ORIGINAL ARTICLE

Diagnostic value of whole-exome sequencing in Chinese pediatric-onset neuromuscular patients

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Background: Neuromuscular disorders (NMDs) comprise a group of heterogeneous genetic diseases with a broad spectrum of overlapping the clinical presentations that makes diagnosis challenging. Notably, the recent introduction of whole-exome sequencing (WES) is introducing rapid changes on the genetic diagnosis of NMDs. We aimed to investigate the diagnostic value of WES for pediatric-onset NMDs.

Methods: We applied integrated diagnostic approach and performed WES in 50 Chinese subjects (30 males, 20 females) with undiagnosed pediatric-onset NMDs despite previous specific tests. The patients were categorized in four subgroups according to phenotyping and investigation findings. Variants on NMDs gene list and open exome analysis for those with initial negative findings were identified.

Results: WES identified causative variants in *ACTA1* (n = 2), *POMT1*, *COL6A1* (n = 2), *MTMR2*, *LMNA*, *SELENON*, *DNM2*, *TGFB1*, *MPZ*, *IGHMBP2*, and *LAMA2* in 13 patients. Two subjects have variants of uncertain significance (VUSs) in *TTN* and *SCN11A*, unlikely to be pathogenic due to incompatible phenotypes. The mean interval time from symptom onset to genetic diagnosis was 10.4 years (range from 1 month to 33 years). The overall diagnostic yield of WES in our cohort was 26%. Open exome analysis was necessary to identify the pathogenic variant in *TGFB1* that caused skeletal dysplasia with neuromuscular presentation.

Conclusion: Our study shows a clear role of WES in the pathway of integrated diagnostic approach to shorten the diagnostic odyssey in patients with rare NMDs.

KEYWORDS

integrated approach, neuromuscular disorders, pediatric-onset, whole-exome sequencing

Mandy H. Y. Tsang and Annie T. G. Chiu are co-first authors.

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1 | INTRODUCTION

Neuromuscular disorders (NMDs) comprise a group of heterogeneous diseases of the peripheral nervous system. These diseases are rare, with prevalence rates of 1–10 per 100,000 people worldwide (Deenen, Horlings, Verschuuren, Verbeek, & van Engelen, 2015) and an estimated prevalence of 1 per 4,669 residents in Hong Kong (Chung, Wong, & Ip, 2003). The diagnosis of NMDs is challenging as the phenotypic spectrum is broad, frequently overlapping, and the symptom onset, the clinical features and disease progression are often variable. Nevertheless, establishing a diagnosis is important to enable early treatment, recruitment of patient registries and clinical trials, and genetic counseling.

However, a precise diagnosis remains challenging. Sequential targeted gene Sanger sequencing is the conventional approach in our current clinical setting. This method is tedious and ineffective due to the genotypic heterogeneity of NMDs and huge size of the disease-associated genes. Moreover, many NMDs-related genes have not been identified yet.

Most of the above challenges may be resolved by the next generation sequencing (NGS)-based gene panel test or whole-exome sequencing (WES). NGS-based gene panel has the advantage of high coverage, and it is able to detect various types of pathogenic variants including single nucleotide variants (SNVs), small indels, deletions, and duplications, in both the coding region and noncoding region in the known disease-associated genes. Ankala et al. performed a study in a cohort of NMDs patients using a comprehensive NMDs gene panel. Comparing with other clinical tests, for example, single gene testing and disease-targeted panels, comprehensive NMDs gene panel has the highest diagnostic rate of 46%. Further, they proposed that 11%–18% of the pathogenic variants would be missed by WES due to the low coverage (Ankala et al., 2015).

Moreover, due to gene-disease association discovery over the years, gene panel test has its limitation. The study by Park et al. illustrated that a gene panel which includes a more comprehensive list of genes has a higher diagnostic yield when compared to the commercially available gene panel (Park et al., 2019). Frequent comprehensive literature search is required for designing the gene panel up to date. Also, there may be non-NMDs with phenotypes mimicking NMDs, which would probably be missed by NGS-gene panel approach. It was believed that WES can overcome these limitations as all the coding regions in the entire human genome is sequenced at one time.

The diagnostic value of WES in NMDs has been demonstrated in previous studies. Haskell et al. performed WES in 93 NMDs pediatric and adult patients with overall diagnostic yield of 12.9%, and only 63% prior phenotyping testing, including invasive muscle biopsy, is informative to reach the diagnosis (Haskell et al., 2018). Waldrop et al. performed trio WES in 31 pediatric patients yielded a diagnostic rate of 39%. Two rare genetic cases, Vici syndrome associated with EGP5, infantile hypotonia with psychomotor retardation, and characteristic facies 3 caused by *TBCK* pathogenic variants, were identified. With positive genetic diagnosis and proper surveillance, treatment could be provided (Waldrop et al., 2019).

In this study, we report the diagnostic approach applied to a cohort of Chinese patients with undiagnosed pediatric-onset NMDs and the diagnostic yield associated with WES.

2 | METHODOLOGY

2.1 | Ethical compliance

The study was approved by the Institutional Review Board of the University of Hong Kong (UW 15-603). Written informed consent was obtained from all patients or parents of patients recruited in this study.

