review

Molecular profiling of rare thymoma using next-generation sequencing: meta-analysis

Jelena Kostic Peric[1], Andja Cirkovic[2], Sanja Srzentic Drazilov[1], Natalija Samardzic[3], Vesna Skodric Trifunovic[3,4], Dragana Jovanovic[5], Sonja Pavlovic[1]

[1] Institute for Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[2] Department for Medical Statistics and Informatics, Faculty of Medicine, University of Belgrade, Serbia
[3] University Hospital of Pulmonology, Clinical Centre of Serbia, Belgrade, Serbia
[4] Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[5] Internal Medicine Clinic "Akta Medica", Belgrade, Serbia

Radiol Oncol 2023; 57(1): 12-19.

Received 21 October 2022
Accepted 31 January 2023

Correspondence to: Assist. Prof. Jelena Kostic Peric, Ph.D., Institute for Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia. E-mail: jelena.kostic@imgge.bg.ac.rs

Disclosure: No potential conflicts of interest were disclosed.

Background. Thymomas belong to rare tumors giving rise to thymic epithelial tissue. There is a classification of several forms of thymoma: A, AB, B1, B2, B3, thymic carcinoma (TC) and thymic neuroendocrine thymoma. In this meta-analysis study, we have focused on thymoma using articles based on the disease's next-generation sequencing (NGS) genomic profiling.

Materials and methods. We conducted a systematic review and meta-analysis of the prevalence of studies that discovered the genes and variants occurring in the less aggressive forms of the thymic epithelial tumors. Studies published before 12th December 2022 were identified through PubMed, Web of Science (WoS), and SCOPUS databases. Two reviewers have searched for the bases and selected the articles for the final analysis, based on well-defined exclusion and inclusion criteria.

Results. Finally, 12 publications were included in the qualitative as well as quantitative analysis. The three genes, *GTF2I*, *TP53,* and *HRAS*, emerged as disease-significant in the observed studies. The Odds Ratio for all three extracted genes *GTF2I* (OR = 1.58, CI [1.51, 1.66] $p < 0.00001$), *TP53* (OR = 1.36, CI [1.12, 1.65], $p < 0.002$), and *HRAS* (OR = 1.02, CI [1.00, 1.04], $p < 0.001$).

Conclusions. According to obtained data, we noticed that the *GTF2I* gene exhibits a significant prevalence in the cohort of observed thymoma patients. Moreover, analyzing published articles NGS has suggested *GTF2I, TP53,* and *HRAS* genes as the most frequently mutated genes in thymoma that have pathogenic single nucleotide variants (SNV) and Insertion/Deletion (InDel), which contribute to disease development and progression. These variants could be valuable biomarkers and target points specific to thymoma.

Key words: thymoma; next-generation sequencing (NGS); SNVs/InDels; meta-analysis

Introduction

Thymic epithelial tumors (TETs) are localized in the anterior mediastinum and comprise several forms of thymomas with different malignant potentials, aggressive forms of thymic carcinoma (TC), and thymic neuroendocrine thymoma.[1] Thymomas originate from thymic epithelial tissue. Thymoma and TC are similar and overlapping in many characteristics, but despite these histopathological and cytological features, thymomas could be considered a more benign form of TETs in comparison with TC with aggressive forms. That is the reason why thymomas and TC, exhibit

main differences in therapy approach.[2,3] Here we analyze thymoma including the following subtypes A, AB, B1, B2, and B3.[4] A and AB subtypes belong to *GTF2I*, B1, and B2 are the T-cell signaling group, B2 is as well chromosomal stability group, and B3 (atypical thymoma because is the most aggressive, if we exclude TC) belongs to the chromosomal instability group. Thymomas are classified as rare thoracic tumors with an overall incidence of 0.15 per 100 000 persons per year.[5,6] The majority of thymomas are less aggressive forms and their treatment is based on surgery, while in the case of more aggressive TC treatment approach includes multimodal therapy. This tumor characterizes high heterogeneity which is the reason for precise molecular profiling to choose an adequate precision therapy approach.[7,8] Due to the rarity of the disease, there is still a lack of information about external factors that cause diseases, such as smoke or alcohol. Moreover, epidemiological studies suggest that it is poorly known if various environmental impacts have any specific impact on disease development.[8] The role of mutated genes is one of the crucial factors for thymoma formation and development. Identification of potentially pathogenic variants is obligatory to better explain the molecular milieu of the disease. Our latest study published in January 2020 related to this pathology exhibited variants that play an important role in thymoma.[9]

