ataxia telangiectasia
8	AR AR	*LAMA2* *TMPRSS4*	Muscular dystrophy, congenital merosin-deficient Cerebral atrophy
9	AR AR	*NEB* *GALC*	Nemaline myopathy Krabbe disease
10	AD AD (candidate)	*EVC* *TUBA3E*	Ellis–van Creveld syndrome Global developmental delay, primary microcephaly, lissencephaly, epilepsy (candidate gene)
11	AR AR (candidate)	*RPIA* *LAMTOR4*	Ribose-5-phosphate isomerase deficiency Candidate gene

5 | CONCLUSIONS

CES is a powerful tool for ending the diagnostic odyssey in cases with an unsolved/undiagnosed genetic disorder after traditional molecular diagnostic approaches have been exhausted, and may even be better deployed as a first step approach. CES has the potential to identify potential novel candidate genes and variants in multiple genes (dual diagnoses) resulting in a more complex disease phenotype. Our results support the adoption of CES as routine clinical diagnostic services locally in Qatar and perhaps to other populations with similar characteristics.

ACKNOWLEDGMENTS

The authors would like to thank patients and their families described in this article, the healthcare providers who were involved in their patient care and extend our thanks to Mr. Patricio Santos Ayroso and Ms. Magi Martin for their administrative support. The authors also acknowledge the work of the clinical exome team at GeneDx.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

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How to cite this article: Al-Dewik N, Mohd H, Al-Mureikhi M, et al. Clinical exome sequencing in 509 Middle Eastern families with suspected Mendelian diseases: The Qatari experience. *Am J Med Genet Part A*. 2019;179A: 927–935. https://doi.org/10.1002/ajmg.a.61126