2.2 | Patient cohort

This prospective study recruited 50 Chinese patients with undiagnosed pediatric-onset hereditary NMDs referred to our hospital between September 2016 and August 2018. All recruited patients were examined by a pediatric neurologist and neuromuscular specialist at one or more clinic visit(s) to gather information on disease presentation, family history, cardiac and lung function assessment, and for a thorough physical examination and investigation analysis including blood assay, imaging, muscle and nerve electrophysiological study, genetic testing, and muscle biopsy.

After evaluation, the patients were categorized into four subgroups: hereditary congenital myopathy subgroup (n = 24), hereditary muscular dystrophy subgroup (n = 11), hereditary peripheral neuropathy subgroup (n = 11), and complex condition with neuromuscular involvement subgroup (n = 4).

2.3 Whole-exome sequencing: Genomic analysis, NGS data processing, and variant analysis

WES was performed for all recruited subjects as described previously (Tsang et al., 2019). Genomic DNA was extracted from the peripheral blood samples using a Qiagen Blood Mini Kit (Qiagen). Subsequently, an exome library was prepared using the TruSeq Rapid Exome Library Prep Kit (Illumina Inc.). All DNA preparations and library quality control protocols were performed according to the manufacturers' instructions. The DNA libraries were sequenced using the Illumina NextSeq500 sequencing platform. Our in-house-developed bioinformatics pipeline was used for variant calling and data analysis. The raw reads were aligned to the hg19 reference

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human genome (University of California Santa Cruz, UCSC) using BWA 0.7.10 software (Li & Durbin, 2009). Variant calling workflow was performed according to the GATK best practices, v3.4-46 (DePristo et al., 2011). The output files were annotated using ANNOVAR software.

The 2018 version of the gene table of monogenic NMDs (nuclear genome) was used for the first-tier analysis for all patients (Table 1; Bonne, Rivier, & Hamroun, 2017). Subsequently, cases with negative findings were subjected to open exome analysis. Here, we targeted variants located in the coding and canonical splice-site regions with population frequencies of <1% in control population databases such as the Exome Aggregation Consortium (ExAC) and Genome Aggregation Database (gnomAD). The pathogenicity of the remaining rare variants was assessed using the American College of Medical Genetics and Genomics (ACMG) guideline (Richards et al., 2015). Finally, the potential disease-causing variants were confirmed and segregated using Sanger sequencing.

3 | RESULTS

3.1 | Overall

Fifty Chinese patients (30 males and 20 females, male: female ratio: 1.5:1) of ages ranging from 1 month to 37 years old, were recruited. Thirty-six patients (72%) were below 18 years old at the time of recruitment.

An average coverage of 89× was achieved by WES. Twelve patients (12/50, 24%) were found to harbor either pathogenic or likely pathogenic variants compatible with the clinical diagnosis using the NMDs gene panel. One additional diagnosis was made using open exome analysis (1/50, 2%). The overall WES diagnostic yield was 26% (13/50; Table 2). Two patients were found to harbor VUSs incompatible with the disease phenotype (Table S1).

3.2 | Hereditary congenital myopathy subgroup

Four out of 24 patients in congenital myopathy subgroup were found to have disease-causing variants in *ACTA1* (OMIM 102610) (n = 2), *SELENON* (OMIM 606210), and *DNM2* (OMIM 602378), giving a diagnostic yield of 17%.

One of the patients with *ACTA1* variant had a mild motor phenotype (NMD006; Figure 1a,b), whereas the other subject presented with antenatal onset fetal akinesia with poor prognosis and was electively extubated after detailed discussion with parents (NMD037; Figure 1c,d). One patient has *SELENON* rigid spine syndrome with typical disease presentation (NMD016; Figure 1e). One patient has *DMN2*-related congenital myopathy with progressive disease course (NMD033).

3.3 | Hereditary muscular dystrophy subgroup

Among the 11 patients in this subgroup, WES identified four disease-causative genes, including *COL6A1* (OMIM 120220; n = 2), *LMNA* (OMIM 150330), *POMT1* (OMIM 607423), and *LAMA2* (OMIM 156225), in five patients giving a diagnostic yield of 45% (5/11).

One girl has *POMT1*-related asymptomatic hyperCKaemia with no major weakness but mild joint hyperlaxity (NMD007). She also has mild intellectual disability, autism spectrum disorder, and microcephaly, although her brain MRI findings were normal. WES found compound heterozygous variants in *POMT1* (NM_007171.3) The nonsense variant c.685C>T, p.(Gln229Ter), which has a population frequency of 0.000004061, was classified as likely pathogenic. The missense variant c.1024C>T, p.(His342Tyr) was a VUS with multiple in silico prediction as damaging.

Another young girl with *LAMA2*-related congenital muscular dystrophy (NMD048), she has infantile onset hypotonia with isolated gross motor delay. Brain MRI revealed diffuse cerebral white matter changes (Figure 1f,g) characteristic of merosin-deficient congenital muscular dystrophy.(Kumar, Aroor, Mundkur, & Kumar, 2014) WES identified compound heterozygous variants in *LAMA2* (NM_000426.3). The variant c.250C>T, p. (Arg84Ter) was classified as likely pathogenic, whereas c.4157A>T, p.(Tyr1386Phe) was considered as a VUS with a population frequency of 0.00009693 and a contradictory in silico prediction.

