ARTICLE

HTAADVar: Aggregation and fully automated clinical interpretation of genetic variants in heritable thoracic aortic aneurysm and dissection

Wei-Zhen Zhou¹,*, Yujing Zhang¹, Guoyan Zhu¹, Huayan Shen¹, Qingyi Zeng¹, Qianlong Chen¹, Wenke Li¹, Mingyao Luo², Chang Shu², Hang Yang²,*, Zhou Zhou¹,*

ARTICLE INFO

Article history:
Received 18 May 2022
Received in revised form
24 August 2022
Accepted 24 August 2022
Available online xxxx

Keywords:
Bioinformatics
Clinical significance
Genetic variant interpretation
Next-generation sequencing
Thoracic aortic aneurysm and dissection

ABSTRACT

Purpose: Early detection and pathogenicity interpretation of disease-associated variants are crucial but challenging in molecular diagnosis, especially for insidious and life-threatening diseases, such as heritable thoracic aortic aneurysm and dissection (HTAAD). In this study, we developed HTAADVar, an unbiased and fully automated system for the molecular diagnosis of HTAAD.

Methods: We developed HTAADVar (http://htaadvar.fwgenetics.org) under the American College of Medical Genetics and Genomics/Association for Molecular Pathology framework, with optimizations based on disease- and gene-specific knowledge, expert panel recommendations, and variant observations. HTAADVar provides variant interpretation with a self-built database through the web server and the stand-alone programs.

Results: We constructed an expert-reviewed database by integrating 4373 variants in HTAAD genes, with comprehensive metadata curated from 697 publications and an in-house study of 790 patients. We further developed an interpretation system to assess variants automatically. Notably, HTAADVar showed a multifold increase in performance compared with public tools, reaching a sensitivity of 92.64% and specificity of 70.83%. The molecular diagnostic yield of HTAADVar among 790 patients (42.03%) also matched the clinical data, independently demonstrating its good performance in clinical application.

Conclusion: HTAADVar represents the first fully automated system for accurate variant interpretation for HTAAD. The framework of HTAADVar could also be generalized for the molecular diagnosis of other genetic diseases.

© 2022 The Authors. Published by Elsevier Inc. on behalf of American College of Medical Genetics and Genomics. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
introduction

With the rapid development of next-generation sequencing technologies, genetic testing has been extensively used clinically for Mendelian diseases because it plays substantial roles in clinical management, including diagnosis, treatment decisions, family screening, and reproductive guidance. A major challenge is obtaining an efficient and accurate interpretation of variant pathogenicity owing to the time-consuming and laborious processes involved and the inconsistencies across different laboratories. In 2015, the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) promulgated their guidelines to address the issue of variant interpretation standardization. However, inconsistencies still remain in that researchers have subjective understandings of the ACMG/AMP guidelines and have varying abilities to access information from the literature and databases.

InterVar was first developed to facilitate variant interpretation for the full spectrum of diseases, but it does not perform well for specific diseases. Thus, several pilot studies have been conducted to develop disease-specific interpretation tools, such as Variant Interpretation Platform for genetic Hearing Loss, Variant Interpretation for Cancer, neXtProt for BRCA1, CardioClassifier and Cardio Variant Interpreter for cardiovascular diseases; however, these semiautomated tools still interpret variants largely dependent on user-curated data. Moreover, public databases such as ClinVar, Human Gene Mutation Database (HGMD), and Universal Mutation Database (UMD) are widely used for current tools. However, these databases have inevitable limitations, such as nonstandard sequencing variant nomenclature, inconsistent variant classifications generated by different criteria, and a lack of key information for interpretation, such as family- and case-level data and functional assay results.

Thoracic aortic aneurysm (TAA) is an insidious and life-threatening vascular disease that is difficult to detect and diagnose before catastrophic complications, aortic dissection or rupture. Approximately 95% of the patients with TAA are asymptomatic. When dissection or rupture occurs abruptly, approximately 22% of patients die before reaching the hospital. However, if TAA is promptly recognized and managed surgically before dissection, patients have excellent survival rates with limited complications. Therefore, early detection and diagnosis are crucial for disease monitoring and surgical management to prevent devastating events. Because it is difficult to detect TAA based on symptoms alone, genetic testing is used to facilitate the establishment of a definitive diagnosis in patients and the identification of at-risk relatives.

