中文題目：心肌橋之基因定序研究

英文題目：Gene Sequencing Study of Myocardial Bridge

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**Background:** Myocardial bridge (MB) is a common coronary artery anomaly and an important cause of chest pain. The clinical presentation of MB mimics that of true obstructing coronary artery disease (CAD). Cardiologists could not distinguish between MB and CAD without invasively diagnostic modalities. In the ear of next-generation sequencing and precision medicine, the genetic basis of this vasculopathy is still lacking. We aimed to investigate the possible underlying gene variants associated with MB and hopefully develop an easier method for diagnosis.

**Method:** Whole-exome sequencing technique was exploited to collect genetic information from MB subjects and normal controls. Genotypic differences were analyzed with CLC. Functional annotations were investigated via GO, KEGG, GTEx Portal and knockout mouse databases. Pathogenicity prediction were performed by the ClinVar, CADD, REVEL scoring systems. Amino acid and protein changes were predicted with MutPred2 and the ELM databases.

**Results:** Eight pairs of angiographically-identified MB subjects and normal controls were enrolled for analysis. One hundred and thirty-three candidate variants were identified, functional annotation of which indicated significant association with abnormal skeletal muscle mass, cardiomyopathy, and transmembrane cation proteins. After filtering through ClinVar, CADD and REVEL databases and excluding variants in normal subjects, four non-synonymous single nucleotide variants in four genes were identified potentially pathogenic for myocardial bridge (rs773695263 A>G in SCN1A, rs201137087 C>T in PCDH15, rs397514715 T>C in SMAD9 and rs186669379 C>T in SGCA), all of which resulted in amino acid substitutions statistically significant for myocardial bridge pathogenicity. Among the genes potentially associated with myocardial bridge, the SGCA gene has the highest expression titer and putatively plays the most important role in the development of MB.

**Conclusion:** Myocardial bridge is associated with genetic variants. Among the

potentially pathogenic variants associated with MB, the SGCA gene variant has the highest probability for causality off MB. Further studies would be needed to clarify the underlying mechanisms of gene SGCA in the formation of myocardial bridge.