3.4 | Hereditary peripheral neuropathy subgroup

WES identified disease-causing variants in *MTMR2* (OMIM 603557), *MPZ* (OMIM 159440), and *IGHMBP2* (OMIM 600502) in three patients, giving a diagnostic yield of 27% (3/11). One patient had a VUS in *SCN11A* (OMIM 604385). Two related patients with negative WES had subsequent neuropathy gene panel revealed a reported disease causing variant in the noncoding region of *GJB1* (OMIM 304040).

Here we reported the first Charcot Marie Tooth Disease (CMT) type 4B1 in Chinese (NMD010) (Figure 1h,i). WES found compound heterozygous loss-of-function variants over the *MTMR2* gene (NM_016156.5), c.1794_1797dup, p.(Leu600Argfs*5), and c.1387-2A>G. Both variants were novel and absent in the control population. Both variants are classified as pathogenic, supported by in vivo studies of mtmr2-null mice revealed myelin alterations that produced a less severe version of the neuropathy observed in humans (Bolino et al., 2004; Bonneick et al., 2005).

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TABLE 1	CABLE 1 Genes in the neuromuscular disorders gene panel for first-tier analysis (Bonne et al., 2017)						
AARS	AARS2	ABCC9	ABHD5	ACAD9	ACADVL	ACTA1	ACTC1
ACTN2	ACVR1	ADCK3	ADSSL1	AFG3L2	AGL	AGRN	AHNAK2
AIFM1	AKAP9	ALDH18A1	ALDH3A2	ALG13	ALG14	ALG2	ALPK3
ALS2	AMPD2	ANG	ANK2	ANKRD1	ANO10	ANO5	ANXA11
AP4B1	AP4E1	AP4M1	AP4S1	AP5Z1	APTX	AR	ARHGEF10
ARL6IP1	ASAH1	ASCC1	ATG5	ATL1	ATL3	ATM	ATP13A2
ATP1A1	ATP1A2	ATP2A1	ATP7A	ATXN1	ATXN10	ATXN2	ATXN3
ATXN7	ATXN8OS	B3GALNT2	B4GALNT1	B4GAT1	BAG3	BEAN1	BICD2
BIN1	BSCL2	BVES	C12orf65	C19orf12	Clorf194	C9orf72	CACNAIA
CACNA1C	CACNA1G	CACNA1H	CACNAIS	CACNB2	CACNB4	CALM1	CALM2
CALR3	CAPN1	CAPN3	CASO1	CASO2	CAV3	CCDC78	CCDC88C
CCT5	CFL2	CHAT	CHCHD10	CHKB	CHMP2B	CHP1	CHRNA1
CHRNB1	CHRND	CHRNE	CHRNG	CLCN1	CLN3	CLTCL1	CNBP
CNTN1	CNTNAP1	COA7	COL12A1	COL13A1	COL25A1	COL6A1	COL6A2
COL6A3	COLO	COO2	C004	C006	C007	C009	COX15
COX6A1	COX6A2	CPTIC	CPT2	CRYAR	CSRP3	CTDP1	CTNNA3
CWF19L1	CYP2III	CYP7R1	DAGI	DCAF8	DCTNI	וחשת	
DES	DGAT2	DGUOK			DMPK	DNA2	DNA IR?
DNA IR6	DNM2	DNMTI	DOK7	DOLK	DPAGTI		
DPM3	DSC2	DSG2	DSP	DST	DTNA		DVNC1H1
DVSE		<i>FFF</i> 2	EGR2				
ENO3					EDUNZ		EMD
ENOS	ENTEDI EVOSC3	EXDSC	ENDD4	EADH		EITA EADS2	
	ENUSCO	EAUSCO	EIA4	FA2H EDY2	FAMIIIB	FAR52	FASIKD2
	FBLNJ	F BAL4	FBADI	FDA2	FGD4	FGF14	FHLI
FIG4	FKRP	FKIN	FLADI	FLNA	FLINC	FLVCKI	rUS
FXN	FXRI	GAA	GANI	GARS	GATADI	GBA2	GBEI
GDAPI	GDAP2	GFP11	GJAS	GJBI	GJB3	GJC2	GLEI
GMPPB	GNB4	GNE	GOLGA2	GOSR2	GPDIL	GRID2	GRMI
GYGI	GYSI	HACDI	HACEI	HARS	HCN4	HEXB	HINTI
HK1	HNRNPAI	HNRNPA2B1	HNRNPDL	HOXD10	HRAS	HSPBI	HSPB3
HSPB8	HSPD1	HSPG2	IBA57	IFRD1	IGHMBP2	ILK	INF2
INPP5K	ISCU	ISPD	ITGA7	ITPRI	JPH2	JUP	KARS
KBTBD13	KCNA1	KCNA5	KCNC3	KCND3	KCNE1	KCNE2	KCNE3
KCNH2	KCNJ18	KCNJ2	KCNJ5	KCNQ1	KIAA0196	KIDINS220	KIF1A
KIF1B	KIF1C	KIF21A	KIF26B	KIF5A	KLC2	KLHL40	KLHL41
KLHL9	KY	LICAM	LAMA2	LAMA4	LAMA5	LAMB2	LAMP2
LARGE1	LDB3	LDHA	LIMS2	LITAF	LMNA	LMOD3	LPIN1
LRP12	LRP4	LRSAM1	MAG	MAP3K20	MAPT	MARS	MARS2
MATR3	MB	MEGF10	MET	MFN2	MGME1	MIB1	MME
MORC2	MPV17	MPZ	MRE11A	MRPL3	MRPL44	MRPS25	MSTN
MSTO1	MTM1	MTMR2	MTO1	MTPAP	MURC	MUSK	MYBPC1
MYBPC3	MYH14	MYH2	МҮНЗ	МҮН6	MYH7	MYH8	MYL1
MYL2	MYL3	MYL4	MYLK2	МҮМК	MYO18B	MYO9A	MYOT
MYOZ2	MYPN	NAGLU	NDRG1	NDUFAF1	NEB	NEFH	NEFL
NEK1	NEXN	NGF	NIPA1	NKX6-2	NOP56	NOTCH2NLC	NPPA

