

Review of doctoral dissertation in technical sciences,
discipline: biomedical engineering

**Mathematical modelling in comparative analysis of methylation profiles
of *de novo* and therapy-related AML**

Agnieszka Cecotka, M.Sc. Eng.

Ph.D. dissertation of Agnieszka Cecotka, M.Sc. Eng., entitled „Mathematical modelling in comparative analysis of methylation profiles of *de novo* and therapy-related AML”, provides an interesting and unique analysis of DNA methylation in acute myeloid leukaemia (AML) of various aetiologies. The best approaches were developed, selected, and described based on data preprocessing techniques, statistical analysis, mathematical modelling, and functional analysis of detected features with the objective to validate the results obtained using various platforms.

The hardcover dissertation submitted for review, written in English in an extremely thorough, interesting and logical manner, contains 121 typed pages and has a layout typical of doctoral dissertations. It consists of five main sections, namely Introduction, Background, Material and Methods, Results, and Conclusions. It also includes a list of abbreviations, a summary in both Polish and English, and a list of references. It contains dozens of figures (including multi-panel ones), 28 tables, which are placed in the text, making it easier for the reader to comprehend the presented material and results. The author cited 157 references.

The Introduction section is a rather extensive review of the term “epigenetics” and its role and potential impact of modifying factors, DNA methylation processes with their effects on gene expression, genome stability maintenance, as well as an extensive discussion of the epidemiology, types and classification of AML, and therapeutic regimens used in this disease. Such an in-depth discussion of the presented issues testifies to the very good substantive preparation of the Ph.D. Candidate, who, in undertaking the mathematical modelling in comparative analysis of methylation profiles of *de novo* and therapy-related AML, has explored extremely difficult issues in medicine and life sciences – not only in the field of oncology and genetics, but also biochemistry. The introduction to the dissertation discusses all the important aspects of the material analysed in the Ph.D. dissertation, leading to the next section, which describes the purpose of the dissertation.

In the “Objectives” section, the Ph.D. Candidate formulates two main research objectives of the study, namely the development of methods based on mathematical modelling, comparative statistical analysis and the compilation of their results, used for analysis of DNA methylation data, to enable the identification of differences in DNA

methylation levels in genomic regions of various patient groups, and the integration of results obtained using different experimental platforms in order to obtain validated findings that are less error-prone.

In the “Materials and methods” section, the Ph.D. Candidate describes the analysed datasets and methods used for their analysis. In each scenario, the study involved analysis of DNA methylation level distribution, detection of differentiating probes in patient groups evaluated in the study, outcome integration in order to identify genomic regions associated with specific cases, as well as functional analysis. The study of gender-related differences in AML involves a survival analysis, and research into different types of AML requires marker detection and validation, and the use of the unsupervised feature selection technique.

In the next section, “Results,” Agnieszka Cecotka, M.Sc. Eng., presents the results of the conducted analyses with rich illustrative material, and relates these results to published literature data. The author first presents an examination of acute myeloid leukaemia profile on the basis of differences in distribution between AML and a healthy sample. Next, gender-related differences in methylation level of specific genomic regions are evaluated and compared with healthy controls. The author also addresses the issue of detecting genomic regions that can impact survival. Subsequently, the methylation profiles of various groups of patients are discussed in addition to the methods to identify and validate differentially methylated genomic regions in *de novo* AML, chemotherapy-related AML, and radiotherapy-related AML. Finally, the results from all analyses are compiled.

Before the planned publication of the results of the Ph.D. dissertation, it is worth expanding the discussion with emphasis on the clinical implications of the results of this analysis. In the reviewed dissertation, I found no section titled “Discussion” because the Ph.D. Candidate discussed the obtained results in relation to literature data in the “Results” section. Also, in my opinion, the “Conclusions” section should not include the literature citations [156 and 157] – their place is precisely in the discussion. These editorial issues do not diminish the value of the dissertation, and it is suggested that they be addressed in preparation of the paper for future publication.