To improve the reliability and efficiency of genetic testing in heritable TAA and dissection (HTAAD), we developed HTAADVar, an unbiased and fully automated system for variant interpretation, comprising a self-built variant database and interpretation programs. HTAADVar shows multifold higher sensitivity and specificity than other tools and produces a diagnostic yield in a real sequencing cohort that is highly comparable to the manual interpretation and previous reports. To facilitate HTAADVar use, we built a web server with a friendly interactive interface and powerful browse, search, and variant interpretation functions. Stand-alone programs are also provided for customized interpretations and generalization of the HTAADVar framework to other diseases.

Materials and Methods

Data collection

We first selected 18 HTAAD genes as our targeted genes according to the clinical validity of genes for HTAAD evaluated by the expert panel of the Aortopathy Working Group (Supplemental Methods). Then, we retrieved 1564 publications from PubMed using the query statement described in the Supplemental Methods. For completeness, 617 publications collected from ClinVar and/or HGMD but missed in the PubMed query were also retrieved. We excluded all publications reporting only large sequence variations (>50 base-pairs) or performing analyses based on the variants in public databases. Moreover, we also integrated the variants in targeted genes identified in 790 Chinese probands by our laboratory. Comprehensive metadata were manually collected and double-checked independently by experts. Functional annotations for genes were integrated following a previous procedure.

Variant database comparison

To ensure a fair comparison, we compared our variant database with ClinVar (v2021-11-08), HGMD (v2021.2), and UMD (v2021-12-06) based on the single-nucleotide variants and insertions-deletions (≤50 basepairs) from studies published by October 20, 2020. We also excluded studies based on database variants.

Rule optimization and implementation

There are 28 criteria under the ACMG/AMP framework, and the ClinGen Sequence Variant Interpretation working group suggested removing PP5 and BP6. PM3 and BP1 were deemed not applicable because our targeted genes cause HTAAD in a dominant mode, and truncating variants are not the only pathogenicity mechanism of HTAAD. Therefore, we established HTAAD-specific and genespecific rules and implementation methods for 24 criteria of the ACMG/AMP guidelines (Supplemental Methods). The HTAADVar implementation process is also described in the Supplemental Methods.
Benchmarking and comparative analysis

To evaluate the performance of HTAADVar, we used pathogenic or likely pathogenic (P/LP) and benign or likely benign (B/LB) variants by multiple submitters with no conflicts in ClinVar (v2021-11-08) as a benchmark data set.

We performed automated variant interpretation using InterVar and CardioClassifier using default parameters without manual adjustment. To ensure interpretation under the same framework, we recalibrated the InterVar result after removing PP5 and BP6. We compared the final classifications and activated rules using different tools.

Web server and stand-alone programs

We constructed a MongoDB database to store and manage the collected data. For interpretation, we provided a web server and Perl programs to support users with different requirements. The web server has a user-friendly interface developed using Java, HTML, CSS, and PHP.

Results

HTAAD variant database

A professional database dedicated to variant interpretation was first developed to create a fully automated system. We constructed an HTAAD variant database for subsequent interpretation. For 18 HTAAD genes, we searched PubMed, ClinVar, and HGMD to retrieve related studies (Materials and Methods). In total, 2181 research articles were retrieved. After scrutinizing the abstracts to exclude irrelevant studies, 697 articles published between January 1989 and October 2020 were retained to be manually curated. We also integrated variants identified in 790 in-house patients to improve the interpretation power (Supplemental Methods; Supplemental Table 1). Finally, 4373 unique variants were collected in the database (Figure 1).

For each study, comprehensive metadata were extracted and proofread manually, as shown in Supplemental Tables 2 to 8. Particularly, we integrated the key information for variant interpretation, such as variant observations in unrelated patients, the heterozygosity and parental origin of variants, including mosaic and de novo status, patient phenotypes, family history, familial segregation as scored following Jarvik and Browning’s guidelines, variant effect on gene function to which specific PS3 strength level was assigned according to experimental model and phenotypic expressions (Supplemental Methods), and variant effect on splicing to which specific PVS1 strength level was assigned according to ClinGen PVS1 guidelines. To avoid repeatedly recording the same variant because of the variety of naming conventions, variants were standardized into a format with the chromosome and position (in GRCh37/hg19), reference allele, and alternative allele and then annotated at transcript-based DNA and protein levels following the nomenclature recommendations of the Human Genome Variation Society by Ensembl Variant Effect Predictor.