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TABLE 1	(Continued)						
NT5C2	NTRK1	NUP155	NUP88	OPA1	OPTN	ORAII	PABPN1
PAX7	PCNA	PDK3	PDYN	PEX7	PFKM	PFN1	PGAM2
PGK1	PGM1	PHKA1	PHOX2A	РНҮН	PIEZO2	PIP5K1C	PKP2
PLD3	PLEC	PLEKHG5	PLN	PLP1	PMP2	PMP22	PNKP
PNPLA2	PNPLA6	PNPLA8	POGLUT1	POLG	POLG2	POMGNT1	POMGNT2
РОМК	POMT1	POMT2	PPP2R2B	PRDM12	PRDM16	PREPL	PRKAG2
PRKCG	PRPH	PRPS1	PRUNE1	PRX	PSEN1	PSEN2	PTRF
PTRH2	PUM1	PUS1	PYGM	PYROXD1	RAB7A	RAF1	RAPSN
RBCK1	RBM20	RBM7	REEP1	REEP2	RETREG1	RFC1	RNASEH1
RNF216	RPH3A	RRM2B	RTN2	RUBCN	RXYLT1	RYR1	RYR2
RYR3	SACS	SBF1	SBF2	SCN11A	SCN1B	SCN2B	SCN3B
SCN4A	SCN4B	SCN5A	SCN9A	SCO2	SCYL1	SDHA	SELENON
SEPT9	SETX	SGCA	SGCB	SGCD	SGCE	SGCG	SGPL1
SH3TC2	SIGMAR1	SIL1	SLC12A6	SLC16A1	SLC18A3	SLC1A3	SLC22A5
SLC25A1	SLC25A20	SLC25A4	SLC25A42	SLC25A46	SLC33A1	SLC52A2	SLC52A3
SLC5A7	SLC9A1	SMCHD1	SMN1	SNAP25	SNTA1	SNX14	SOD1
SPAST	SPEG	SPG11	SPG20	SPG21	SPG7	SPTAN1	SPTBN2
SPTBN4	SPTLC1	SPTLC2	SQSTM1	STAC3	STIM1	STUB1	SUCLA2
SUCLG1	SURF1	SYNE1	SYNE2	SYT14	SYT2	TARDBP	TAZ
TBK1	TBP	TCAP	TDP1	TDP2	TECPR2	TECRL	TFG
TGFB3	TGM6	TIA1	TIMM22	TK2	TMEM240	TMEM43	TMEM65
TMPO	TNNC1	TNNI2	TNNI3	TNNT1	TNNT2	TNNT3	TNPO3
ТОРЗА	TOR1A	TOR1AIP1	TPM1	TPM2	ТРМЗ	TPP1	TRAPPC11
TRDN	TRIM2	TRIM32	TRIM54	TRIM63	TRIP4	TRPC3	TRPV4
TSFM	TTBK2	TTN	TTPA	TTR	TUBA4A	TUBB3	TWNK
TYMP	UBA1	UBA5	UBAP1	UBQLN2	UCHL1	UNC13A	VAMP1
VAPB	VCL	VCP	VMA21	VPS13D	VPS37A	VRK1	VWA3B
WARS	WDR73	WNK1	WWOX	XRCC1	YARS	YARS2	ZFHX2
ZFYVE26	ZFYVE27						

3.5 | Complex condition with associated neuromuscular involvement

Among the four patients in this subgroup, WES identified one disease-causative variant in, *TGFB1* (OMIM 190180) by open exome analysis, giving a diagnostic yield of 25% (1/4).

The female patient (NMD034) had persistent motor clumsiness and limb pain, mild girdle weakness. WES analysis using NMDs gene panel did not identify any pathogenic variant. We subsequently proceeded with open exome analysis, which revealed a previously reported pathogenic variant, c.653G>A, p.(Arg218His), in *TGFB1* (NM_000660.6). Segregation study using Sanger sequencing confirms the variant is de novo. This variant was associated with Camurati– Engelmann disorder (Kinoshita et al., 2000). Patients with this condition exhibit progressive diaphyseal dysplasia with thickening of the diaphyseal bones, sclerosis of the skull base, and bone pain. Many cases are initially classified as neuromuscular disease because this condition often manifests with features of myopathy. Her subsequent skeletal survey confirmed skeletal dysplasia (Figure 1j).

