Genetic variations in 40 different genes are reported to be implicated in the pathogenesis of dilated cardiomyopathy (DCM). Genome-wide linkage analysis in a large pedigree should be ideally performed to identify the causative mutation of monogenic diseases, including DCM; however, in many cases, researchers have no choice but to adopt a candidate gene approach, mainly because of the small size of pedigrees available. Establishment of the pathogenicity/causality of genetic variants identified through a candidate gene approach in small pedigrees is often challenging because the current criteria to establish the causal relationship between a genetic variant and monogenic disease basically rely on family evaluation.

Approximately 30% of DCM patients are found to have disease-associated genetic variants. There are no universal standards for interpreting the significance of these variants. How should we correctly distinguish a true pathogenic mutation capable of causing disease from a disease-modifying variant or a merely benign rare variant? Metrics for assessing the pathogenicity include the type or extent of amino acid change (e.g., missense, insertion/deletion, truncation), rarity in the population, evolutionary conservation of affected amino acid residues, functional significance in cellular or animal models, and com-

**Figure.** Genetic testing in family members. In this small pedigree, variant A identified in an index DCM patient (arrow) cannot be defined as a causative mutation. If this variant A is carelessly classified as such, his daughter carrying this variant will be regarded as at risk for developing DCM, conversely, his son without variant A will be free from the need for longitudinal follow-up. Suppose an unidentified variant B is found to be the true causative mutation in this index patient, the follow-up strategy for his son and daughter will be entirely changed. Circles denote female family members, squares are male family members, a solid symbol shows a family member clinically affected by DCM, and open symbols are clinically unaffected family members. DCM, dilated cardiomyopathy.
putational prediction, all of which are supportive of pathogenicity. In order to verify causality, using segregation analysis, cosegregation of the genetic variant of interest with the disease phenotype in multiple relatives within a kindred should be demonstrated. The presence of the variant in affected relatives and its absence in unaffected ones are powerful evidence that the variant is the cause of the disease. The availability of multiple large families to assess segregation increases the strength of evidence. In that sense, the causality of some genetic variants identified through a candidate gene approach in DCM families that are of insufficient size to provide significant segregation analyses remains inconclusive.

In this issue of the Journal, Arimura et al report a heterozygous FHOD3 variant Tyr1249Asn in a Japanese patient with familial DCM. FHOD3, a type of sarcomere protein, plays an essential role in the regulation of actin assembly and sarcomeric organization during myofibrillogenesis. In vitro functional analysis demonstrated that this variant impairs the ability to induce actin dynamics-dependent activation of serum response factor, suggesting the relevance of this variant in the pathogenesis of DCM. Because of the small size of the pedigree, segregation analysis could not be performed, so it is hard to define this novel rare genetic variant as a DCM-causing mutation. In other words, it would be appropriate to regard this variant as a DCM-associated rare variant, not a DCM-causing mutation. Whether causative or not, understanding the genetic basis of DCM will advance our knowledge concerning the fundamental pathophysiology of DCM, and in this sense, genetic studies such as this are valuable. More effort to identify other DCM-associated variants should be made. However, when this variant is added to the DCM-related genetic variant database, the fact that this variant could not be definitively identified as causative using segregation analysis should be carefully noted. Essentially, the extent to which each variant is validated as related to the pathogenesis of DCM should be clearly stated. Within the genomic research arena, insisting on such dogmatism may appear to be excessive. Nevertheless, considering the application of genetic variants to genetic testing for clinical diagnosis, any ambiguity should be carefully eliminated from genetic variant databases.

In the clinical setting, determining precisely if a genetic variant is the cause of a disease or not is very critical. When genetic testing identifies a causative variant in the index patient (probands), clinical screening can be restricted to family members who have inherited that variant. Family members without that variant would not require longitudinal clinical evaluation. Therefore, we should be very cautious in deciding if a genetic variant identified in a proband is causative. A true causative mutation might be present elsewhere, possibly in a non-tested gene. Missclassification of genetic variants without careful evaluation will needlessly confuse cascade genetic testing, the goal of which is to identify at-risk family members (Figure). Concluding falsely that a non-causative benign rare variant in a proband is the cause of a disease means healthy relatives carrying that benign variant could be mislabeled as being at risk for developing disease. At the same time, because the true causative mutation has been overlooked, truly at-risk relatives could be falsely reassured. Taken together, “to be or not to be causative” is the big question, at least in clinical genetic testing.

Nowadays, next-generation sequencing technologies are being increasingly used to identify DCM-related genetic variants. Genetic variation can be present anywhere throughout the entire genome. Some variants have no effect on the phenotype, and other variants affect the phenotype to different extents. From among an ocean of variants, excluding genetic noise (common variants and non-causative rare variants), only true causative mutations must be skillfully identified. Generally, as a larger number of rare variants are detected in deep-resequencing, the unprecedented complexity of adjudicating the pathogenicity/causality of variants is being uncovered. In a report on the Exome Sequencing Project database of more than 2,400 individuals, it was shown that 33 out of 197 previously published rare variants reported as being DCM-causative were present also in this public genomic database, raising the possibility that several non-causative rare variants were previously published as DCM-causing. A comprehensive analysis of TTN (encoding a giant protein titin), which is a major source of DCM-causing variants, demonstrated that rare truncating variants are prevalent in control subjects without cardiomyopathy (in total, 3%), suggesting that even TTN truncating variants, which are theoretically predicted to be pathogenic, if each of them is rare, cannot necessarily be defined as pathogenic. Even when interpreting the deep-resequencing data, segregation analysis remains the gold standard for the acquisition of robust evidence for pathogenicity/causality. In practical terms, however, because of the small size of the pedigrees, we often have no choice but to use genetic databases, bioinformatics tools, and functional studies to determine the variant pathogenicity/causality. In such a situation, when each variant is added into a genetic variant database, the extent to which that variant is validated should be carefully annotated. At present, that is the best scheme available to avoid confusion in real-world genetic diagnosis. In the next-generation sequencing era, interpretations of variant pathogenicity/causality as well as genetic disease paradigms may be continuously updated, but still, to be causative or not will remain a big question.

Disclosures
The author has no conflicts of interest to declare.

References