Trieste, 14/7/2025

Dear Evaluation Commission,

This is my report on the thesis presented by Ismail Gdamabosi entitled **"TDP-43-Metabolism Interplay in Neurodegenerative Disorders"** in the Laboratory for Translational Research in Neuropsychiatric Disorders, BRAINCITY - Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences in Warsaw. The supervisor of the thesis was **Prof. Leszek Kaczmarek, Ph.D., D.Sc.** with **Dr. Ali Jawaid, M.D., Ph.D** as auxiliary supervisor.

Evaluation of Scientific quality

This dissertation presents a comprehensive investigation into the complex interplay between TDP-43 dysfunction and cellular energy metabolism in the context of neurodegenerative disorders, particularly Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD).

Taken together, the scientific quality of the dissertation is **very high**, demonstrating a rigorous and systematic approach to systematically investigates the metabolic impact of TDP-43 dysfunction using multiple, complementary approaches including transcriptomic, metabolic, and functional analyses. Through this wide array of experimental techniques, the thesis demonstrates that TDP-43 dysfunction profoundly disrupts cellular energy metabolism.

All the sections of the thesis are quite informative, with the **Introduction** providing the background of ALS and FTLD, the role of TDP-43, and the disease-modifying effects of metabolic disorders and physical activity, setting the stage for the study's hypotheses. Following, the **Material and Methods** are highly detailed, providing sufficient information for reproducibility. It covers cell culture, genetic manipulations (RNAi, plasmid transfection, rescue experiments, mutant selection), various biochemical and metabolic assays, omics techniques (RNA-seq, qPCR, Western blot, GC-MS metabolic mapping). The **Results** are presented in several parts (I to VII) that examine specific experimental sections while the Discussion nicely Integrates all findings. Overall, the data presentation is excellent and data are consistently presented at publication-ready quality with mean ± SEM, and statistical significance is clearly denoted. Most importantly, the Results section includes summary figures that integrate complex findings into coherent models, enhancing understanding. Finally, the work provides a **Summary** and a very important section on **Study Limitations** and **Future Research Directions.** The thesis then ends with the **References & Appendices** which represent a omprehensive list of cited literature and useful supplementary information like TDP-43 sequences, primer lists, and a detailed record of scientific contributions.

Evaluation of the Scientific Content

The **Aims** are generally clear, well-defined, and highly relevant to the field of neurodegeneration. They have included to: 1. Determine the impact of TDP-43 dysfunction on cellular energy metabolism in motor neurons, 2. Compare metabolic responses across motor neurons, microglia, and neuroblastoma cells following TDP-43 dysfunction, 3. Investigate the role of cellular metabolic sensing in TDP-43-associated metabolic dysregulation, 4. Assess the influence of systemic metabolic factors on TDP-43-driven metabolic adaptations, and 5. Evaluate the translational relevance of NSC-34 TDP-43 signatures by comparing them with RNA-seq profiles from ALS and FTLD post-mortem cortex. All aims are ambitious and address critical gaps in understanding the role of metabolism in TDP-43 proteinopathies. The **Research Methodology** is robust, comprehensive, and employs state-of-the-art techniques. It utilizes three distinct mouse cell lines (NSC34 motor neuron-like, BV2 microglia, N2A neuroblastoma) to explore cell-type-specific effects and an impressive list of techniques that center on multi-omics assays, and metabilic mapping. The **Presented Results:** The results are extensive, detailed, and largely consistent, although some nuances and inconsistencies are openly acknowledged by the author. They show that TDP-43 dysfunction fundamentally disrupts cellular energy metabolism, with a distinctive impact on motor neurons. The **Discussion:** The discussion is insightful at integrating the complex findings, relating

them to existing literature, and developing a coherent "metabolic perspective" on TDP-43 proteinopathies. Finally, the **Literature** literature cited is extensive from epidemiological observations on metabolic factors in ALS/FTLD to mechanistic studies on TDP-43, mitochondrial function, and AMPK signaling and demonstrates a thorough understanding of the field.

Information about Detected Mistakes/Shortcomings, Errors, and Wrong or Inaccurate Wording

The dissertation itself provides a transparent and critical "Study Limitations" section, which is a strong point.In this section, the author explicitly states that NSC34 cells do not fully mimic mature motor neurons and that in vitro systems lack the complex cellular interactions (e.g., neuron-glia crosstalk) present in vivo. Also, the study acknowledges that it primarily captures early-stage metabolic adaptations, and long-term consequences are unresolved, suggesting the need for longitudinal studies. Finally, a crucial limitation identified was the very small sample size of ALS patient-derived RNA-seq data (n=2), which significantly reduces statistical power and generalizability.

Assessment of Whether the Dissertation Provides an Original Solution to a Scientific Problem

The dissertation provides original solutions to a significant set of scientific problems.First, the thesis provides evidence that TDP-43 dysfunction intrinsically drives a hypermetabolic state in motor neurons that makes them vulnerable to oxidative damage. Most importantly, it has shown that and systemic metabolic factors can modulate this intrinsic vulnerability. The cell-type-specific metabolic adaptations to TDP-43 dysfunction, particularly the clear divergence between motor neurons, microglia, and neuroblastoma cells, is a novel finding that helps explain the selective vulnerability observed in ALS. This could be linked with dysregulated AMPK signaling whose activation occurs even under energy-replete conditions. As a result, The thesis has already led to or is in press for peer-reviewed publications, indicating that its originality has been recognized by the scientific community.