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4 | DISCUSSION

4.1 | Our study confirms the importance of WES in the diagnosis of patients with NMDs

4.1.1 | Diagnostic yield

Our study achieved an overall diagnostic yield from WES of 26% (13/50), with 12 cases (24%) by NMDs gene panel analysis, and one (2%) by open exome analysis. These cases were diagnostically challenging as prior investigations failed to give clues on specific genetic diagnosis. Our findings are compatible to previous studies where the diagnostic yield

				Open Access		
		Reported/Novel (diagnosis)	Novel (Congenital myopathy)	Reported (Laing et al., 2009) Nemaline myopathy (Congenital myopathy)	Reported (Bioun et al., 2007) Centronuclear myopathy) (Congenital myopathy)	Novel (Rigid spine syndrome)
		ACMG CLN Post_P value by Bayesian calculation	Likely pathogenic (0.975)	Likely pathogenic (0.975)	Likely pathogenic (0.975)	Likely pathogenic and VUS (0.994 and 0.900)
			c.874A>G, p.(Arg292Gly) de novo	c.1001C>G, p.(Pro334Arg) de novo	c.1852G>A, p.(Ala618Thr) de novo	c.238delG, p.(Axp80Thrfs#20) unknown inheritance c.1304G5-A, p.(Arg435Gin) maternal inherited
		WES findings	ACTAI (NM_001100.3)	ACTAI (NM_001100.3)	D/M/2 (NM_001005360.2)	<i>SELENON</i> (NM_206926.1)
egiimiii		Prior normal gene studies	I	SMNI	1	
ov mreud n		NCS & EMG	NCV: Absent bilateral peroneal CMAP responses EMG: Myopathic	Q	Refused	NCV: Small CMAPs EMG: ND
pauruus win		Muscle biopsy	Nonspecific myopathic change	Ĝ	congenital fiber-type dispropor-tion	Nonspecific myopathic change
		CK	54-200	298	21-72	116-360
aguoig	tigations	MRI muscles	ABN	Q	ĝ	ABN
In ni	Invest	MRI brain	NAD	Ð	Q	NAD
ulligo, W EG ICoull a		Systemic involvement (respiratory, cardiac, cognition, musculoskeletal)	Respiratory: Restrictive lung function Cardiac: Mitral valve prolapse and regurgitation with left ventricular dilatation on Lisinopril Cognition: Normal Musculoskeletal: rigid spine, pes cavus	Respiratory: Intubated and ventilated since birth Cardiac: Normal Cognition: Uncertain Musculoskeletal: Bilateral shoulder, wist, finger, knee, ankle contractures	Respiratory: Noctumal NIV since 14 years; Continuous NIV since 19 years Cardiae: Normal Cognition: Normal Musculoskeletai: Cavovarus feet with surgeries: Scoliosis with initial brace use: refused scoliosis surgery	Respiratory: Noctumal NIV since 13 years Cardiae: Normal Cognition: Normal Musculoskeletal: Progressive rigid spine, endrems, ankles
11101 111 VCSUBAUOII 1111		Clinical presentation	Arthrogyposis multiplex congenita and delay walking, followed by stabilization of motor performance since then. Exam showed facial, neck flexor and limb girdle weakness, pectus exeavatum, rigid spine, and club feet.	Antenatal polyhydramnios and decrease fetal movement. Bom prematurely at 32 weeks of gestation, required intubation, and resuscitation ab birth. Ex am showed generalized weakness with poor respiratory effort. Developed chytothoras required chest drain. Died at 46 days old.	Neonatal-onset hypotonia and mild generalized weakness. Independent walking at a young age with waldling and easy falling. Lost annbulation at 13 years and required mocturnal NIV since aged 14 and continuous use since aged 19. Developed progressive scoliosis, and lost progressive scoliosis, and lost progressive scoliosis, and lost progressive scoliosis, and lost generalized weakness, muselik westing, marked scoliosis, anselik	Delayed walking and persistent motor clumsiness with increased walking difficulty at 10 years. Developed type II tespiratory failure required BIPA at 13 years. Rigd spine with ned, hyperetension observed at 14.5 years. Exam showed limb girdle weakness (lower limb > upper limb) and contractures.
		Motor	Delayed walking since 24 months	Non- ambulatory Minimal active movement	Walking before 18 months Fell easily at early age Lost ambulation at 13 years	Delayed walking since 21 months
CIIIICAI		ub group	CM	CM	CM	CM
Ctall (Onset	٣	AN	а	CH
		Sex/ Age	F/18	F/1 month	M/22	W/16
		Project No.	dmn 900	037 037	033 033	016 016

(Continues)

