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CLINICAL REPORT

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Differing disease phenotypes of Duchenne muscular dystrophy and Moyamoya disease in female siblings of a Korean family

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Abstract

Background: Variable disease phenotypes can be influenced by several factors such as allelic variation, environmental factors, genetic modifiers, and genotype–environment interaction. Herein to the best of our knowledge, this is the first report of the coexistence of *DMD* and *RNF213* gene mutations in a Korean family with differing disease phenotypes of Duchenne muscular dystrophy (DMD) and Moyamoya disease (MMD) in each female sibling.

Methods: Deletion or duplication of the exon in *DMD* was screened using multiplex ligation-dependent probe amplification (MLPA). Subsequently, single exon deletion or duplication identified by MLPA was confirmed by Sanger sequencing. On the other hand, a common missense mutation [NM_001256071.2:c.14429G>A (p.Arg4810Lys)] related to MMD in exon 60 of *RNF213* was also identified by Sanger sequencing.

Results: Three female family members carried the same disease-causing mutations, c.9953_9954delAG of *DMD* and c.14429G>A of *RNF213*. Two (II-2 and II-3) of these siblings suffer from the disease but exhibited different DMD or MMD symptoms, while the mother (I-2) seemed almost unaffected.

Conclusion: This report illustrates the difficulty that might be encountered in the interpretation of complex clinical manifestations when different genetic defects affecting neuromuscular and vascular diseases coexist.

KEYWORDS

disease phenotypes, *DMD* gene, Duchenne muscular dystrophy, Moyamoya disease, *RNF213* gene

1 | INTRODUCTION

Duchenne muscular dystrophy (DMD, OMIM#310200) is X-linked recessive myopathy caused by mutations in the dystrophin gene located on Xp21. However, certain cases of female DMD carriers show clinical symptoms or manifestations of the disease, which vary from presenting with elevated serum creatine kinase level to evidence of calf hypertrophy, scoliosis, or myopathic patterns on electromyography. (Papa et al., 2016). The frequency of symptomatic female carriers has been estimated to be in the range from 2.5% to 7.8% according to skeletal muscle

Joonhong Park and Woori Jang contributed equally to this work.