Figure 1. The Study Flow Chart of Genetic Study of Myocardial Bridge

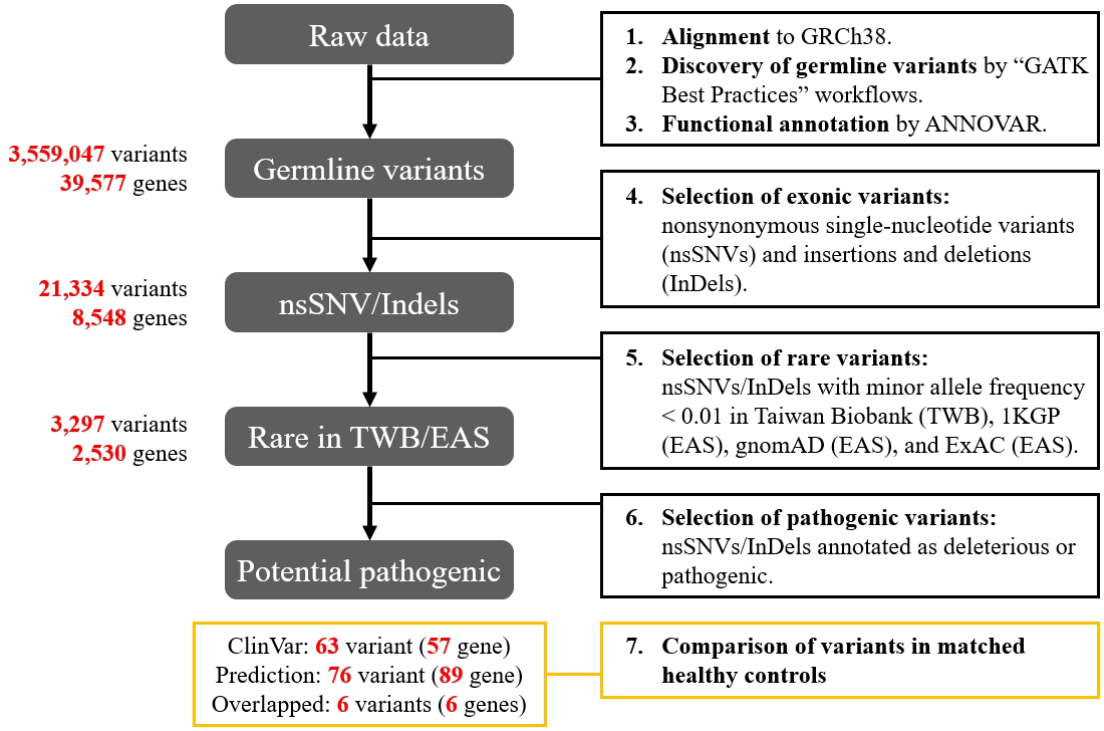

Table 1. Functional Annotation of Myocardial Bridge Gene Set

| Functional Annotations | No. of Reference Genes in the Category | No. of MB candidate genes in the category | p Valve | FDR |
|---|---|---|---|---|
| **Knockout Mouse Phenotypes** | | | | |
| decreased skeletal muscle mass | 107 | 9 | $7.01 \times 10^{-7}$ | $4.27 \times 10^{-3}$ |
| impaired skeletal muscle contractility | 38 | 6 | $1.33 \times 10^{-6}$ | |
| abnormal skeletal muscle mass | 121 | 9 | $1.98 \times 10^{-6}$ | |
| **KEGG pathway** | | | | |
| arrhythmogenic right ventricular cardiomyopathy | 72 | 7 | $4.68 \times 10^{-6}$ | $1.53 \times 10^{-3}$ |
| **GO term categories** | | | | |
| inorganic cation transmembrane transport | 722 | 21 | $1.79 \times 10^{-7}$ | $1.23 \times 10^{-3}$ |
| cation transmembrane transport | 810 | 22 | $2.87 \times 10^{-7}$ | |
| muscle contraction | 339 | 14 | $4.14 \times 10^{-7}$ | |

Table 2. Potentially Pathogenic Variants for Myocardial Bridge

| Chr. | Position | Ref. | Alt. | Gene | Type | avSNP150 | Allelic Frequency | | | | Pathogenicity | | | Subj. No. (MB) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1KGP (EAS) | gnomAD (EAS) | ExAC (EAS) | TWB | ClinVar | CADD | REVEL | |
| 2 | 166041286 | A | G | SCN1A | nsSNV | rs773695263 | - | - | 0 | - | Conflicting interpretations of pathogenicity | 25.9 | 0.884 | 5 |
| 10 | 53857257 | C | T | PCDH15 | nsSNV | rs201137087 | - | 0.0074 | 0.0054 | 0.0065 | Conflicting interpretations of pathogenicity | 34 | 0.818 | 4 |
| **10** | **86717984** | **T** | **G** | **LDB3** | **nsSNV** | **rs566463138** | **0.001** | **0.0026** | **0.0016** | **0.0035** | **Conflicting interpretations of pathogenicity** | **26.6** | **0.78** | **7** |
| 13 | 36879563 | T | C | SMAD9 | nsSNV | rs397514715 | - | 0.0013 | 0.0015 | 0.0005 | Pathogenic | 24.8 | 0.902 | 6 |
| **13** | **51949723** | **G** | **A** | **ATP7B** | **nsSNV** | **rs750019452** | - | **0.0003** | **0.0023** | **0.002** | **Pathogenic/Likely pathogenic** | **34** | **0.842** | **5** |
| 17 | 50167954 | C | T | SGCA | nsSNV | rs186669379 | 0.006 | 0.0045 | 0.0036 | 0.006 | Conflicting interpretations of pathogenicity | 23.5 | 0.864 | 3 |

*Variants shown in bold were also identified in healthy controls.