	Reported/Novel (diagnosis)	Reported (Giusti et al., 2005) Ulhrich CMD) (Ulhrich CMD)	Novel (Bethlam myopathy)	Reported (Bonne et al., 1999) EDMD (EDMD)	Novel (Merasin deficient CMD)
ACMG CLN Post_Pvalue by Bayesian calculation (1.000) (1.000)		Pathogenic (1.000)	Pathogenic (1.000)	Likely pathogenic (0.999)	Likely puthogenic and VUS 0.994 and 0.325
		c.850G>A. p.(Gly284Arg) de novo de novo	c. 1056 + 2dupT de novo de novo	c.1357C>T, p.(Arg453Trp) de novo	c.230C>T, p(Arg84*) unknown inheritance c.4157A>T, p(Tyr1386Phe) unknown inheritance
	WES findings	COL6A1 (NM_001848.3)	COL6A1 (NM_001848.3)	(NM_170707.4)	(NM_00426.3)
	Prior normal gene studies		DMD FKTN FKRP	SEIENON	I
	NCS & EMG	NCV: Normal EMG: Myopathic	NCV: Normal EMG: Myopathic	NCV: Normal EMG: Myopathic	NCV: Small CMAPs EMG: ND
	Muscle biopsy	Dystrophic change, severe debtion of collagen VI in sarcdemma	Dystrophic changes 4 alpha- dystrogly- can no collagen VI staining done	Myopathic change Core-like structures	QN
	CK	61-299	7 299– 1,311	699 1,259	1 2,253– 2,887
igations	MRI muscles	Ê	ABN	ABN	Ą
Invest	MRI brain	NAD	NAD	NAD	ABN
	Systemic involvement (respiratory, cardiac, cognition, musculosketetal)	Respiratory: No need for ventilator Cardiac: Normal Cognition: Normal Musculosketetal: Distorated high before Distorated high before long finger flexor, elbow flexor and knee flexor, elbow flexor and knee flexor, without bace scoliosis without bace	Respiratory: Mild obstructive sleep apnea syndrome Cardiac: Normal Cognition: Normal Muscubeslenti Progressive long finger Record clow flexion and Alexor, elbow flexion and Alexor, elbow flexion and Alexor, elbow flexion and Record elbow flexion and Record elbow flexion and Alexor, elbow flexion and Alexor	Respiratory: Normal Cardiac: Normal Cognition: Normal Musculoskeletail: Contractures at clows and ankles	Respiratory: Normal Cardiac: Normal Cognition: Normal Musculoskeletal: No contractures
	Clinical presentation	Noted hypotonia & dislocated hip in first year of fife. Delayed walking at 18 months old. Required orthopaedic intervention to both hip dislocation at aged 2. Pensistent waddling gait and failed to climb stairs unassisted at 6 years of age. Exam revealed facial rash, pilaris keratosis, distal hyperfaxity, marked limb gitule weakness, elbow, and finger flexor contracture. marked equinovants.	Normal walking at 1 years old, but persistent easy falling and motor clumsiness after 6 years. Examination revealed mild limb girdle weakest (lower limb > upper limb), distal hyperlatity. Facial rash, follicular hyperkeratosis, contractures over finger flexors, elbows, and tendoachilles, pes cavus.	History of persistent clumsiness since walking began. Noted lower limb weakness at 2 years. Exam showed thin body buid, limb girdle weakness (lower limb > upper limb), elbow & finger contractures, and tight tendoachilles.	Noted hypotonia, hyporeflexia, significant head lag and decreased active limb movement in the first month of life. Persistent gross motor delay. Sat unsupported at 9 months.
	Motor	Delayed walking since 18 months	Walking since 12 months	Walking since 12 months	Sitting Walking with support
	NMD sub group	đ	MM	đM	QW
	Onset	Z	CH	CH	ZZZ
	Sex/ Age	F/19	8 IVW	8/W	F/1
	Project No.	CIM 046	CIMN 800	NMD 013	048 048

TABLE 2 (Continued)

(Continues)

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	Reported/Novel (diagnosis)	Novel (Asymptomatic HyperCKaemia)	Novel (CMT 4B1)	Reported (Giannini et al., 2006; Liu et al., 2017) SMARD type I (CMT 2)	Novel (CMT1B)	
	ACMG CLN Post_P value by Bayesian calculation	Likely pathogenic and VUS (0.997 and 0.812)	Pathogenic (0.994 and 0.994)	Likely pathogenic (0.900 and 0.994)	Likely pathogenic (0.975)	
		c.685C>T, p.(Gln229%) maternally inherited c.1024C>T, p.(His342Tyr) paternally inherited	c.1797_1798insAGAA, p.(Leu600fs ⁴ 5) maternal inberited c.13877_2A>G unknown inberitance	c. 1060G>A, p.(Gly354Ser) patemally inherited c.2356delG, p.(Arg786fs ⁴ 45) unknown inheritance	c.737A>G, p.(Asp246Gly) de novo	
	WES findings	<i>POMT1</i> (NM_007171.3)	MTMR2 (NM_016156.5)	IGHMBP2 (NM_002180.2)	MPZ (NM_00630.8)	
	Prior normal gene studies	awa	COL6A1, COL6A2, COL6A3	I	PMP22	
	NCS & EMG	Both NCV and EMG normal	NCV: Refused imitially, agreed after genetic result available – no SNAP, CMAP responses EMG: ND	NCV: SM axonal polyneuro- pathy EMG: Neurogenic	NCV: SM demyelina- ting polneuro- pathy EMG: ND	
	Muscle biopsy	QN	End strage changes suspected decrease collagen VI	QN	â	
	CK U/L	11408– 3,883	30-91	83	48-127	
gations	MRI muscles	AB	Ê	Q	Q	
Investi	MRI brain	NAD	Q	Ð	R	
	Systemic involvement (respiratory, cardiac, cognition, musculoskeletal)	Respiratory: Normal Cardiac: Normal Cognition: Mild intellectual disabilities, autism spectrum disorder Musculoskeletal: Normal	Respiratory: Nocturnal NIV since 29 years Cardiac: Normal Cognition: Normal Musculoskeletal: Contractures at fingers, knees and ankles, mild scoliosis	Respiratory: Nomal Cardiac: Normal Cognition: Unknown Musculoskeletal: Normal	Respiratory: Normal Cardiac: Normal Cognition: Normal Musculoskaletal: Contractures at ankles	
	Clinical presentation	History of developmental delays & mild joint laxity. Asymptomatic hyperCKaemia with no clinical weakness. Microcephaly. Mild autism spectrum disorder.	History of easy falling and foot deformity requiring orthopedic surgeries during childhood. Progressive weakness with loss of ambulation at 17 years, nocturnal NIV since 29 years. Exam revealed ptosis, facial weakness, soft & hoarse voice, generalized limb weakness (distal > proximal), muscle wasting in hands, and calves.	History of easy falling and unsteady gait since walking began. Exam showed mild proximal girdle weakness but significant distal weakness with muscle wasting and mild tightness in Achilles tendons.	History of intermittent lower limb pain and motor clumsiness since 8 years. Stable motor performance with mild progressive distal muscle weakness, especially weak ankle dorsiflexors with varus deformity of the feet over time.	
	Motor	Walking since 16 months	Walking since normal age Independent walking until 17 years	Walking since 15 months Independent walking	Normal walking age Independent walking	
	ub dub group	QW	Z	Nd	Z	
	Onset	СН	CH	СН	СН	
	Sex/ Age	F/12	F/33	F/7	M/14	
	Project Vo.	CIMIN 007	CIMD 010	4MD 960	442 442	