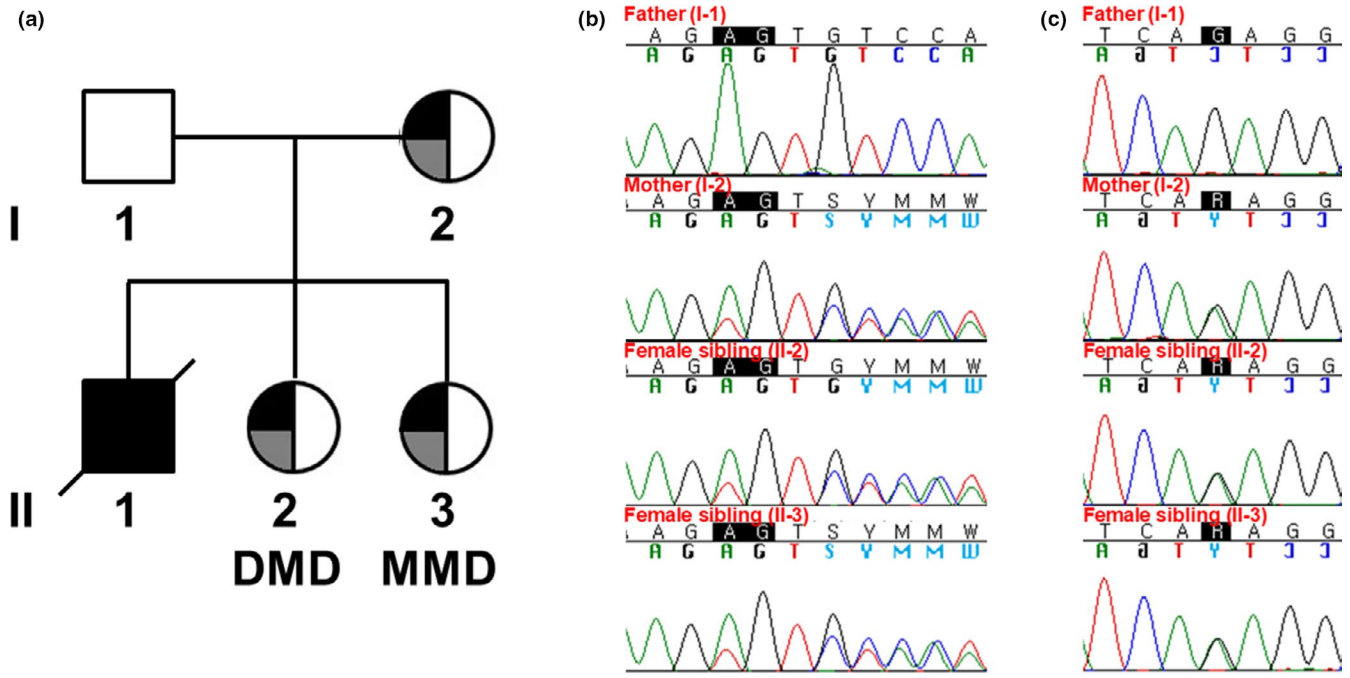

FIGURE 1 (a) Pedigree analysis of a Korean family diagnosed with Duchenne muscular dystrophy and Moyamoya disease with DMD and *RNF213* mutations. Solid black and gray symbols indicate c.9953_9954delAG (p.Glu3318Valfs*15) of DMD and c.14429G>A (p.Arg4810Lys) of *RNF213* mutations, respectively. Individual II-2 showed clinical symptoms of Duchenne muscular dystrophy only, whereas II-3 presented clinical manifestations of Moyamoya disease only, even though both female siblings carried the same *DMD* and *RNF213* mutations originating from the mother in the heterozygous state. (b) Results of Sanger sequencing for c.9953_9954delAG (p.Glu3318Valfs*15) of DMD. (c) Results of Sanger sequencing for c.14429G>A (p.Arg4810Lys) of *RNF213*. DMD, Duchenne muscular dystrophy

involvement (Hoogerwaard, Ginjaar, Bakker, & de Visser, 2005). The frequency of female carrier tended to be decreased in mothers of DMD patients with deletion mutations (53.5%) than in those with duplications (66.7%) and small mutations (67.9%) (Lee et al., 2014). It was suggested that de novo deleterious mutations occur more frequently rather than other mutations (Zimowski, Pawelec, Purzycka, Szirkowiec, & Zaremba, 2017).

On the other hand, Moyamoya disease (MMD, OMIM #607151) is a cerebral arteriopathy which is predominantly characterized by progressive stenosis of the bilateral internal carotid arteries (ICAs) and their collateral branches. Genetic studies of *RNF213* revealed a founder mutation, p.Arg4810Lys (rs112735431), in 95% of MMD families, 73% of nonfamilial MMD patients, and 1.4% of controls; this mutation greatly increases the risk of MMD (Kamada et al., 2011). In Korean MMD patients, the minor allele frequency of the c.14429G>A (p.Arg4810Lys) of *RNF213* was statistically higher ($p < .001$) in the MMD patients (41.8%, 138/330) than in the healthy controls (1.4% 8/588), and the mutation elevated the relative risk of MMD with an odds ratio of 52.1 ($p < .001$) compared to healthy controls (Kim et al., 2016).

Herein to the best of our knowledge, this is the first report of the coexistence of *DMD* and *RNF213* gene mutations in a Korean family with differing disease phenotypes of DMD and MMD in each female sibling.

2 | CLINICAL REPORT

This study was approved by the institutional review board of The Catholic University of Korea, Daejeon St. Mary's Hospital. All participants and their parents provided written informed consent for clinical and molecular analyses. Patient consent for publication of medical information was obtained from the participants' parents. Two other female siblings carrying same genetic mutation status of *DMD* and *RNF213* showed different genetic disease phenotype, respectively.

First female sibling with DMD (Figure 1a, individual II-2) was admitted with complaints of progressive weakness of the bilateral lower extremities that began after high school graduation. Prior to admission, she had an admission history of episodic rhabdomyolysis at the age of 19. Cardiac echocardiography showed normal ejection fractions but suspicious hypokinetic wall movement. Electrocardiography findings revealed deep Q waves in V4–6 leads. The muscle biopsy findings showed variations in fiber size, decreased dystrophin, and mosaic expression of dystrophin protein by