To that end, advances in high-throughput technology (next-generation sequencing-NGS) have enabled assess of the mutational profiling of various types of diseases including cancer. NGS in genomics includes whole-genome sequencing (WGS), whole-exome sequencing (WES), and targeted sequencing (TS).[10] WGS covers all genomes but provides lower sequencing coverage in comparison to TS. Advances in NGS have contributed to a better understanding of molecular events that lead to disease origin as well as the development of various genetic tests and potential targeted therapy. TS approach targets specific regions of interest and is more cost-effective and often suitable for diagnostic panels which include a specific set of genes characteristic of the disease. Thus, TS provides a patient-specific mutational landscape for effective targeted therapy and better patient management.[11,12] Different approaches are used depending on the need required by the type of study. WGS represents the most comprehensive method for genome analysis and covers the entire DNA of interest. The main limitation of WGS is low sequencing coverage (25 - 30x), high cost per sample (which is lower with the increasing role of methodology), and complex and demanding computation analyzed genome, in comparison to WGS or TS.[13]

This study aimed to identify all relevant articles that evaluate the frequency of SNVs and InDels in thymomas using NGS technology through PubMed, Web of Science, and SCOPUS databases, and to perform a meta-analysis of the prevalence to get better insight into their possible involvement in thymomas. The introduction should summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

Materials and methods

This systematic review was performed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[14]

Study selection Systematic review

Publications were screened for inclusion and exclusion in the meta-analysis of prevalence in two phases, and all disagreements were solved by a discussion with the third reviewer. We included studies that analyze the molecular landscape of thymoma using next-generation sequencing (NGS) genomics. Studies were excluded if they: 1) investigated related diseases but not thymoma; 2) evaluated other outcomes (NGS genomics for gene expression); 2) explored populations other than human (animal models, cell lines); 3) were abstracts; 4) were not original articles (reviews, systematic reviews, case reports, etc.).

Database search

Two researchers have conducted a meta-analysis of the prevalence of the genes and variants involved in thymoma disease. The meta-analysis of all published peer-reviewed articles related to the study was performed by searching the PubMed, Web of Science (WoS), and SCOPUS electronic databases, until the 12th of December 2022. Keywords used for article search in all databases were next-generation sequencing (NGS) and thymoma. Only publications written in English were considered. Additionally, reference lists of articles identified through electronic retrieval were manually searched, as well as relevant reviews and editorials. Experts in the field were contacted to identify other potentially relevant articles.

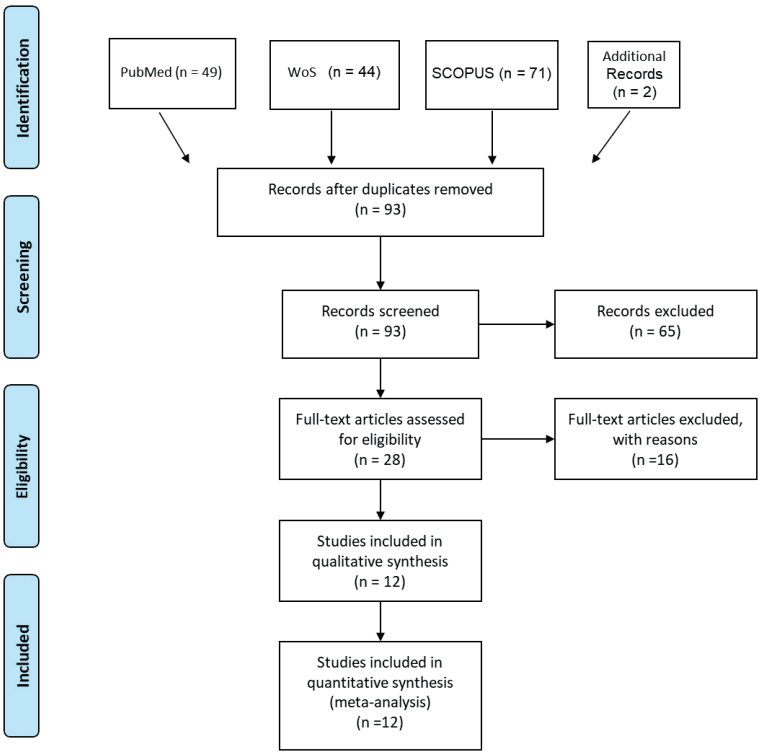

FIGURE 1. Flow chart to illustrate the process by which articles were selected or rejected for inclusion in the study.

