신경근육재활 및 전기진단
발표일시 및 장소 : **10 월 26 일(금) 13:51-14:03 Room E(5F)**

OP- Scientific 1-4

Usefullness of comprehensive next-generation sequencing panel for neuromuscular diseases in Korean

Dae-Hyun Jang[1*†], Dong-Woo Lee[1], Hyun Mi Oh[2], Jihye Park[3], Ja-Hyun Jang[4]

Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Department of Rehabilitation Medicine[1], , National Traffic Injury Rehabilitation Hospital, College of Medicine, The Catholic University of Korea, Department of Rehabilitation Medicine[2], Seoul St. Mary`s Hospital, College of Medicine, The Catholic University of Korea, Department of Rehabilitation Medicine[3], Green Cross Genome, Department of Laboratory Medicine[4]

Purpose
Neuromuscular disorder (NMD) is a very broad term that encompasses many diseases that affect the neuromuscular system. The genetic and clinical heterogeneity, unspecific clinical features, unidentified genes and the implication of large genes give challenges in routine molecular diagnosis, increasing turnaround time and effort to make molecular validation of the diagnosis. Recently, the targeted multi-gene panel sequencing (GPS) has been emerging as a molecular diagnostic tool, which is a part of next generation sequencing (NGS) technique. The multi-GPS generally ensures that all coding exons of the gene of interest are targeted, and all exons exhibit sufficiently high coverage, which is the most appropriate in diagnosing diseases like NMDs that are genetically heterogenous but similar in clinical aspect. There have been several multi-GPS studies of a specific NMD such like congenital myopathy, muscular dystrophy, motor neuron disease and polyneuropathy. However, few researches on the comprehensive NMDs gene panel have been conducted. Therefore, the purpose of the present study was to assess the usefulness of a comprehensive NMDs gene panel.

Methods
We designed two comprehensive NGS panels targeting 293 genes (version 1) and 410 genes (version 2) associated with NMDs. All patients were analyzed by a NGS panel (Version 1 was used from June 2016 to September 2017, and version 2 was used from October 2017 to May 2018), chromosomal microarray and karyotyping tests.

Results
Total 91 patients were enrolled and a genetic diagnosis was possible in 36 of 91 patients (39.5%). Thirty-four patients were diagnosed through the comprehensive NMDs gene panel, and two were confirmed by chromosome microarray test (Fig. 1). Approximately 38.5% (35/91) of the subjects using this panel version 1 and 2 were tested for myopathy as the initial impression, and 18 of 35 patients were diagnosed as myopathy and the

diagnosis rate was as high as 50%. Neuropathy was suspected in 15 patients, of which 7 (46.7%) were diagnosed. In the case of ataxia, four of 12 patients (33.3%) were diagnosed. Spastic paraplegia was suspected in 20 patients, and 5 of them (25.0%) were diagnosed. A diagnostic yield of version 2 was higher than version 1 (14/39; 35.9% vs 20/52; 38.5%, Fig. 2). Total 37 definitive causative and 10 possible causative variants were identified, of which 17 were novel (Table 1).

Conclusions

Comprehensive NMDs gene panel can improve the genetic diagnosis efficiency. Due to the rapid discovery of disease causing genes, an update of gene panel is required.

Table 1. Summary of 34 patients diagnosed with causative/possible causative variants using NGS panel.