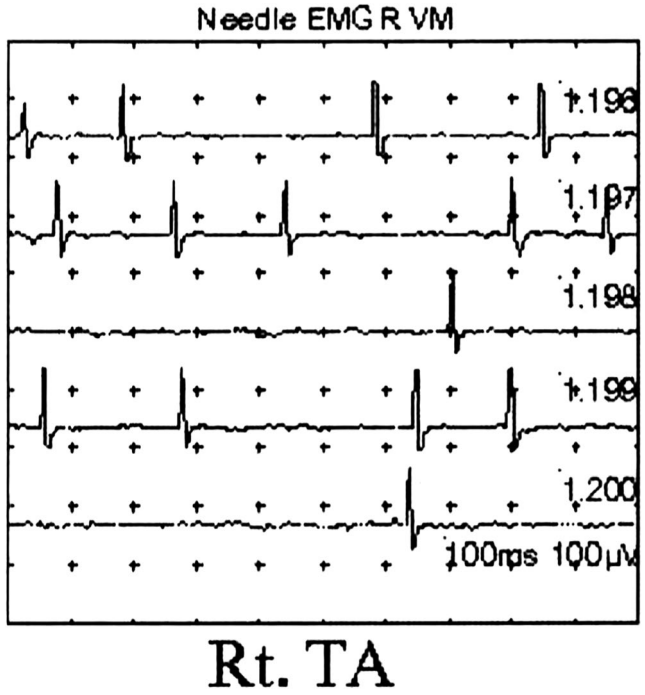

FIGURE 2 In the electromyography study, the patient's (II-2) motor unit action potential showed small amplitude, polyphasic, short duration in the right vastus medialis muscle

immunochemical stain. Electromyography showed small-amplitude, polyphasic, short duration in the right vastus medialis muscle (Figure 2). Serum creatine phosphokinase was found to be elevated (5,120 U/L). Accordingly, she was presumptively diagnosed as being a symptomatic DMD carrier at the age of 20. She showed recurrent headache and underwent brain magnetic resonance imaging (MRI)/magnetic resonance angiography (MRA) examination. No suspicious findings of MMD were found.

Second female sibling with MMD (Figure 1a, individual II-3), a 16-year-old and the third child of nonconsanguineous parents, presented with tingling sensations in both hands and lower legs as well as myalgia for a week after exercise. Two months after the initial visit, another symptom occurred in the form of TIA associated with numbness and weakness of both hands at the age of 17. Brain MRI/MRA confirmed the occlusion of both ICA terminal portions with prominent moyamoya vessels and leptomeningeal collaterals (Figure 3). She underwent successful encepha-duro-arterio-synangiosis with symptomatic reduction. Her postoperative course was uneventful. She continues to experience mild tingling sensations in both hands after working with normal cognitive academic performance for 2 years.

Female siblings' mother (Figure 1a, individual I-2), a 46-year-old healthy DMD carrier, did not show any medical problems upon visiting our hospital. Her neurological examination was normal, however, despite no association symptom of MMD, she was stressed about the possibility of MMD and wanted to undergo brain MRI/MRA examination. No suspicious findings of MMD were observed.

Male sibling (Figure 1a, individual II-1) could not walk until much later than other children of his age. He often fell down and had trouble climbing stairs or getting up from the floor. He was diagnosed with DMD at the age of 5 and expired due to cardiopulmonary problems at the age of 23.

3 | GENETIC ANALYSIS

Deletion or duplication of the exon in *DMD* was screened using multiplex ligation-dependent probe amplification (MLPA). MPLA was conducted according to the manufacturer's protocol using the SALSA P034 DMD mix 1 Kit and P035 DMD mix 2 Kit (MRC Holland). The resulting peak heights were normalized, and a deletion or duplication was considered in the case that a normalized peak ratio was under 0.7 or above 1.3. As a result, single apparent single exon deletion was detected in exon 68 of *DMD*. Subsequently, single exon deletion or duplication identified by MLPA was confirmed by Sanger sequencing. A novel frameshift mutation [NM_004006.2: c.9953_9954delAG (p.Glu3318Valfs*15)] causing premature codon termination in exon 68 of *DMD* was identified in the male sibling (II-1) in the hemizygous state and in the mother and female siblings in the heterozygous state, respectively (Figure 1b). On the other hand, a common missense mutation [NM_001256071.2:c.14429G>A (p.Arg4810Lys)] related to MMD in exon 60 of *RNF213* was detected in the mother and female siblings in the heterozygous state, but not in the male sibling (Figure 1c). Biologic paternity and kinship were confirmed by a short tandem repeat multiplex assay on 16 loci across the genome using AmpFLSTR® Identifiler (Applied Biosystems).