Compared with ClinVar, HGMD, and UMD, as shown in Table 1, our variant database compiles a larger set of published variants in HTAAD genes. UMD contains the fewest variants because it has been adapted to only 7 targeted genes. In contrast to the user submission of some databases, we manually curated comprehensive information from the literature and double-checked it to ensure the high quality of the data. Notably, the standardized information critical to variant interpretation is available only in our variant database. Furthermore, more comprehensive annotations for variants, such as the alternative allele frequency with sequencing depth in Genome Aggregation Database (gnomAD), predictive algorithms for variant effects, protein domain, and pathogenicity classifications generated by our unified automated interpretation programs, were also integrated into our database. Overall, the HTAAD variant database can provide a reliable resource to support the in-depth interpretation of HTAAD variants.

Substantial improvement in the performance of HTAADVar

Given that the ACMG/AMP framework can fully exert its power in a disease- and gene-specific manner, optimizing rules for specific disease–gene pairs is particularly important to achieve accurate classification. Our panel consists of experts in genetics, molecular biology, clinical diagnosis, and treatment for HTAAD. The experts in this panel are members of the Precision Medicine Group, established in 2019 and affiliated with the National Society of Vascular Surgery. In the same year, our experts issued the “Chinese expert consensus on the genetic testing and clinical management of heritable thoracic aortic aneurysm/dissection” to promote HTAAD molecular diagnosis standardization in China. Accordingly, we refined the ACMG/AMP rules by combining HTAAD- and gene-specific knowledge with released recommendations from expert panels such as ClinGen SVI for PVS1, PS2, PM5, PM6, PP1, PP5, and BP6 (Supplemental Methods; Supplemental Tables 9 and 10) and developed a suite of methods to implement the rules, leveraging the HTAAD variant database and public data sets (Figure 1; Supplemental Figure 1). Using our automated interpretation system, 4373 variants in our database were assessed, and their classifications are summarized in Supplemental Table 11.

To evaluate the performance of our system, we compared HTAADVar with InterVar and CardioClassifier, which are freely accessible and commonly used in ACMG/AMP-based interpretation for HTAAD variants. Among our targeted genes, only FBN1 can also be analyzed by CardioClassifier; thus, we selected 611 nonconflicting variants in FBN1 from ClinVar as a benchmark set, consisting of 299 P/LP and 312
B/LB variants. For a fair comparison, we recalibrated InterVar classifications after removing the reputable source criteria (PP5 and BP6) because of their questionable utility \(^{19}\) rendering them inapplicable for HTAADVar and CardioClassifier. Based on an automated interpretation step with default parameters, InterVar and CardioClassifier classified 109 (36.45\%) and 113 (37.79\%) P/LP variants, respectively, as having the same pathogenicity, whereas HTAADVar reproduced the classifications for 277 P/LP variants with a sensitivity of 92.64\%. InterVar and CardioClassifier classified 52 (16.67\%) and 17 (5.45\%) B/LB variants, respectively, as having the same pathogenicity, whereas HTAADVar reproduced the classifications for 221 B/LB variants with a specificity of 70.83\% (Figure 2A). Notably, all misclassified variants were interpreted as variant of uncertain significance (VUS) by the tools.