Article screening and selection

Two reviewers (JKP, AC) independently evaluated the eligibility of all titles and abstracts. Studies were included in the full-text screening if either reviewer identified the study as being potentially eligible, or if the abstract and title did not include sufficient information. Studies were eligible for full-text screening if they included NGS genomics analysis of thymoma. TETs include thymoma and TC forms, which have been distinguished. The same reviewers independently performed full-text screening to select articles for inclusion according to the criteria listed under the Inclusion and Exclusion Criteria. Disagreements were resolved by consensus (JKP, AC) or arbitration (SP).

Data extraction and quality assessment

Two reviewers independently abstracted the following data: author(s), country of research, year of publication, study design, sample size, study population, type of thymoma, inclusion and exclusion criteria used in the original articles, method of NGS variant detection, genes harboring variants, number of patients having genes with SNVs/InDel variants. Each reviewer independently evaluated the quality of selected manuscripts.

Statistical analysis

The primary outcome was the number of patients harboring SNVs/InDels variants per gene. The odds ratio was evaluated as the ratio between patients with variants in a specific gene per total number of observed patients with thymoma.

Heterogeneity was assessed using the Chi-square Q and I2 statistics. I2 presents the inconsistency between the study results and quantifies the proportion of observed dispersion that is real, i.e., due to between-study differences and not due to random error. The categorization of heterogeneity was based on the Cochrane Handbook and states that I2 < 30%, 30% to 60%, or > 60%, corresponds to low, moderate, and high heterogeneity, respectively.[15] Funnel plots were used to evaluate publication bias. Forest plots were constructed for each analysis showing the Odds Ratio (box), 95% confidence interval (lines), and weight (size of box) for each trial. The overall effect size was represented by a diamond. A p-value < 0.05 was considered to be statistically significant. For graph plots (Forest and Funnel) we used Cohrain's RevMan 5.4 version.[16]

Results

Systematic review

The literature search for original articles was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) statement. A total of 166 potentially eligible publications were found in search of PubMed, WoS, and SCOPUS electronic databases PubMed 49 articles, WoS 44 articles, and SCOPUS 71. Moreover, we included 3 additional studies from an additional PubMed search (Additional records). After duplication removal, and exclusion of review articles, case reports, abstracts, investigations on animals, cell lines, and articles with unsuitable outcomes, 65 articles were excluded. After exclusion, according to previously defined criteria, 44 articles were assessed in full text. Totally, 12 full-text articles were considered for final analyses. The flow diagram represents the selection workflow of publications (Figure 1).

Selected studies have been presented with the names of studies' authors, year of published works, NGS approach, type of tumor, and genes that har-

bored any single nucleotide variants (SNVs) or small insertions/deletions (InDels) in Table 1.

The number of patients harboring or not genes with variants, as well as a total number of patients is shown in the same table. Finally, selected studies were published from 2014 to 12th December 2022. The total number of patients analyzed for *GTF2I* was 433 and 193 patients harbored SNVs/InDels variants in this gene. The total number of cases with SNVs/InDels variants was 58 in *TP53* in the group of 309 thymoma patients. The number of analyzed patients for variants in *HRAS* was 187, while 15 among them had SNVs/InDel variants. The maximum sample size per group was 270. Four studies were from China (4), Japan (2), the USA (1), Italy (1), Austria (1), France (1), Poland (1), and Serbia (1). Analyzed studies used different study designs, mostly cross-section, and case-control designs.

Meta-analysis of prevalence

The analyses of the original articles related to the next-generation sequencing genomics of thymoma have suggested the prevalence of three genes, namely, *GTF2I, TP53*, and *HRAS*. The results of analyzed genes have been represented on Forest plots (Figure 2., Figure 3., and Figure 4.) and Funnel plots (Figure 5., Figure 6., and Figure 7.) with corresponding Odds Ratio (box), Confidence Interval (CI, lines), weight (size of box), and overall size effect in diamonds.