4 | DISCUSSION

In this report, three family members carried the same disease-causing mutations, c.9953_9954delAG of *DMD* and c.14429G>A of *RNF213*. Two (II-2 and II-3) of these siblings suffer from the disease but exhibited different DMD or MMD symptoms, while the mother (I-2) seemed almost unaffected. The same mutations do not always manifest in all individuals who share certain hereditary diseases. Furthermore, when these mutations do manifest, they do not always manifest in terms of the same clinical symptoms. These findings have good grounds for the examples of expressivity and penetrance. Expressivity refers to the extent to which a genotype exhibits its clinical phenotype. On the other hands, penetrance refers to the proportion of a population of individuals who harbor a disease-related genotype and express the related disease manifestation. While estimation of

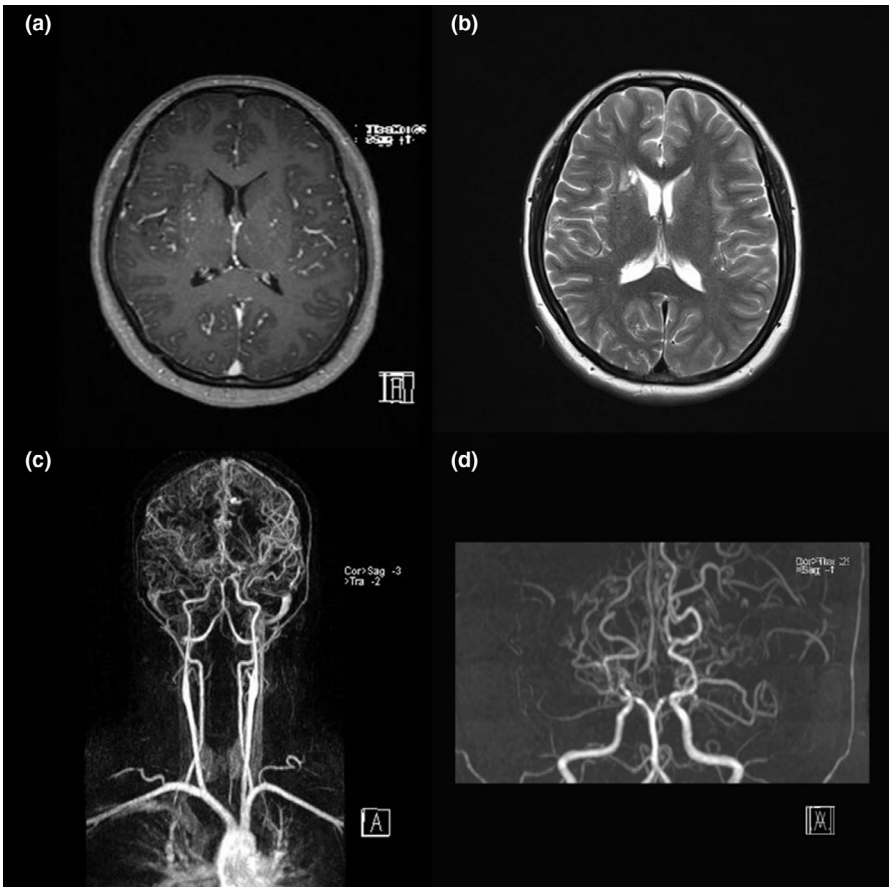

FIGURE 3 (a, c, and d) Occlusion of both ICA terminal portions with prominent moyamoya vessels and leptomeningeal collaterals. (b) Encephalomalacic change in the right striatocapsular region. ICA, internal carotid artery

penetrance concentrates upon whether a disease is expressed or not in a population, the frequency to which a genotype is revealed phenotypically in individuals can be estimated. Both the penetrance and expressivities of certain diseases can be understood partially through the influence of genetic modifiers (Table 1).

Variable disease phenotypes can be influenced by several factors such as allelic variation, environmental factors, genetic modifiers, and genotype–environment interaction. In most cases with a particular genotype is inherited, it is not fully understood why the same allele can cause insignificantly or obviously different phenotypes. In certain cases, a genetic modifier can change the threshold for trait expression, which intends that the genetic modifier causes a less or more proportion of individuals to express the disease phenotype. On the one hand, a genetic modifier can influence the degree of penetrance by altering the gene transcript expression, or affecting phenotypes at other organizational levels according to modifying phenotypes at the organismal or cellular levels (Nadeau, 2001). For example, some individuals carrying homozygous for the *DFNB26* allele are not deaf, which results in incomplete penetrance, however most individuals with homozygous status of the gene are deaf (Riazuddin et al., 2000).