Figure 2. The Tissue Expressions of the Potential Gene Variant for Myocardial Bridge

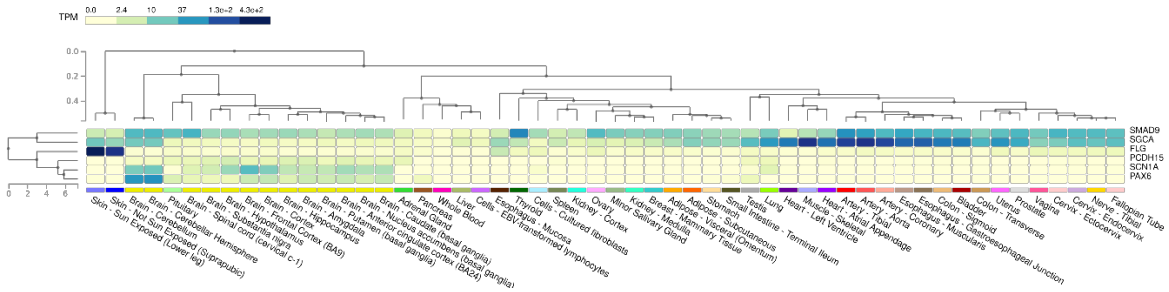


Figure 3. The Expression of the Gene SGCA among Human Tissues

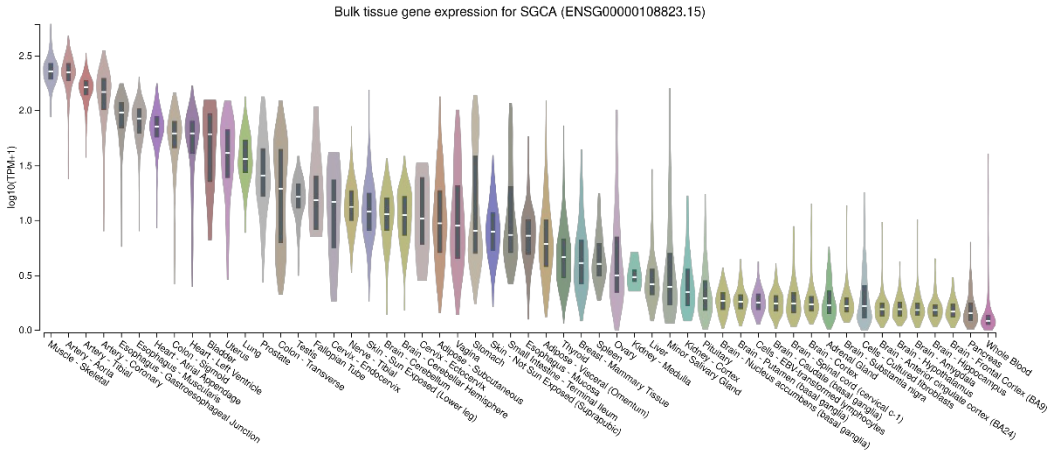

Table 3. Protein Structure Prediction of Pathogenic Variants for Myocardial Bridge

| Gene | Substitution | MutPred2 score | Affected ELM motifs | Affected PROSITE | Molecular mechanism with most significance | Probability of pathogenicity | p Value |
|---|---|---|---|---|---|---|---|
| SGCA | A107V | 0.669 | ELME000147 | Plk phosphorylation site | Altered transmembrane protein | 0.34 | $4.9x10^{-5}$ |
| PCDH15 | V1242M | 0.710 | ELME000193 | Nucleus Export Signal | Altered transmembrane protein | 0.31 | $1.1x10^{-4}$ |
| SCN1A | M787T | 0.880 | none | - | Gain of allosteric site at F784 | 0.26 | $8.3x10^{-3}$ |
| SMAD9 | K43E | 0.785 | ELME000064<br>ELME000146<br>ELME000271<br>ELME000278<br>PS 00006 | CK2 phosphorylation site<br>PCSK cleavage site<br>Nuclear Localization signal<br>Nuclear Localization signal<br>Casein kinase II phosphorylation site | Altered disordered interface | 0.28 | 0.02 |