Figure 1 The framework overview of HTAADVar. HTAADVar consists of a self-built variant database that integrates manually curated literature and in-house data and interpretation programs that implement automated interpretation based on all ACMG criteria to obtain a final classification under the 5-tier system. In the interpretation process, the criteria above the dotted line are scored on the basis of the variant database and in-house controls, whereas those under the dotted line are scored based on the external data sets. ACMG, American College of Medical Genetics and Genomics; AAF, alternative allele frequency; gnomAD, Genome Aggregation Database.
The high performance of HTAADVar is not limited to \textit{FBN1}. In comparison with InterVar, we further extended the benchmark set to 1764 nonconflicting variants in ClinVar across all targeted genes. Based on this larger set, HTAADVar still reached 2 to 3 times the sensitivity (HTAADVar: 85.10\% vs InterVar: 35.10\%) and specificity (HTAADVar: 76.41\% vs InterVar: 28.12\%) compared with InterVar (Supplemental Table 12), although, the InterVar classifications might be slightly biased toward those of ClinVar because InterVar refers to ClinVar information during interpretation.3

As shown in Figure 2B, Supplemental Figure 2, and Supplemental Table 13, the evidence, such as \textit{de novo} observations, functional studies, familial segregation, and phenotype data for PS2/PM6, functional evidence for PS3/BS3, number of unrelated probands with the variant for PS4, familial segregation for PP1/BS4, phenotype specificity for PP4 and family history for PS2/PM6, but without a family history.4

We compared the classifications of 547 variants from 790 in-house probands obtained by HTAADVar and manual interpretations.
interpretation to further assess the clinical utility of HTAADVar. Of the 300 P/LP variants interpreted using HTAADVar, the pathogenicity was the same as the manual interpretation in 99.33% of variants. Among the 55 B/LB variants, the consistency was lower (58.18%), largely because of the different strategies used; manual interpretation applied more stringent BP4 rules requiring all 4 categories of predictions, including the conservation, function, meta, and splicing site predictions, to support the benign classification, whereas HTAADVar assigns a variant as benign only if the predictions from most of these categories are supportive. To evaluate which rule is more appropriate, we used the aforementioned 1348 B/LB benchmark variants for testing. Using manual interpretation rules resulted in the application of only 38 (2.82%) B/LB variants to BP4, which is far below that of HTAADVar (80.12%), indicating that the BP4 rule of manual interpretation may be too stringent. Finally, of the 192 VUS variants in HTAADVar, 20 were manually upgraded to LP, and 2 were downgraded to LB. This change is mainly because (1) HTAADVar adopted the ClinGen SVI recommendations to refine the ACMG/AMP criteria. However, some of these recommendations are generally too complex to follow manually, such as the loss-of-function PVS1 criterion. In addition, some of them were released recently, such as the \textit{de novo} PS2 and PM6 criteria, and were typically not used for the manual interpretation.
interpretation. (2) Manual interpretation primarily uses ClinVar and UMD to quickly retrieve the necessary evidence, including unpublished user-submitted data. HTAADVar only reviewed and included published and our in-house data. Although, the HTAADVar strategy better ensures data quality, some evidence are missed. Therefore, we will continually update HTAADVar with data from our laboratory and the user community to alleviate this problem.

Among 790 in-house patients, P/LP variants were associated with 332 by HTAADVar, resulting in an overall molecular diagnostic yield of 42.03%. This yield is highly consistent with that obtained by manual interpretation (44.56%) and comparable with previously reported data consistent with that obtained by manual interpretation. (2) HTAADVar substantially improved the efficiency of the interpretation on the premise of the reliability of classification.

Comparative analysis with ClinVar

ClinVar is a freely accessible archive of genetic variants and their clinical significance to disease and is thus widely used in variant interpretation. To assess the concordance rate and investigate the reasons for discordance, we compared variant classifications made by HTAADVar with those in ClinVar based on 1977 shared variants. For 1160 P/LP variants in HTAADVar, ClinVar also annotated 1096 (94.48%) as P/LP and none as B/LB. For 93 B/LB variants in HTAADVar, 65 (69.89%) were also annotated as B/LB in ClinVar, and none were annotated as P/LP (Figure 3). This shows that ClinVar is highly consistent with HTAADVar in P/LP interpretation but lower in B/LB interpretation. For the variants annotated as B/LB in HTAADVar but VUS in ClinVar, most of them were applied to BP5 by HTAADVar because the allelic and alternative locus data available in our database enable HTAADVar to automatically assess BP5, which is difficult in manual assessment.