The prevalence of the *GTF2I* gene was the highest (58%), a bit lower was for the *TP53* gene (36%) and the lowest was the *HRAS* gene (2%), for the thymoma population. According to obtained data, we pointed out genes *GTF2I* and *TP53* that exhibit the prevalence in the cohort of observed thymoma patients.

Discussion

The aim of this study was to explore genomic background of indolent forms of thymoma using genomic high-throughput approach. Thymoma epithelial tumors (TETs) comprise thymoma, thymic carcinoma (TC), and thymic neuroendocrine thymoma, as we said previously. In this meta-analysis, we take into consideration several forms of thymomas, including A, AB, B1, B2, and B3 subtypes, which have diverse invasive (malignant) potential. Thymoma is a tumor type that belongs to rare types of thoracic tumors, which is the reason for a lower number of studies that we have

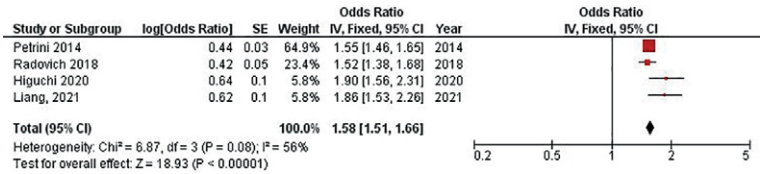

FIGURE 2. Forest plot of *GTH2I* gene in thymoma.

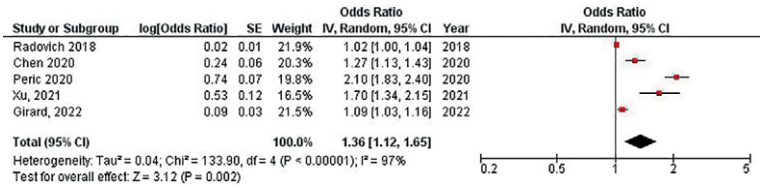

FIGURE 3. Forest plot of *TP53* gene in thymoma.

FIGURE 4. Forest plot of *HRAS* gene in thymoma.

finally selected as eligible for analysis, along with the relatively recently developed method of analysis that we have applied.

As well, keywords used in this research are limited to the NGS genomics approach to analyzing genes included in thymoma. The potential of high-throughput sequencing or NGS enables the detection of the molecular profile, typical for specific tumors or a variety of diseases. All genomic sequencing methods, including WGS, WES, and TS have been applied in the research or in diagnostics. The selection of methodology depends on all advantages or disadvantages suitable for appropriate use. Applying previously explained inclusion/exclusion criteria we were able to select and analyze articles related to this topic, and we were able to find genes associated with thymoma.

Meta-analysis of the prevalence of the gene variants that we performed using selected studies, has indicated that the majority of patients exhibited variants in *GTF2I, TP53*, and *HRAS* genes. The number of analyzed articles was relatively low, due to the rarity of the disease. Moreover, most of the studies have been published from 2014 to 2022.

The molecular background of this pathology is still poorly understood, including all types of A,

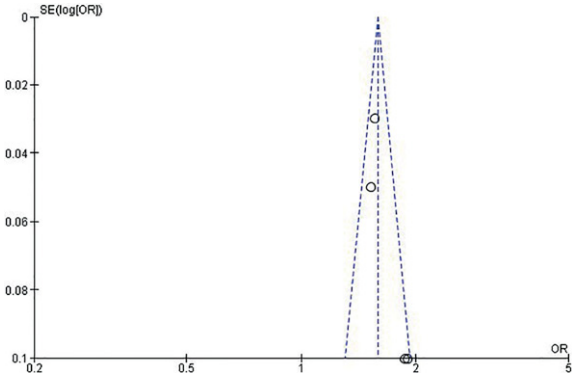

FIGURE 5. Funnel plot of *GTF2I* gene in thymoma.

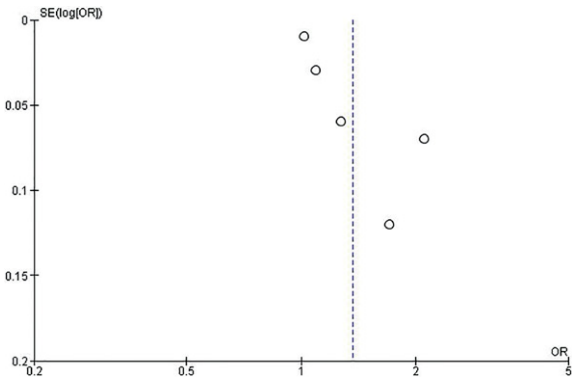

FIGURE 6. Funnel plot of *TP53* gene in thymoma.

