

Adam Kuzdraliński, D.Sc., Eng.,
Associate Professor
Head of the Department of Bioinformatics
and Artificial Intelligence Applications,
Polish-Japanese Academy
of Information Technology,
86 Koszykowa Street
02-008 Warsaw, Poland
e-mail: akuzdralinski@pjwstk.edu.pl

Warsaw, 02 September 2026

Review of the doctoral dissertation
submitted by Ms Ranjini Ghosh Dastidar,
entitled

“A Dynamic Regulatory Model of *In Vivo* Vitamin D Responsiveness: From
Transcriptomic Discovery to Systems Identification in Human Immune Cells”,
prepared under the supervision
of Prof. Carsten Carlberg

Formal basis and scope of the review

This review was prepared following my appointment as a reviewer by the Scientific Council of the InLife Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, on 11 June 2026, and pursuant to the letter of the Director of the Institute, Prof. Monika M. Kaczmarek, dated 3 July 2026. The doctoral proceedings concern the award of the degree of Doctor of Agricultural Sciences in the discipline of food technology and nutrition. The proceedings were initiated on 12 May 2026.

In accordance with Article 187 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended), this review addresses three essential questions: whether the dissertation demonstrates the candidate's general theoretical knowledge in the relevant discipline, whether it demonstrates her ability to conduct scientific research independently, and whether it presents an original solution to a scientific problem.

General characteristics of the dissertation

The dissertation combines three published research papers and one manuscript under review, accompanied by a detailed commentary, and comprises 201 pages. It includes English and Polish abstracts, a list of abbreviations, a guide to the studies, a general introduction, clearly stated objectives and hypotheses, descriptions of the four component studies, combined observations, a general discussion, conclusions, limitations, recommendations, lists of figures and tables, references, declarations concerning authorship and individual contributions, official documents, and the appended papers.

The dissertation is based on the following four works:

1. **Ghosh Dastidar, R.**, Jaroslawska, J., Malinen, M., Tuomainen, T. P., Virtanen, J. K., Bendik, I., & Carlberg, C. (2024). In vivo vitamin D targets reveal the upregulation of focal adhesion-related genes in primary immune cells of healthy individuals. *Scientific Reports*, 14(1), 17552. DOI: 10.1038/s41598-024-68741-9. The candidate is the first author.
2. AlGhamdi, S. A., **Ghosh Dastidar, R.**, Rybiński, M., Alsufiani, H. M., Khoja, S. O., Enaibsi, N. N., Saif, S. F., & Carlberg, C. (2024). Evaluation of the vitamin D response index in a Saudi cohort. *Saudi Pharmaceutical Journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, 32(8), 102137. DOI: 10.1016/j.jsps.2024.102137. The candidate is designated as an equal-contributing author.
3. Gospodarska, E., **Ghosh Dastidar, R.**, Jaroslawska, J., Rybiński, M., Raczyk, M., Tokarczyk-Malesa, K., Romaszko, J., & Carlberg, C. (2025). Transcriptomic profiling of immune modulation induced by vitamin D₃ in the VitDPAS and VitDHiD cohort studies. *Scientific Reports*, 15(1), 17334. <https://doi.org/10.1038/s41598-025-02495-w>. The candidate is again designated as an equal-contributing author.
4. **R. Ghosh Dastidar** et al., "Dynamic System Identification Defines a Minimal Transcription Factor Control Architecture of Human Endocrine Gene Regulation", a first-authored manuscript submitted to Nucleic Acids Research, the documentation appended to the dissertation confirms receipt by the journal and the initiation of reviewer invitations in April 2026.