Half of the 724 VUS variants classified by HTAADVar (369, 50.97%) were upgraded to P/LP and 21 (2.90%) were downgraded to B/LB in ClinVar (Figure 3). The possible reasons for the discrepancy are as follows: (1) ClinVar submitters obtained the variant classifications based on other criteria, such as Blueprint, Baylor, and Laboratory for Molecular Medicine genetics variant classification, or with no explicit criteria. (2) If ACMG/AMP guidelines were adopted, submitters may not have adjusted the strength level for specific criteria following the expert panel recommendations. For example, in ClinVar, PVS1 may be incorrectly applied for genes in which the loss-of-function is not a well-established disease mechanism, such as MFAP5 and TGFB3, or submitters did not determine PVS1’s appropriate strength level following the ClinGen SVI recommendations, such as NM_000138.4:c.164+1G>A of FBN1. PP1 may be activated for variants that segregate with disease in a family, but the segregation score does not meet the minimum requirement of PP1 quantitative criteria, such as NM_003238.6:c.1013C>A (p.Pro338His) of TGFB2, with a segregation score of 1/4. (3) The thresholds for allele frequency evidence (BA1, BS1, and PM2) and the annotations of gene functional regions used by the submitters might differ from those of HTAADVar. In our interpretation, PS4 and PP1 must be applied together with PM2. If the variant is observed in gnomAD even at a low frequency, PS4 and PP1 cannot be applied. ClinVar submitters may not use such a stringent threshold; thus, rare variants may be applied to PM2, PS4, and PP1 and upgraded to P/LP, such as NM_000138.4:c.7754T>G (p.Gly1838Asp), which is outside the well-established functional domains of FBN1. The submitter still applied PM1 because several variants in its nearby residues have been associated with Marfan syndrome. (4) Finally, submitters may include additional evidence from their unpublished data during assessment as mentioned above. For example, NM_001613.4:c.772C>A (p.Gly1838Asp), which is outside the well-established functional domains of FBN1. The submitter still applied PM1 because several variants in its nearby residues have been associated with Marfan syndrome. (5) Finally, submitters may include additional evidence from their unpublished data during assessment as mentioned above. For example, NM_001613.4:c.772C>T (p.Arg258Cys) of ACTA2 has been reported as de novo in a TAAD family by Guo et al, which HTAADVar also collects. Another de novo event of this variant in the submitter’s laboratory further supports its pathogenic role. Overall, when using HTAADVar or ClinVar to interpret variants, users should pay attention to interpretation and classification differences and their possible reasons.

Web interface and customized running using stand-alone programs

HTAADVar provides a simple-to-use web server (http://htaadvvar.fwgenetics.org) to facilitate access to our variant database and interpret any variant of interest. In this article, the powerful browse, search, and interpretation functions of the HTAADVar webserver are introduced (Figure 4).

Users can browse the gene, variant, and study levels as mentioned above. For example, NM_001613.4:c.772C>T (p.Arg258Cys) of ACTA2 has been reported as de novo in a TAAD family by Guo et al, which HTAADVar also collects. Another de novo event of this variant in the submitter’s laboratory further supports its pathogenic role. Overall, when using HTAADVar or ClinVar to interpret variants, users should pay attention to interpretation and classification differences and their possible reasons.
gene–HTAAD associations, the total number of variants in the gene reported in HTAAD, and the gene function summary. Variant distributions grouped by transcript, exon/domain, and pathogenicity are sequentially shown. Furthermore, users can click on the “Gene Annotation” label in the top left to view comprehensive gene annotations. On the Variant page, users can browse the variant information flexibly. Notably, HTAADVar interprets the variants at the transcript level. The “IC” column indicates the consistency of classifications between different transcripts. Users can click on the “+” sign to view the classifications on hidden transcripts. Users can scrutinize the reasoning behind the interpretation and all supporting data by clicking on the evidence codes in the “Evidence” column. The sample information will be shown when users click on the “Probands” column. The Source page summarizes literature information, including the PubMed identifier, title, authors, journal, publication date, study type, and count of variants reported. By clicking on the “Study Type” column, users can view the detailed information for “Sequencing Study” and “Functional Study.”

In the web server, 2 search modes are available. The basic search box on the home page and the top navigation bar enables users to search for genes, variants, and literature of interest in the variant database. The gene symbol and aliases, variants in the format of “chromosome: start position-end position: reference allele > alternative allele,” Human Genome Variation Society expression at the complementary DNA and protein levels, and PubMed identifier can be recognized. Fuzzy matching is allowed for variant and literature queries. On the Variant and Source pages, search boxes under the table header enable users to filter data using an advanced mode.