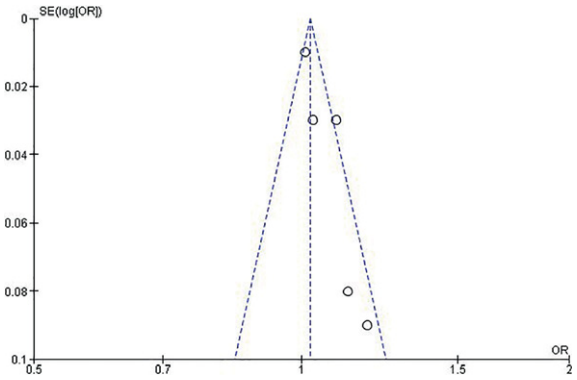

FIGURE 7. Funnel plot of *HRAS* gene in thymoma.

AB, B1, B2, and B3 thymoma. We have analyzed only NGS genomics-based articles. Further in the discussion will also be considered other methodological approaches to better explain obtained results. The newest data that appeared 2022, have indicated that *KIT* gene is an important factor in different processes related to thymoma disease. Unfortunately, we have not had enough data to demonstrate the statistical significance of this gene to put it in a group of prevalent genes for thymoma.[17,18]

GTF2I (General Transcription Factor IIi) encodes a phosphoprotein that binds to the initiator element (Inr) and Inbox element in promoters regulating transcription.[20] *GTF2I* gene is a member of a B cell receptor signaling pathway, the AKT signaling pathway (genecards.org), and is involved in crucial processes particularly, cell proliferation, cell cycle, and development.[19] Finally, the advances in next-generation sequencing characterized *GTF2I* as a master gene in TETs pathology.[20] The frequency of *GTF2I* variants is typical for less aggressive forms of thymoma.[21] *GTF2I* is a gene that embryonic aberration could lead to lethality, which indicates its essential role in embryo development.

The most pathogenic variants in this gene are Chr 7 c.1211T > A and c.1271 (COSM5095139) which could be used in targeted therapy that could lead to successful clinical application.[21,22] Despite *GTF2I* pathogenicity in thymomas, *GTF2I* mutations are very rare (< 1%) in other types of cancer, according to TCGA. Moreover, many types of molecular events lead to thymoma genesis. TCGA's studies of thymoma have shown recurrent mutations in the *GTF2I* gene and suggested this gene as a potential drug target for this disease.[23-25] Our findings confirm the prevalence of the SNVs/InDels variants in *GTF2I* in thymoma. Results have been reported in several articles which have been presented in the Forest and Funnel plots. Petrini and colleagues, using WES have discovered missense mutation Chr 7 c.1211T > A and subsequent protein change p. Leu404His in all types of thymoma A, AB, B1, B2, B3, and TC.[19,26] In the publications that we selected for analysis, *GTF2I* harbored p. Leu404His and p. Leu424His (COSM5095139) pathogenic variants, recognized as a disease-marker gene in thymoma.[22,26-28]

TP53 is a crucial tumor suppressor gene regulating the most important processes in the cell including cell cycle regulation, DNA repair, senescence, and apoptosis.[29] *TP53* has been described as the gene responsible for disease pathogenesis. A recent study has demonstrated common loss-of-function in *TP53* in type B thymomas and TCs.[30] In a study by Peric *et al*., two variants with stop codon have been identified R213* and V91*, as well as four pathogenic missense changes namely, D281N, G244S, R158C, E221K.[22,31,32] One of the newest studies by Xu S *et al*., included in our meta-