The Ph.D. Candidate has achieved the set research objectives. In the “Conclusions” section, she included thoroughly described conclusions that correspond to the study objectives, relating to their achievement.

The aims of the dissertation included the detection of differentially methylated probes and genomic regions among various groups of patients, and the integration of results obtained using various experimental platforms. These objectives have been achieved with regard to acute myeloid leukaemia methylation profile, gender-related differences in DNA methylation in AML, and DNA methylation aberrations in *de novo* and treatment-related AML.

The study confirmed that acute myeloid leukaemia is associated with changes in DNA methylation. AML-related methylation changes vary depending on genomic region types: TSS, gene body, and intergenic. The number of detected hypermethylated probes and regions was significantly higher compared to those hypomethylated. Genes whose genomic regions (in particular TSS) were found to be hypo- or hypermethylated in AML were confirmed as directly associated with leukaemia. The relationship between the identified genes and processes altered in the course of AML was confirmed in a functional analysis.

The results revealed gender-related differences in methylation profile in AML. Corresponding differences were not seen in healthy individuals. The gender differences in AML

were found in probes of CpG-rich Regulatory Sequence genomic regions. DNA methylation changes that occur in these regions have the greatest effect on gene expression. In other genomic regions, gender-related differences in AML are insignificant.

Methylation profile analysis identified differences in methylation level distribution among genomic region types. The analysis involved a combination of statistical methods and the Illumina annotation system, in which probes are assigned to specific genomic regions.

These two approaches used to identify differentially methylated probes and genomic regions provided consistent results. Methods corrected for multiple pairwise comparisons turn out to be more restrictive. The results from the first and third studies were combined in order to find differentially methylated features for AML. Only a few common genomic regions with hypo- and hypermethylation were identified. Nevertheless, the differences in methylation level distribution among genomic region types were similar in both analyses. It was confirmed that the frequency of hypermethylation was higher compared to hypomethylation. The low similarity of results could be due to different definitions of the compared regions (TSS in the first study and CpG-rich RS in the third study) as well as small numbers of study subjects, as well as intra-group variation.

Combined results from the second and third studies confirmed that in most cases the methylation level was gender-dependent. The consistency of observations was confirmed visually.

The summary, which is included in both Polish and English, is excellently written.

The last part of the work ends with a list of cited, extremely comprehensive global literature, including publications of Polish authors (a total of 157 items).

The presented Ph.D. dissertation is of a high scientific level in terms of statistical analysis. The Ph.D. Candidate has well managed the compilation of an extensive and complex research material, modern statistical methods, interpretation of numerical results and their clear graphical presentation. Noteworthy is the fact that she used a sequence of consecutive computational techniques, which demonstrated her superior skills and practical experience as a researcher. Moreover, these are indeed substantively difficult matters that must be supported by extensive knowledge gained, given the scientific age, in a fairly short period of time. And the smooth cooperation with the medical field of the analysed research issues proves the skill and independence in overcoming scientific difficulties, as well as the high intellectual level of this dissertation. The above observations deserve high appreciation and recognition from the scientific community, and a special distinction for the Ph.D. Candidate.

Also worth highlighting is the perfect editorial form of the dissertation, which has been written entirely in English (except for the included abstract in Polish).

The Ph.D. dissertation submitted for review complies with the current conditions set forth in the Act on academic degrees and academic title and degrees and title in art, as amended (Art. 13(1) of the Act of 14 March 2003 on academic degrees and academic title and degrees and title in art, consolidated text in Journal of Laws of 2017, item 1189).

In view of the above, I have the honour to submit to the Academic Board for the Discipline of Biomedical Engineering at the Silesian University of Technology in Gliwice a request to admit Agnieszka Cecotka, M.Sc. Eng., to the further stages of the proceedings.

In addition, due to the innovative nature of the dissertation, and the uniqueness of the mathematical analyses carried out, as well as its extremely high level of scientific content, I kindly recommend that this dissertation be awarded with distinction.

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