The four works form a logical sequence. The first study characterises the in vivo transcriptional response to vitamin D₃ in peripheral blood mononuclear cells (PBMCs) using RNA sequencing and classifies individuals as high, mid, or low vitamin D responders on the basis of their transcriptional response. The second investigates whether low responsiveness is also observed in a genetically and environmentally distinct Saudi population. The third compares the Finnish VitDHiD and Polish VitDPAS cohorts and identifies a conserved core of 26 vitamin D-responsive genes. The fourth uses longitudinal transcriptomic data from VitDPAS to develop a model based on ordinary differential equations (ODEs) and a reduced network of 11 transcription factors connected by 117 regulatory interactions.

The clinical interventions, sample collection, and laboratory preparation were carried out by collaborating teams, while RNA sequencing for the Finnish and Polish transcriptomic studies was performed at the EMBL Gene Core in Heidelberg, Germany. The candidate states that she did not perform the wet-laboratory work and describes her scientific contribution as computational and bioinformatic. According to the contribution statements, participant recruitment, sample collection, PBMC isolation, RNA isolation, RNA-seq library preparation, and other experimental procedures were performed by co-authors and collaborating teams. Her declared contributions include data analysis, software, visualization and conceptualization, as well as methodology in the fourth study and contributions to manuscript preparation.

Assessment of the candidate's general theoretical knowledge

The dissertation demonstrates theoretical knowledge in nutrigenomics, molecular nutrition, immunology, transcriptomics, bioinformatics, and systems biology. The introduction provides a well-structured account of vitamin D metabolism, VDR-mediated transcriptional regulation, the immune functions of vitamin D, the rationale for using PBMCs, the distinction between *in vivo* intervention studies and *in vitro* cell-culture studies and interindividual heterogeneity in responses to supplementation. The candidate also explains the progression from targeted molecular assays to genome-wide RNA-seq and ultimately to mechanistic modelling of transcriptional regulation.

The dissertation is relevant to the discipline of food technology and nutrition because it examines molecular responses to a controlled nutritional intervention, interindividual differences in vitamin D responsiveness, and their possible relevance to personalised nutrition. The work is interdisciplinary, combining nutritional science with molecular biology, immunology, bioinformatics, and mathematical modelling.

The objectives and five research hypotheses are logically connected. The dissertation progresses from the identification of *in vivo* vitamin D responsive genes and response classification, through cross-population and cross-cohort comparisons, to modelling of the underlying regulatory network. The candidate shows that she understands the differences between vitamin D status, gene-expression levels, transcriptional changes, and regulatory network behaviour.

The dissertation also acknowledges several important limitations, including the relatively small cohort sizes, the restriction of transcriptomic analyses to PBMCs, heterogeneity in PBMC cellular composition, the simplifications inherent in the ODE model, the lack of direct experimental evidence for causality of the inferred regulatory interactions.

Some conclusions are stated more strongly than the evidence supports. In particular, the available data support the occurrence of heterogeneous vitamin D responsiveness in different populations, but do not establish that the response index is a “universal biological

phenomenon” or rule out pharmacokinetic contributions to interindividual variability. Likewise, the observed temporal amplification of the transcriptional response is consistent with epigenetic priming but does not constitute direct evidence of this mechanism. Finally, the modelling results support further development of a predictive approach, but prospective prediction of an individual’s responder status before supplementation has not yet been validated. These reservations concern the interpretation of the results and do not alter my positive assessment of the candidate’s theoretical knowledge.

Assessment of the candidate’s ability to conduct scientific research independently

According to the declarations included in the dissertation, her work included processing and quality control of RNA-seq data, differential expression and pathway analyses, development of response-scoring algorithms, k-means clustering, cross cohort comparison, identification of the conserved 26gene core, pharmacokinetic model fitting, construction and reduction of the DoRothEA based regulatory network, formulation of the ODE model, parameter optimisation by differential evolution in Julia, cross cohort validation, visualisation, and contributions to manuscript preparation.

Paper IV extends the computational work by combining pharmacokinetic modelling with regulatory-network and ODE modelling. The candidate reports using R, Python, and Julia for different parts of these analyses. This range of computational methods is a strong aspect of her contribution.