TABLE 1. Characteristics of 12 selected studies

Author	Year	Study design	Thymoma	Gene (SNV)	Number of cases	Total number of cases	NGS genomics
Chen K, China	2020	Cross-sectional	Type A, AB, B1, B2, B3	*APC* *TP53* *ATM* *AKT1* *SMAD4* *ALK* *KRAS* *NRAS*	23 12 22 5 12 19 2 2	50	TS
Enkner F, Austria	2017	Cross-sectional	Type A, B	*HRAS* *SMARCB1* *STK11*	3 1 1	19	TS
Higuchi R, Japan	2020	Cross-sectional	Type A, AB, B	*GTF2I*	14	22	TS
Petrini I, Italia	2014	Cross-sectional	Type A, AB	*GTF2I*	119	270	ES
Radovich M, USA	2017	Case-control	Type A, AB	*GTF2I* *HRAS* *TP53*	44 10 2	105	MOPA
Sakane T, Japan	2020	Cross-sectional	Type A, B2, B3, B4, B5	*HRAS* *PIK3CA* *AKT1* *RAS pathway* *EGFR pathway*	1 2 1 1 3	33	SNaPshot Multiplex
Song ZB, China	2016		Type B2, B3	*PIK3CA* *EGFR*	1 1	37	TS IonAmpliSeq
Peric J, Serbia	2020	Case-control	Type A, B1, B2, B3	*SMAD4* *APC* *ATM* *ERBB4* *TP53*	27 27 26 24 26	35	TSACP
Xu S, China	2021	Cross-sectional	Type A, AB B1, B2, B3	*TP53* *HRAS*	9 2	17	ES
Liang N, China	2021	Cross-sectional	Type A, AB, B1, B2, B3	*GTF2I*	15	24	SureSelectXT TS
Szpechcinski A, Poland	2022	Cross-sectional	Type B2B3	KIT ERBB2	1 1	19	TS
Girard N, France	2022	Cross-sectional	Type A, B1, B2, B3	KIT TP53 HRAS Other genes	1 8 1 80	90	WES

MOPA = multi-omics platform analysis; SNaPshot Multiplex = Snapshot multiplex assay for point mutation; TS = targeted enrichment-based sequencing: TSACP = TruSeq amplicon cancer panel; (WES) = whole exome sequencing

analysis, has analyzed the genomic profile of TETs that has indicated *TP53* as the most mutated gene especially in both B3 and C thymoma causing worse prognosis.[33]

HRAS gene belongs to the Ras signaling pathway, commonly mutated in many tumors harboring pathogenic variants, for instance, G13V in thymoma. One study suggests gain-off function mutations in *HRAS*, especially in type A and AB thymomas.[30] A recent study by Jovanovic D *et al.* has identified recurrent mutations in *HRAS*, marked as a thymoma-specific oncogene.[34] TCGA's studies of thymoma observed enrichment in the *HRAS* gene, as well as *NRAS* and *TP53*.[24,25] Our meta-analysis of prevalence has reported the *HRAS* gene to be involved in the pathogenesis of thymoma.[22,33,35,36] Genes that have shown the prevalence in less aggressive forms of thymoma, especially thymoma-specific oncogene *GTF2I* (transcription factor), TP53, and members of Ras family *HRAS* have been involved in crucial processes including cell cycle regulation, cell proliferation, cell differentiation, apoptosis, and cell survival. Therefore, pathogenic variants within these genes could be important

disease markers and potential therapeutic targets for thymoma.

Conclusions

Our meta-analysis of articles that analyze a mutational portrait using NGS of thymoma has pointed out *GTF2I, HRAS,* and *TP53* genes as thymoma-specific oncogenes. These genes harbor variants SNVs/InDels which contribute to disease development. Moreover, this study indicated the highest prevalence of the *GTF2I* gene (58%). Some of the identified variants are driver mutations, for instance, *GTF2I* (Chr7 c.1211T > A, p. Leu404His and c.1271T > A, p. Leu424His), associated with thymoma pathology. In addition, the majority of detected molecular changes are classified as passenger variants, unable to cause disease and further progression, without the presence of other molecular events. However, the thymoma molecular landscape is still insufficiently understood and explored. Therefore, there is a need for additional analysis and information to get a comprehensive genomic picture for better precision treatment of the patients.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development Republic of Serbia, EB: 451-03-68/2022-14/200042.