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TABLE 2 (Continued)

(Continues)

Reported/Novel

ACMG CLN by Bayesian Post_P value calculation

(diagnosis)

(Kinoshita et al. 2000) Camurati-

pathogenic

p.(Arg218His) c.653G>A, de novo

(NM_000660.6)

WES findings TGFBI

tudies INWS

Muscle biopsy

NCV: Normal neurogenic

g

NAD

Respiratory: Normal

Musculoskeletal: Skeletal

dysplasia

limb girdle weakness noted

on examination.

2 years. Very mild lower

Cognition: Normal

Cardiac: Normal

had persistent easy falling and motor clumsiness after

Walked at a normal age but

Walking since 12 months

CN

СН

F/8

UMD 034

EMG:

gene

NCS & EMG

СĶ UL 100

MRI

ĺRI orain NAD

cardiac, cognition,

musculoskeletal)

Clinical presentation

Motor

group

Onset

Sex/ Age

No.

Project

muscles

Prior normal

(0.975) Likely

Reported

Engelmann (Camurati-

disease

NN, neonatal; PN, hereditary peripheral neuropathy subgroup; SM, sensorimotor; SMARD, spinal muscular atrophy with respiratory distress; SNAP, sensory nerve action potentials; VUS, variant of unknown sequencing; WES, complex condition with neuromuscular involvement subgroup; EDMD, Emery Dreifuss muscular dystrophy; EMG, electromyography; F, female; IN, infantile; LGMD, limb-girdle muscular dystrophy; MD, hereditary nerve conduction study; NCV, nerve conduction study; ND, not done; NIV, noninvasive ventilation; NMDs, Neuromuscular disorders; Engelmann Abbreviations: 1, increased; ABN, abnormal; ACMG CLN, American College of Medical Genetics and Genomics Classification; AD, adulthood; Adol, adolescence; AN, antenatal; BIPAP, Bilevel positive airway lisease) pressure; BMD, Becker muscular dystrophy; CH, childhood; CK, creatine kinase; CM, hereditary congenital myopathy subgroup; CMAP, compound muscle action potentials; CMD, Congenital muscular dystrophy; CN, normal; NCS, muscular dystrophy subgroup; MRI, magnetic resonance imaging; NAD, whole-exome sequencing. Molecular Genetics & Genomic Medicine

tends to be lower in cohorts that have undergone prior comprehensive evaluations, with diagnostic yields as low as 12.9% (Haskell et al., 2018) comparing to 79% in a newly diagnostic cohorts without prior extensive investigation (Schofield et al., 2017). The integrated approach, with deep phenotyping complemented by investigation findings, support the categorization of four distinct subgroups, with the muscular dystrophy subgroup had the highest diagnostic vield (45%).

4.2 Finding of pathogenic variants previously unreported in Chinese populations

Few studies based on WES have been performed in Chinese NMDs patients. To our knowledge, this is the first cohort study to report pathogenic variants in a Chinese patient with MTMR2-related CMT type 4B1. This finding highlights the usefulness of WES in such cases and expands the genetic spectrum relevant to the Chinese population.

Impact on clinical management 4.3

A timely diagnosis enables accurate prognostication and provides guidance regarding the clinical management and family counseling. For example, the confirmed genetic diagnosis of antenatal-onset ACTA1 myopathy in our patient within the first month of life enabled an accurate prognostication to support parents to understand the infant's condition. This led to timely counseling on redirection of care, and provided the parents with relevant knowledge needed to prepare for future pregnancies. A genetic diagnosis also enables relevant surveillance and early detection of NMDrelated complications. As in our child with Emery-Dreifuss muscular dystrophy, though he has not yet developed any cardiac involvement, the genetic diagnosis highlighted the need for close monitoring of cardiac symptoms to enable the early detection of arrhythmia or cardiomyopathy. In another child with Camurati-Engelmann disease, the finding of bony dysplasia that mimicked a neuromuscular condition allowed a timely referral for orthopedic monitoring of skeletal complications. At the same time, routine echocardiogram was discontinued.

Expanding the phenotype of known 4.4 pathogenic variant

POMT1 variant has not been reported to be associated with asymptomatic hyperCKaemia. The findings of our patient have expanded the clinical spectrum of this known pathogenic variant.