The candidate’s contribution was computational rather than experimental. The assessment of her scientific independence should therefore focus on her role in the design, implementation, and interpretation of the computational analyses..

The candidate’s individual declarations describe a broader and more detailed contribution than the author-contribution statements in some of the appended papers. This is particularly apparent with respect to manuscript preparation and, in Paper IV, the conceptual and methodological contribution to the modelling. These differences do not in themselves indicate a contradiction, but the candidate should clarify during the defence the scope of her own contribution to the collaborative work.

The documented scope of the candidate’s computational contribution provides sufficient evidence of her ability to conduct scientific research independently. I therefore assess this criterion positively.

Assessment of the original solution to the scientific problem

The central scientific problem concerns the molecular basis of interindividual variation in the response to vitamin D₃ supplementation. The dissertation addresses this problem by analysing

data from human nutritional interventions using transcriptomic profiling, response classification, cross-cohort comparison, and ODE modelling.

Paper I provides the transcriptomic basis for the subsequent studies. In the 25 participants included in the transcriptomic analysis of the Vitamin D Human in vivo Dose Trial (VitDHiD), RNA-seq analysis of samples collected before and 24 hours after an 80,000 IU vitamin D₃ bolus identified 452 differentially expressed genes at a false discovery rate (FDR) < 0.05. Focal adhesion emerged as a major upregulated pathway. The transcriptional response was also used to classify participants as high, mid, or low vitamin D responders. In the stable responder subgroups, baseline HLA-C expression was more than fourfold higher in high responders than in low responders.

Paper II extends the analysis to a Saudi cohort and shows that a low-response phenotype can also be identified in this population. It should not, however, be regarded as a direct validation of the RNA-seq response index, because responder classification in the full cohort was based on cytokine changes, while a subset of 37 participants was additionally classified using a seven-gene qPCR panel. These findings support the presence of heterogeneous vitamin D responsiveness in the Saudi cohort, but they do not by themselves establish the universality of the same response index across populations.

Paper III provides the most direct cross-cohort transcriptomic comparison in the dissertation. It analyses an independent Polish cohort and identifies a high-confidence core of 26 genes shared between VitDPAS and VitDHiD. Of these 26 genes, 22 were downregulated and four were upregulated, with several genes associated with TNF and NFκB signalling.

Paper IV is the most methodologically ambitious component of the dissertation and also requires the greatest caution in interpretation. It develops a dynamic regulatory model of the vitamin D response from longitudinal transcriptomic data and proposes an 11-transcription-factor network, including a TP53-AP1-MYC regulatory axis distinguishing high and low responders. The mechanistic interpretation of these results, however, requires careful consideration.

The evidence does not uniquely support a single causal regulatory mechanism. Despite reduction of the regulatory network to 11 transcription factors, the resulting ODE model still contains 373 optimizable parameters per supplementation cycle. Good agreement between simulated and observed expression trajectories therefore does not by itself demonstrate that the inferred interaction strengths are uniquely determined by the data. The low sensitivity of most model parameters is consistent with substantial parameter redundancy. Since network reduction is not equivalent to a formal identifiability analysis, the inferred regulatory interactions should be interpreted primarily as hypothesis-generating rather than as uniquely established causal relationships.

The model was evaluated in an independent VitDHiD cohort, but this validation concerned the prediction of cohort-level mean transcription-factor expression rather than prospective classification of individual responders. The reported R^2 values above 0.94 therefore demonstrate cross-cohort transferability of the model at the group level, but not individual-level predictive performance. Prospective classification of previously unseen individuals would require a separate validation study specifically designed for that purpose.

These limitations concern the interpretation of some of the strongest claims rather than the originality of the research as a whole. The combined transcriptomic and modelling work constitutes an original approach to investigating interindividual variation in the response to vitamin D supplementation. I therefore assess positively the criterion that the dissertation presents an original solution to a scientific problem.