References

1. Cowen D, Richaud P, Mornex F, Bachelot T, Jung GM, Mirabel X, et al. Thymoma: results of a multicentric retrospective series of 149 non-metastatic irradiated patients and review of the literature. FNCLCC trialists. Federation Nationale des Centres de Lutte Contre le Cancer. *Radiother Oncol* 1995; **34:** 9-16. doi: 10.1016/0167-8140(94)01493-m
2. Levine GD, Rosai J. Thymic hyperplasia and neoplasia: a review of current concepts. *Human Pathology* 1978; **9:** 495-515. doi: 10.1016/s0046-8177(78)80131-2
3. Falkson CB, Bezjak A, Darling G, Gregg R, Malthaner R, Maziak DE, et al. The management of thymoma: a systematic review and practice guideline. *J Thorac Oncol* 2009; **4:** 911-9. doi: 10.1097/jto.0b013e3181a4b8e0
4. Okumura M, Ohta M, Tateyama H, Nakagawa K, Matsumura A, Maeda H, et al. The World Health Organization histologic classification system reflects the oncologic behavior of thymoma: a clinical study of 273 patients. *Cancer* 2002; **94:** 624-32. doi: 10.1002/cncr.10226
5. Engels EA, Pfeiffer RM. Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies. *Int J Cancer* 2003; **105:** 546-51. doi: 10.1002/ijc.11099
6. Tomaszek S, Wigle DA, Keshavjee S, Fischer S. Thymomas: review of current clinical practice. *Ann Thorac Surg* 2009; **87:** 1973-80. doi: 10.1016/j.athoracsur.2008.12.095
7. Chau NG, Kim ES, Wistuba I. The multidisciplinary approach to thymoma: combining molecular and clinical approaches. *J Thorac Oncol* 2010; **5:** S313-7. doi: 10.1097/JTO.0b013e3181f20d9a
8. Miyamoto K, Acoba JD. Thymomas and thymic carcinomas: a review on pathology, presentation, staging, treatment, and novel systemic therapies. *EMJ Respir* 2017; **5:** 100-7. doi: 10.33590/emjrespir/10310358
9. Peric J, Samaradzic N, Skodric Trifunovic V, Tosic N, Stojsic J, Pavlovic S, et al. Genomic profiling of thymoma using a targeted high-throughput approach. *Arch Med Sci* 2020. doi: 10.5114/aoms.2020.96537
10. Slatko BE, Gardner AF, Ausubel FM. Overview of next-generation sequencing technologies. *Curr Protoc Mol Biol* 2018; **122:** e59. doi: 10.1002/cpmb.59
11. Duncavage EJ, Abel HJ, Szankasi P, Kelley TW, Pfeifer JD. Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia. *Mod Pathol* 2012; **25:** 795-804. doi: 10.1038/modpathol.2012.29
12. Xu H, DiCarlo J, Satya RV, Peng Q, Wang Y. Comparison of somatic mutation calling methods in amplicon and whole exome sequence data. *BMC Genomics* 2014; **15:** 244. doi: 10.1186/1471-2164-15-244
13. Chakravarty D, Gao J, Phillips SM, Kundra R, Zhang H, Wang J, et al. OncoKB: A Precision Oncology Knowledge Base. *JCO Precis Oncol* 2017; **2017:** PO.17.00011. doi: 10.1200/PO.17.00011
14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidem* 2009; **62:** e1-34. doi: 10.1016/j.jclinepi.2009.06.006
15. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page M J, et al. A Cochrane handbook for systematic reviews of interventions. *Cochrane handbook for systematic reviews of interventions*. 2019. (cited 2022 Dec 19). Available at: https://training.cochrane.org/handbook
16. Cohrain Training. RevMan 5. (accessed 2021 Aug 3). Available at: https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman/revman-5-download
17. Girard N, Basse C, Schrock A, Ramkissoon S, Killian K, Ross JS. Comprehensive genomic profiling of 274 thymic epithelial tumors unveils oncogenic pathways and predictive biomarkers. *Oncologist* 2022; **27:** 919-29. doi: 10.1093/oncolo/oyac115
18. Szpechcinski A, Szolkowska M, Winiarski S, Lechowicz U, Wisniewski P, Knetki-Wroblewska M. Targeted next-generation sequencing of thymic epithelial tumours revealed pathogenic variants in KIT, ERBB2, KRAS, and TP53 in 30% of thymic carcinomas. *Cancers* 2022; **14:** 3388. doi: 10.3390/cancers14143388
19. GeneCards®: The Human Gene Database. (Updated: 2023 Jan 10). Available at: https://www.genecards.org/
20. Müllauer L. GTF2I gene mutation – a driver of thymoma pathogenesis. *Mediastinum* 2017; **1:** 11. doi: 10.21037/med.2017.11.03
21. Feng Y, Lei Y, Wu X, Huang Y, Rao H, Zhang Y, et al. GTF2I mutation frequently occurs in more indolent thymic epithelial tumors and predicts better prognosis. *Lung Cancer* 2017; **110:** 48-52. doi: 10.1016/j.lungcan.2017.05.02022
22. Radovich M, Pickering CR, Felau I, Ha G, Zhang H, Jo H, et al. The integrated genomic landscape of thymic epithelial tumors. *Cancer Cell* 2018; **33:** 244-58.e10. doi: 10.1016/j.ccell.2018.01.003
23. Liu D, Zhang P, Zhao J, Yang L, Wang W. Identification of molecular characteristics and new prognostic targets for thymoma by multiomics analysis. *BioMed Res Int* 2021; **2021:** Article ID 5587441, 1-15. doi: 10.1155/2021/5587441
24. TCGA's Study of Thymoma. (cited 2022 Dec 5). Available at: https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/studied-cancers/thymoma
25. Venuta F, Rendina EA, Anile M, de Giacomo T, Vitolo D, Coloni GF. Thymoma and thymic carcinoma. *Gen Thorac Cardiovasc Surg* 2012; **60:** 1-12. doi: 10.1007/s11748-011-0814-0
26. Petrini I, Meltzer PS, Kim IK, Lucchi M, Park KS, Fontanini G, et al. A specific missense mutation in GTF2I occurs at high frequency in thymic epithelial tumors. *Nature Genetics* 2014; **46:** 844-9. doi: 10.1038/ng.3016