FIGURE 1 (a, b) Patient with heterozygous novel variant in *ACTA1* has rigid spine and pes cavus. (c, d) Patient with heterozygous reported variant in *ACTA1* had flaccid posture with minimal skin creases. Chest X-ray demonstrated bilateral chest drains with residual chylothorax on the right side. (e) Patient with compound heterozygous variants in *SELENON* has rigid spine. (f, g) Patient with compound heterozygous variants in *LAMA2* has the axial view of her T2 weighted MRI brain images shown diffuse cerebral white matter signal changes compatible to merosindeficient congenital muscular dystrophy. (h, i) Patient with compound heterozygous reported pathogenic variants in the *MTMR2*, with facial weakness and marked finger contractures with distal hand wasting. (j) Patient with heterozygous reported pathogenic variant in *TGFB1* with thickening of the diaphyseal bones (white arrows) on her X-ray of bilateral femurs

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FIGURE 2 Summary of patients in each phenotypic category with prior neuromuscular investigations including metabolic testing, MRI scan, muscle biopsy, nerve conduction study/ electromyography, genetic testing, and creatine kinase testing. CK, creatine kinase; EMG, electromyography; MRI, magnetic Resonance Imaging; NCS, nerve conduction study



FIGURE 3 Proposed integrated diagnostic approach of neuromuscular disorders



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4.5 | Role and limitations of WES

WES has shortened the diagnostic odyssey for our patients. The time from symptom onset to achieve a genetic diagnosis from WES of our 13 patients ranged from 1 month to 30 years with a mean interval time of 10.4 years. All our recruited patients are diagnostically challenging with no causative genetic diagnosis despite highly specialized tests including muscle biopsy, gene test, and imaging (Figure 2). WES also identifies new conditions that mimic neuromuscular disorders, as observed in our patient with skeletal dysplasia due to *TGFB1* pathogenic variant.

However, WES also has its limitations. One major challenge is the interpretation of VUSs, which represents a potential bottleneck in the genetic diagnostic pipeline (Bertier, Hétu, & Joly, 2016). In our cohort, we identified 2 VUSs not compatible with the patients' phenotypes. The patient with heterozygous *TTN* variant might be either carrying a second undetected *TTN* disease-causing variant and therefore has a recessive titnopathy, or is simply carrying this heterozygous variant that does not explain his condition (Table S1).

Short-read WES is of limited usefulness for detecting variants other than SNVs and small indels, such as copy number variations (CNVs), expansions, or contractions in repetitive regions, chromosomal rearrangements and deep intronic variants. CNVs include exon deletion in *SMN1* in spinal muscular atrophy, exon deletion, or duplication in dystrophinopathy, *PMP22* duplication in Charcot-Marie-tooth diseases, could be evaluated by multiple ligation probe analysis (MLPA) or whole-genome sequencing. Expansion or contraction in repetitive regions include CTG triplet repeat in myotonic dystrophy and contraction of the D4Z4 macrosatellite repeat in *DUX4* in facioscapulohumeral muscular dystrophy could be evaluated by fragment analysis. Correct clinical diagnosis of these distinctive NMDs guiding the appropriate target gene study would avoid unnecessary WES that could not detect these variants.

WES may also miss the variant outside the exome that arise in the deep intronic or untranslated regions (UTR). This is illustrated by the two brothers, with heterozygous variant in the 5' UTR of *GJB1* with c.-103C>T variant have been identified as causative for X-linked CMT (Tomaselli et al., 2017) that could not be picked up by WES but revealed by neuropathy gene panel that specifically looked up this region. Furthermore, pseudogenes may lead to WES errors and WES coverage is nonoptimal at the beginnings or ends of exons, in GC-rich regions, and homopolymeric regions.

5 | CONCLUSION

Deep phenotyping is needed to direct the WES analysis. While there are challenges in using WES, its application has shortened the diagnostic odyssey for patients with monogenic neuromuscular disorders. Given the declining cost, we recommend considering WES in the diagnostic workflow in patients with NMDs (Figure 3).

CONSENT FOR PUBLICATION

All subjects in this study provided signed consent for the publication of their data.

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CONFLICT OF INTEREST

All authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

Mandy Ho Yin Tsang and Annie Ting Gee Chiu should be considered joint first author. Brian Hon Yin Chung, Sophelia Hoi Shan Chan should be considered joint corresponding authors. Mandy Ho Yin Tsang designed and performed the WES analysis and prepared the manuscript. Annie Ting Gee Chiu prepared the manuscript. Bernard Ming Hong Kwong designed and performed the WES analysis. Rui Liang assisted patient recruitment, data entry, DNA extraction, and WES preparation. Wetor Hok Lai Ho helped with the DNA extraction and WES preparation. Mullin Ho Chung Yu, Kit San Yeung, Christopher Chun Yu Mak, Gordon Ka Chun Leung, Steven Lim Cho Pei, and Jasmine Lee Fong Fung assisted the WES analysis. Virginia Chun Nei Wong and Francesco Muntoni reviewed the manuscript. Brian Hon Yin Chung design and conducted the WES analysis. Sophelia Hoi Shan Chan recruited the patients, designed the study, and prepared the manuscript. All co-authors approved the submitted manuscript.

DATA AVAILABILITY STATEMENT

The dataset analyzed during the current study is not publicly available for privacy reasons. However, the corresponding authors will provide access upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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