In addition to searching for preinterpreted variants in the database, users can interpret any variant of interest. The web server provides a friendly interactive interface to enable users to submit data easily for single-variant interpretation. The interpretation of multiple variants supports variant submission in variant call format. The results are rendered as HTML pages and/or Excel files.

In additional, HTAADVar can be run in Perl, which is more suitable for large tasks. In the stand-alone version,
users can refine the thresholds of PVS1, PS2, PS4, PM2, PM5, PM6, BS1, BA1, and computational predictors in the configuration file and update the variant classifications in the database. Users can interpret any variant on the basis the updated database using newly defined thresholds.

**Discussion**

Genetic testing has been widely used in clinical practice, and variant interpretation is a crucial but challenging step. In this study, we developed an unbiased interpretation system that can automatically implement all applicable ACMG/AMP guideline criteria. HTAADVar was demonstrated to be superior in accuracy and efficiency, indicating that it can be effectively applied for molecular diagnostics.

Compared with existing tools, HTAADVar has the following advantages: (1) It is the first fully automated system of variant interpretation specific to HTAAD that does not require users to manually curate the items of evidence from massive publications in variant annotations. Metadata from publications, in-house sequencing data, and public data sets have been integrated and organized to support a comprehensive annotation in HTAADVar. (2) Based on the 2015 ACMG/AMP guidelines, the refined rules combining disease- and gene-specific knowledge with expert panel recommendations for PVS1, PS2, PM5, PM6, PP1, PP5, and BP6 substantially increase the interpretation power. (3) HTAADVar allows users to interpret variants with customized criteria, which is useful in such a rapidly evolving field of variant interpretation. (4) Finally, HTAADVar provides a user-friendly web interface, and users can interactively interpret the variants or search for metadata for variants of interest in our expert-reviewed variant database.

However, HTAADVar has the following limitations: (1) HTAADVar only supports automated interpretation for variants in HTAAD genes. However, its framework could be generalized to other inherited diseases and genes. Users
would need to build a variant database and define the interpretation parameters specific to their targeted diseases and genes of interest. Then, the interpretation program can be easily applied. (2) The current rules and thresholds were determined according to previous recommendations and our expert panel’s knowledge, but a consensus has not yet been reached in the field. Users can update the thresholds in the configuration file to interpret variants according to their preferences. Alternatively, the transparent result report enables users to manually adjust the classification.

We will continuously improve HTAADVar as genetic studies on HTAAD progress. In the future, we will update HTAADVar regularly by curating new publications, incorporating new in-house data, including HTAADVar user community data, updating the list of disease-causing genes, and adjusting rules, thresholds, or methodologies for interpretation according to new recommendations from expert panels. HTAADVar is a valuable system that can greatly facilitate clinician and researcher assessment of variant pathogenicity. Its framework and methodologies could substantially improve standardization for the molecular diagnosis of genetic diseases.

Data Availability

All data collected for the HTAAD variant database in this study are available on the web server without login requirements (http://htaadvar.fwgenetics.org/).

Acknowledgments

We acknowledge Yandong Cao, Kaituo Mi, Kupeng He, Yulei Liu, and Wuqiang Zhang at Anngene Technology Co Ltd for their support and contribution to the web server building and Ge Gao at Peking University for assistance with database comparison. This work was supported by the Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (CIFMS) (2021I2M1008) and the Young Scientists Fund of the National Natural Science Foundation of China (31801103).

Author Information


Ethics Declaration

The study was approved by the Ethics Committee of Fuwai Hospital (approval number: 2017-877). All patients and their relatives in the in-house cohort signed informed consent forms.

Conflict of Interest

The authors declare no conflict of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.08.024) contains supplementary material, which is available to authorized users.

Affiliations

1Center of Laboratory Medicine, State Key Laboratory of Cardiovascular Disease, Beijing Key Laboratory for Molecular Diagnostics of Cardiovascular Diseases, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; 2Center of Vascular Surgery, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

References