27. Higuchi R, Goto T, Hirotsu Y, Yokoyama Y, Nakagomi T, Otake S, et al. Primary driver mutations in GTF2I specific to the development of thymomas. *Cancers* 2020; **12:** 1-12. doi: 10.3390/cancers12082032

28. Liang NX, Liu L, Huang C, Liu HS, Guo C, Li J, et al. Transcriptomic and mutational analysis discovering distinct molecular characteristics among chinese thymic epithelial tumor patients. *Front Oncol* 2021; **11:** 647512. doi: 10.3389/fonc.2021.647512

29. Hafner A, Bulyk ML, Jambhekar A, Lahav G. The multiple mechanisms that regulate p53 activity and cell fate. *Nat Rev Mol Cell Biol* 2019; **20:** 199-210. doi: 10.1038/s41580-019-0110-x

30. Marx A, Belharazem D, Lee DH, Popovic ZV, Reissfelder C, Schalke B, et al. Molecular pathology of thymomas: implications for diagnosis and therapy. *Virchows Arch* 2021; **478:** 101-10. doi: 10.1007/s00428-021-03068-8

31. Chen K, Che JM, Zhang XF, Jin RS, Xiang J, Han DP, et al. Next-generation sequencing in thymic epithelial tumors uncovered novel genomic aberration sites and strong correlation between TMB and MSH6 single nucleotide variations. *Cancer Letters* 2020; **476:** 75-86. doi: 10.1016/j.canlet.2020.02.001

32. Xu S, Li XF, Zhang HY, Zu LL, Yang LQ, Shi T, et al. Frequent genetic alterations and their clinical significance in patients with thymic epithelial tumors. *Front Oncol* 2021; **11:** 667148. doi: 10.3389/fonc.2021.667148

33. Jovanovic D, Markovic J, Ceriman V, Peric J, Pavlovic S, Soldatovic I. Correlation of genomic alterations and PD-L1 expression in thymoma. *J Thorac Dis* 2020; **12:** 7561-70. doi: 10.21037/jtd-2019-thym-13

34. Enkner F, Pichlhöfer B, Zaharie AT, Krunic M, Holper TM, Janik S, et al. Molecular profiling of thymoma and thymic carcinoma: genetic differences and potential novel therapeutic targets. *Pathol Oncol Res* 2017; **23:** 551-64. doi: 10.1007/s12253-016-0144-8

35. Rajan A, Girard N, Marx A. State of the art of genetic alterations in thymic epithelial tumors. *J Thorac Oncol* 2014; **9:** S131-S6. doi: 10.1097/JTO.0000000000000298

36. Sakane T, Murase T, Okuda K, Saida K, Masaki A, Yamada T, et al. A mutation analysis of the EGFR pathway genes, RAS, EGFR, PIK3CA, AKT1 and BRAF, and TP53 gene in thymic carcinoma and thymoma type A/B3. *Histopathology* 2019; **75:** 755-66. doi: 10.1111/his.13936