Patient ID	Sex	Current age, y	Onset age, y	Gene	Panel	Variant	ACMG score	Final diagnosis	Causative/Possible causative	References
26	F	33	NB	*MYH7*	1	NM_000257.3: c.1498_1500del (p.Glu500del)	Likely pathogenic	Laing Distal Myopathy	Possible causative	This study
27	M	58	25	*REEP1*	1	NM_022912.2: c.603delC (p.*202Argfs*21)	VUS	SPG31/HMN5B	Possible causative	This study
28	M	53	31	*SPG4*	1	NM_014946.3: c.1307C>T (p.Ser436Phe)	pathologic	Spastic paraplegia 4	Causative	Neurology. 2000;55:1388-90
29	F	10	NB	*RYR1*	1	NM_000540.2:c.4496_4497delTT (p.Phe1499Cysfs*47) NM_000540.2:c.9716T>A (p.Met3239Lys)	Pathogenic Likely pathogenic	Minicore myopathy with external ophthalmoplegia	Causative Causative	This study This study
30	M	1.3	NB	*LMNA*	1	NM_005572.3:c.745C>T (p.Arg249Trp)	Pathogenic	LMNA related congenital muscular dystrophy	Causative	Ann Neurol. 2008;64:177-86
31	M	24	17	*CACNA1A*	1	NM_000068.3:c.3855C>G (p.Tyr1285*)	Pathogenic	Episodic ataxia type 2	Causative	This study Eur J Neurol. 2017 Jul;24(7):e43-e44
32	M	42	42	*CYP27A1*	1	NM_000784.3:c.1420C>T (p.Arg474Trp)	Pathogenic	Cerebrotendinous xanthomatosis	Causative	J Lipid Res. 1994;35:1031-9
33	F	46	20	*LMNA*	1	NM_170707.3:c.1412G>C (p.Arg471Pro)	VUS	LMNA related limb girdle muscular dystrophy	Possible causative	This study
34	M	32	20	*GNE*	1	NM_001128227.2: c.131G>C (p.Cys44Ser) NM_001128227.2: c.258-8G>A	Pathogenic VUS	GNE myopathy	Causative Possible causative	Hum Mutat. 2014;35:915-26 This study
35	M	53	40	*GJB1*	1	NM_000166.5:c.590C>T (p.Ala197Val)	Pathogenic	Charcot-Marie-Tooth Neuropathy X Type 1	Causative	Clin Genet 2012;81:142-9
36	M	55	20	*EMD*	1	NM_000117.2:c.101dupA (p.Tyr34*)	Pathogenic	Emery-Dreifuss muscular dystrophy 1	Causative	Neuromuscul Disord 1999;9:159-65
37	F	10	NB	*CACNA1A*	1	NM_001127221.1: c.4991G>A (p.Arg1664Gln)	Pathogenic	non-progressive congenital cerebellar ataxia	Causative	J Neurol Sci. 2006;241(1-2):13-7
38	F	23	15	*CACNA1A*	1	NM_001127221.1: c.5035C>T (p.Arg1679Cys)	Pathogenic	Episodic ataxia, type 2	Causative	J Neurol Sci 2010;291(1-2):30-6
39	F	45	25	*CAPN3*	1	NM_000070.2: c.1118G>A (p.Trp373*) NM_000070.2: c.1795dupA (p.Thr599Asnfs*33)	Pathogenic Pathogenic	Muscular dystrophy, limb-girdle, type 2A	Causative Causative	This study Muscle Nerve 1998;21:1493-501
72	M	35	35	*DYSF*	2	NM_003494.3: c.1284+2T>C NM_003494.3: c.5303G>A (p.Arg1768Gln)	Pathogenic likley Pathogenic	Muscular dystrophy, limb-girdle, type 2B	Causative Causative	J Neurol Sci 2003;211(1-2):23-8 This study
73	F	7	2	*SACS*	2	NM_014363.5: c.12973C>T (p.Arg4325*) NM_014363.5: c.11101T>C (p.Trp3701Arg)	Likely pathogenic VUS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay	Causative Possible causative	J Neurol 2006;253:1372-3 This study
74	M	50	4	*MFN2*	2	NM_014874.3: c.1090C>T (p.Arg364Trp)	Pathogenic	Charcot-Marie-Tooth disease, axonal, type 2A	Causative	Neurology 2011;76:1690-6