Meanwhile, genetic modifiers may also move the range of disease phenotypes or the trait distribution, which influence more individuals harboring a disease-associated allele to express a less or more severity of the disease phenotype. For example, beta thalassemia, an inherited blood disorder caused by defective beta-globin synthesis, demonstrates variable clinical manifestations and diverse expressivities. The diversity of these phenotypes is influenced by a number of factors such as the involvement of numerous genetic modifiers in other loci affecting the production of globin. (Thein, 2005).

The female carriers with *DMD* mutations are directly correlated with a skewed inactivation in X chromosome. X chromosome containing the normal *DMD* is primarily inactivated, resulting in moderate to severe muscle involvement (Viggiano, Ergoli, Picillo, & Politano, 2016). The relationship between relative proportion of the wild type transcript or total DMD transcript level and DMD phenotype in DMD females is not well known. Moreover, the absence of an association between X-inactivation and transcriptional pattern of

TABLE 1 Genotype and phenotype findings in a Korean family with Duchenne muscular dystrophy and Moyamoya disease

	Mother (I-2)	Male sibling (II-1)	Female sibling (II-2)	Female sibling (II-3)
p.Glu3318Valfs*15 of *DMD*	Heterozygous	Hemizygous	Heterozygous	Heterozygous
p.Arg4810Lys of *RNF213*	Heterozygous	—	Heterozygous	Heterozygous
Brain MRI/MRA	—	—	Not abnormal	Abnormal
Thigh MRI	—	—	Not abnormal	—
Myoglobin, ng/mL (25–58)	—	—	143	—
CK-MB, ng/mL (0.3–5)	4.86	86.16	33.56	2.15
Troponin T, ng/mL (<0.014)	0.010	0.031	0.016	< 0.003
hsCRP, mg/dL (< 0.3)	0.44	0.05	2.39	0.69
AST, IU/L (8–32)	17	54	40	14
ALT, IU/L (5–33)	12	50	51	16
LDH, IU/L (135–250)	180	4,810	757	184
LDH electrophoresis	—	—	Normal	Normal
CPK, IU/L (26–190)	178	10,820	5,120	122
CK electrophoresis	—	Abnormal	Abnormal	Normal
CK-MM, U/L	—	7,682 (71%)	4,864 (95%)	122 (100%)
Macro CK, U/L	—	3,130 (29%)	256 (5%)	0

Note: Abbreviation: —, not done; CPK, creatine phosphokinase.

dystrophin, suggesting that *DMD* avoids, to some degree, X chromosome inactivation (Brioschi et al., 2012).

On the other hand, the penetrance of autosomal dominant MMD is very low, as indicated by discordant identical twins or the idea of "skipping a generation" (Mineharu et al., 2006). The Japanese p.Arg4810Lys founder mutation in *RNF213* showed diminished penetrance prominently in spite of its very high effect size (217, addictive model) (Moteki et al., 2015). Pedigree analysis of MMD families suggests autosomal dominant inheritance fashion with low penetrance or in a polygenic manner (Mineharu et al., 2006). However, the concordance rate of MMD in monozygote twins can be as high as 80% (Mukawa, Nariai, Matsushima, & Ohno, 2013). Recurrence rates in offspring and relatives were also reported to be 34- and 42-fold higher, respectively, than they were in the healthy controls (Kuriyama et al., 2008). In Korean MMD patients, the homozygous c.14429G>A (p.R4810K) variant is particularly associated with early-onset MMD (age at onset <5 years), severe clinical symptoms at diagnosis, and adverse prognosis (Kim et al., 2016). Based on the observed prevalence of 16.1 per 100,000 persons, these proportions are minimum estimates, suggesting that this *RNF213* p.Arg4810Lys variant is not the sole risk factor for MMD. Diverse influencing factors, such as other *RNF213* rare variants, other genetic alleles, or environmental components, may account for the large discrimination between the actually observed prevalence of MMD and the prevalence of carrier with the variant allele (Jang, Shin, Yoon, & Ki, 2015).

In conclusion, this report illustrates the difficulty that might be encountered in the interpretation of complex clinical manifestations when different genetic defects affecting neuromuscular and vascular diseases coexist. Thus, comprehensive evaluation of genotype and phenotype correlation is required in order to identify whether co-inheritance of mutations associated with different hereditary diseases exist in a same family members and can help early diagnosis for family members and identify those at risk for certain diseases.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

JP and WJ performed the experiments and interpreted the data and were involved in drafting the manuscript. JYH was involved in revising the manuscript for intellectual content. All authors read and approved the manuscript and are accountable for all aspects of the study.

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