Splice site mutation of LMNA causes severe dilated cardiomyopathy via strong dominant reduction of total lamin expression

K. Kato[1], S. Ohno[2], K. Sonoda[2], T. Makiyama[3], T. Ozawa[1], M. Horie[4]

[1]Shiga University of Medical Science, Cardiovascular and Respiratory Medicine, Otsu, Japan; [2]National Cerebral and Cardiovascular Center, Department of Bioscience and Genetics, Osaka, Japan; [3]Kyoto University, Kyoto, Japan; [4]Shiga University of Medical Science, Center for Epidemiologic Research in Asia, Otsu, Japan

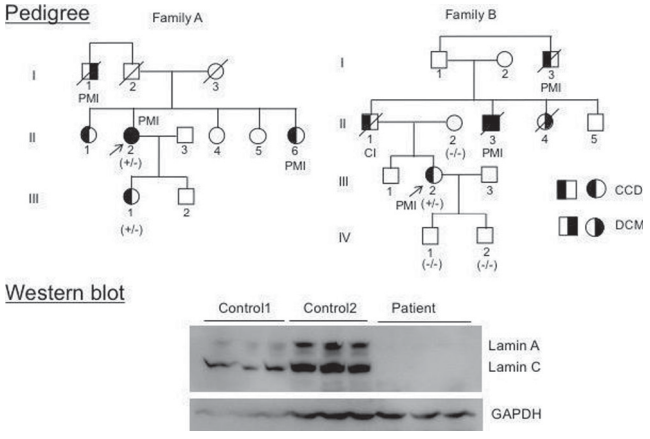
Funding Acknowledgement: Type of funding source: Public grant(s) – National budget only. Main funding source(s): Japan Society for the Promotion of Science KAKENHI

Objective: LMNA is a known causative gene of dilated cardiomyopathy (DCM) and familial cardiac conduction disturbance (CCD). Genetic variants affecting the pre-mRNA splicing process often lead to premature stop codons and result in nonsense-mediated mRNA decay (NMD), followed by degradation of mutated alleles. The misssense variant LMNA c. 936G>C was previously reported in a French family affected by muscular dystrophy, CCD, and DCM, but no detailed analysis has been performed. We so far identified the same variant in two Japanese families affected by CCD and DCM. In this study, we investigated the molecular consequences of the variant located at the last codon of LMNA exon5 to demonstrate its pathogenicity.

Methods: Genomic DNA and total RNA were isolated from patients' peripheral blood lymphocytes or cardiac tissue. LMNA-coding exons were screened by direct sequencing. Complementary DNAs (cDNAs) were generated by reverse transcription PCR from RNA. Quantitative PCR (qPCR) was performed to quantify the LMNA cDNA amount by using specific primers for lamins A and C. The protein expressions of both isoforms were analyzed by western blotting.

Results: We detected the heterozygous LMNA c.936 G>C (p. Q312H) variant at the end of exon 5 by genomic DNA sequencing in two unrelated Japanese families (figure. pedigree) affected by DCM and CCD. In a genomic database survey, we did not find the variant in either gnomAD, TogoVar, or the Human Genetic Variation Database. The two commonly used splice site predictor tools, NetGene2 and FSPLICE, estimated that this site was a splice donor site. Sequencing of cDNA demonstrated that the mutated allele was absent. By qPCR assay, we confirmed a 90% reduction in LMNA cDNA. Western blot analysis revealed that lamin A and C expression was reduced far more than 50% (figure. western blot).

Conclusions: We report a LMNA missense mutation found in two families, which disrupts a normal splicing site, leads to NMD, and resulted in severe cardiac laminopathy. The drastic reductions of lamin expression at the cDNA and protein levels suggested that other co-existing mechanisms may also have suppressed the expression of the healthy wild type allele.



Pedigree and western blot assay