75	M	14	8	*BSCL2*	2	NM_032667.6: c.269C>T (p.Ser90Leu)	Pathogenic	HMN5A (Neuropathy, distal hereditary motor, type VA), SPG17 (spastic paraplegia-17)	Causative	Muscle Nerve 2007;36:384-6
76	F	8	NB	*INF2*	2	NM_022489.3: c.311G>A (p.Cys104Tyr)	Likely pathogenic	Charcot-Marie-Tooth disease, dominant intermediate E	Causative	NEJM 2011;365:2377-88
77	M	9	NB	*SPG4*	2	NM_014946.3: c.1253_1255del(p.Glu418del)	Pathogenic	Spastic paraplegia 4	Causative	J Neurol 2013;260: 906-909
78	F	17	NB	*PMP22*	2	NM_000304.3: c.281delG (p.Gly94Alafs*17)	Pathogenic	Charcot-Marie-Tooth disease type 1E	Causative	Muscle Nerve 1997;20:1308-10
79	F	37	7	*GNE*	2	NM_005476.5: c.2135T>C (p.Met712Thr)	Pathogenic	GNE myopathy	Causative	Nat Genet 2001;29:83-7
80	M	23	20	*ANO*	2	NM_213599.2: c.1158delT (p.Phe386Leufs*41) NM_213599.2: c.1640G>A (p.Arg547Gln)	Pathogenic VUS	Miyoshi muscular dystrophy 3 or Muscular dystrophy, limb-girdle, type 2L	Causative Possible causative	This study Neuromuscul Disord 2013;23:456-60
81	F	10	7	*TTN*	2	NM_133378.4: c.26231-1G>C NM_133378.4: c.85108dup (p.Arg28370Lysfs*15)	Pathogenic Pathogenic	Muscular dystrophy, limb-girdle, type 2J	Causative Causative	This study This study
82	M	33	7	*DMD*	2	NM_004006.2: c.1652G>A (p.Trp551*)	Pathogenic	Duchenne muscular dystrophy	Causative	This study
83	M	58	40	*DES*	2	NM_001927.3: c.1255C>T (p.Pro419Ser)	Pathogenic	Myofibrillar myopathy	Causative	Neuromuscul Disord. 2007;17:443-50
84	M	31	20	*SPG11*	2	NM_025137.3: c.3291+1G>T NM_025137.3: c.5410_5411del (p.Cys1804Profs*25)	Pathogenic Pathogenic	Spastic paraplegia 11	Causative Causative	J Neurol 2009;256:1714-8 J Neurol 2009;256:1714-8
85	M	22	20	*DES*	2	NM_001927.3: c.1043A>C (p.Gln348Pro)	Pathogenic	Myofibrillar myopathy	Causative	PLoS One 2014;9:e115470
86	F	1.8	NB	*ALDH3A*	2	NM_000382.2: c.1291_1292del (p.Lys431Glufs*5) NM_000382.2: c.1309A>T (p.Lys437*)	Pathogenic Pathogenic	Sjogren-Larsson syndrom	Causative Causative	Am J Hum Genet 1999;65:1547-60 J Child Neurol. 2013 Oct;28(10):1259-65
87	F	55	25	*DYSF*	2	NM_003494.3: c.779C>G (p.Pro260Arg) NM_003494.3: c.2997G>T (p.Trp999Cys)	Likely pathogenic Pathogenic	Muscular dystrophy, limb-girdle, type 2B	Causative Causative	This study Proc Jpn Acad 1999;75B;207-212
88	M	3	2	*DMD*	2	NM_004006.2: c.9563+1G>A	Pathogenic	Duchenne muscular dystrophy	Causative	This study
89	F	17	NB	*ACTA1*	2	NM_001100.3: c.739G>C (p.Gly247Arg)	Likely pathogenic	ACTA1 gene related myopathy	Causative	This study
90	M	27	10	*LAMA2*	2	NM_000426.3: c.4640C>T (p.Thr1547Met) NM_000426.3: c.7928_7929del (p.Arg2643Lysfs*9)	VUS Likely pathogenic	LAMA2-Related Muscular Dystrophy	Possible causative Possible causative	Sci Rep 2016;6:29088 This study
91	M	44	41	*GJB1*	2	NM_000166.5: c.394T>C (p.Trp132Arg)	Pathogenic	Charcot-Marie-Tooth Neuropathy X Type 1	Causative	Clin Genet 2012: 81:142-149