Major comments and matters requiring clarification

The following matters should be corrected or discussed:

1. The dissertation reports the VitDHiD response-class distribution as high $n = 4$, mid $n = 12$, and low $n = 9$. This is inconsistent with the published classification of the VitDHiD cohort, corresponding to high $n = 9$, mid $n = 12$, and low $n = 4$. The numbers of high and low responders therefore appear to have been reversed and should be corrected.
2. The dissertation refers to the VitDHiD cohort as comprising either 40 or 25 participants. While 40 participants were enrolled in the study, the published transcriptomic analysis included 25 participants. The dissertation should clearly distinguish between the number of enrolled participants and the cohort included in the transcriptomic analysis.
3. For the Saudi cohort, the dissertation reports 88.6% agreement between cytokine- and qPCR-based classifications among 37 participants. Paper II, however, reports agreement for 30 of 37 participants (81%). The value of 88.6% refers instead to the consistency of response classification in the combined VitDPAS/VitDHiD analysis (62 of 70 participants) reported in Paper III.
4. The conclusion that the vitamin D response index is a “universal biological phenomenon” should be stated more cautiously. The Saudi study provides important evidence that high, mid, and low vitamin D responsiveness is not restricted to European populations. However, the proportion of low responders differed depending on the population and the method used for classification: 39% in the full Saudi cohort using cytokine-based scoring and 24% in the qPCR analysis of a 37-participant subset, compared with 16% in VitDHiD and approximately 20% in VitDPAS. These results support the generalizability of the response-index concept across geographically distinct populations, but do not establish its universality or, by themselves, exclude

pharmacokinetic contributions to interindividual differences in vitamin D responsiveness.

5. The manuscript states that structural identifiability was “enforced” or “enhanced” through iterative kernel reduction and fitting across repeated perturbation cycles. Network reduction decreases model dimensionality, but it is not clear whether formal structural-identifiability analysis was performed. The candidate should clarify the basis for this claim and discuss to what extent the fitted interaction parameters can be considered uniquely determined by the data. This is particularly relevant given the low sensitivity of most model parameters and the authors’ interpretation of this result as evidence of substantial parameter redundancy, together with the subsequent mechanistic interpretation of differences between high- and low-responder models, including the proposed TP53-AP1-MYC regulatory axis.
6. The model was tested in an independent VitDHiD cohort, but the analysis was based on mean gene expression for the cohort rather than predictions for individual participants. The candidate should therefore clarify to what extent these results support the proposed use of the model for predicting individual vitamin D responsiveness. In particular, the current validation does not show that the model can predict whether a previously unseen individual will be a high, mid, or low responder.
7. The statement that temporal amplification provides the first direct evidence of epigenetic priming in humans is too strong. The study shows a stronger transcriptional response after repeated supplementation, but does not directly measure changes in chromatin across the supplementation cycles. These results may support the hypothesis of epigenetic priming, but they do not demonstrate this mechanism directly.

These comments are scientifically important and should be discussed during the public defence. They do not invalidate the main transcriptomic results or the overall originality of the research.

Final conclusion

The doctoral dissertation of Ms Ranjini Ghosh Dastidar entitled “A Dynamic Regulatory Model of In Vivo Vitamin D Responsiveness: From Transcriptomic Discovery to Systems Identification in Human Immune Cells” presents original research combining nutrigenomics, transcriptomics, bioinformatics, and systems biology.

In my assessment, the dissertation demonstrates the candidate’s general theoretical knowledge in the discipline of food technology and nutrition and her ability to conduct scientific research independently within the computational and bioinformatic scope of her contribution. The dissertation also presents an original solution to a scientific problem. I therefore conclude

that the dissertation meets the requirements of Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science.

The comments and concerns identified in this review should be addressed during the public defence. They do not affect my overall positive assessment of the dissertation.

I therefore recommend that the doctoral committee admit Ranjini Ghosh Dastidar to the public defence.