NB, new born; VUS, variant of uncertain significance

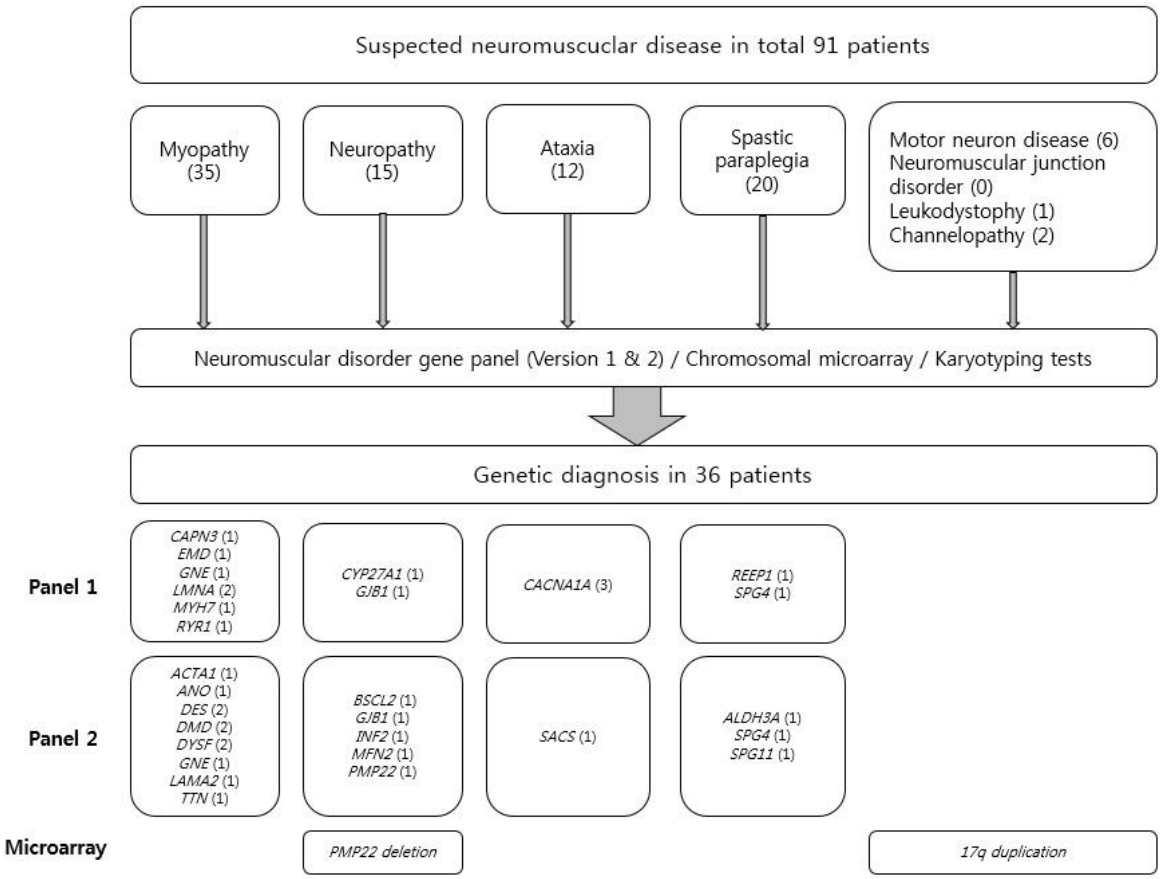

Figure 1. Flow chart of patients enrolled in this study

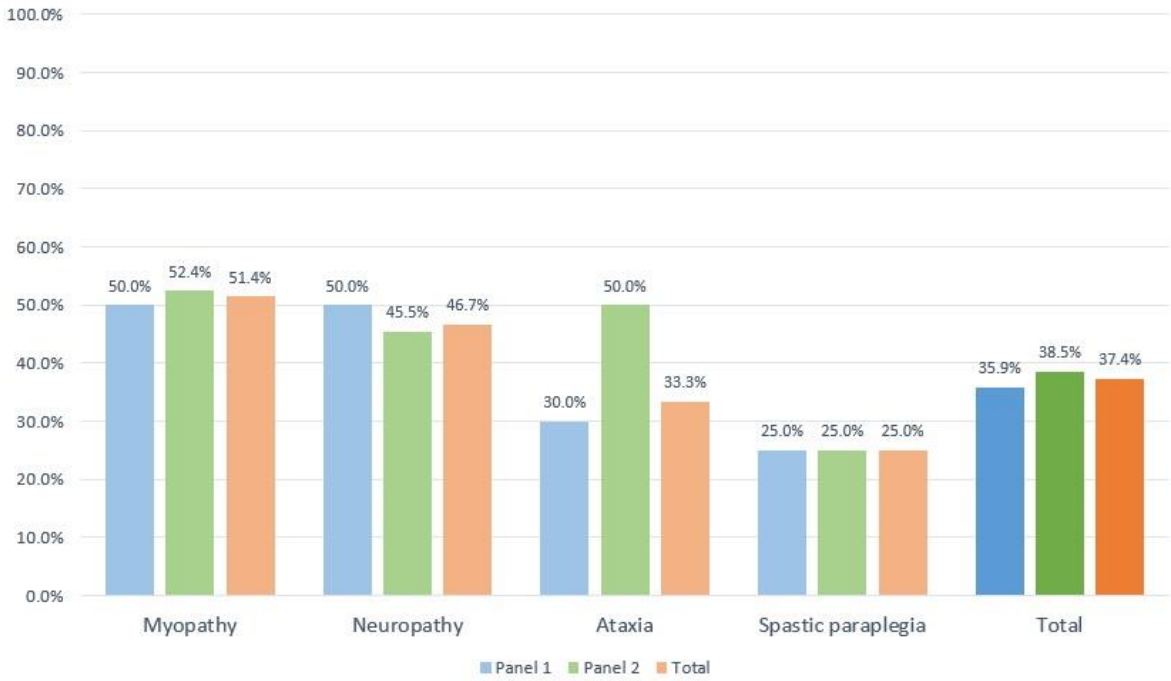

Figure 2. Diagnostic rate for each disease category using NGS panel version 1 and 2.