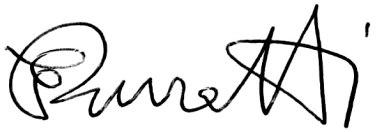
Assessment of Whether the Doctoral Dissertation Demonstrates the Ph.D. Student's Overall Theoretical Knowledge of the Field and Ability to Conduct Independent Scientific Work

The doctoral dissertation unequivocally demonstrates the Ph.D. student's overall theoretical knowledge of the field and outstanding ability to conduct independent scientific work.. In particular, yhe detailed "Future Research Directions" section reflects an awareness of current gaps, a capacity for critical thinking, and the foresight to propose relevant and innovative future studies.

Therefore, I have no hesitation in evaluating this work positively. This doctoral dissertation entitled "TDP-43-Metabolism Interplay in Neurodegenerative Disorders" meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science, and I recommend the admission of M.Sc. Ismail Gbadamosi by the Scientific Council of the Nencki Institute of Experimental Biology PAS to the next stages of the procedure for the award of the doctoral degree.

In addition, this reviewer agrees that the dissertation **goes beyond the standard and deserves to be distinguished**. The reason is that Ismail Gbadamosi's PhD dissertation is a high-quality scientific work that addressed successfully a significant problem in neurodegeneration research (ie. the interconnection between TDP-43 and metabolic dysfunction) with originality and methodological rigor.

Very best,



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Review of the doctoral dissertation of Ismail Gbadamosi

„TDP-43-Metabolism Interplay in Neurodegenerative Disorders"

The doctoral dissertation of Ismail Gbadamosi was carried out under the supervision of Prof. Leszek Kaczmarek, Ph.D., D.Sc., and the auxiliary supervision of Dr. Ali Jawaid, M.D., Ph.D. The work was completed at the Laboratory for Translational Research in Neuropsychiatric Disorders, BRAINCITY – Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences.

The dissertation addresses a very important and thought-provoking issue: the considerable individual variability in the course of amyotrophic lateral sclerosis, a devastating and currently incurable disease. The rather uncommon, direct reflection offered by the doctoral candidate at the beginning of the dissertation is noteworthy. It introduces the reader to a specific clinical situation and explains how the research concept was developed. This approach demonstrates the candidate's sensitivity to the translational relevance of the work and effectively guides the reader from the perspective of the patient to the cellular mechanisms activated in the course of the disease. The doctoral candidate points out an interesting paradox: patients with metabolic disorders such as diabetes, obesity, or hyperlipidemia often have a better prognosis and slower progression of ALS, even though these conditions are usually risk factors in other diseases. He links this paradox to changes in cellular metabolism. In the following chapters, he examines the relationship between TDP-43 - a protein whose abnormal deposits are found in both ALS and frontotemporal dementia (FTD) - and the metabolism of different cell types in the central nervous system. His long-term aim is to identify metabolic pathways that could serve as targets for future drug therapies.

The dissertation is very extensive, comprising 173 typed pages, including 56 figures, 6 tables, and 99 references. Despite its unconventional yet highly valuable introduction titled “A Tale of Two Patients”, the thesis follows a standard structure and consists of: Title Page; Table of Contents; Acknowledgements; List of Abbreviations; Scientific Rationale and Context; four Study Hypotheses; General Objective of the Study; five Specific Aims of the Study; Materials and Methods; Results presented in seven sections; Discussion; Summary; Conclusions; Bibliography; and four Appendices. The thesis is written in clear and fluent English.

The abstracts, published in Polish and English, are concise but very communicative, allowing readers to quickly understand the topic and results of the work.

The Introduction reviews how metabolic disorders can influence neurodegenerative diseases linked to TDP-43 pathology. It also explains the pathological overlap between ALS and FTD. The section then looks at metabolic changes in TDP-43 proteinopathies and gives a clear overview of TDP-43 itself, including its structure, normal functions, and the ways it becomes harmful—from loss of function in the nucleus to toxic gain of function. This part of the dissertation is informative, but it also contains some unclear points, mainly due to the inclusion of clinical aspects, which are somewhat outside the doctoral candidate’s main field of expertise. Therefore, several points in the introduction would benefit from clarification or a more careful formulation.

- It may be useful to note that FUS pathology is very rare, so as not to imply that it represents a common variant.
- The statement regarding “similar neurodegenerative mechanisms” could be considered somewhat speculative.
- The evidence linking T2DM with FTD is limited, and this point would be better presented with greater caution.
- The proposed protective mechanisms remain hypothetical (except BMI) and are not yet supported by direct causal evidence.
- Reported effect sizes for the relationship between BMI and ALS risk vary across studies; presenting them as fixed values may therefore be misleading.

- The claim that obesity strongly predicts a favorable prognosis is still debated, with some evidence suggesting a non-linear relationship between BMI and survival.

- Causal interpretations should be made carefully, as pre-diagnostic weight loss could partly account for the observed association between low BMI and ALS risk.

- The association between intense physical activity and ALS risk remains controversial, with studies reporting inconsistent results.

- The suggestion that moderate exercise exerts therapeutic benefits should be framed more cautiously, as the evidence primarily supports improvements in function and well-being rather than direct disease modification.

- The choice of Neuro2a (N2a) neuroblastoma cells as a 'non-motor neuronal comparator' raises some concerns. Because these cells are tumor-derived, their metabolism differs substantially from normal neurons, which may limit the interpretation of differences observed with NSC-34 cells. A clearer justification for this model would be valuable, and the use of additional non-transformed neuronal models could strengthen the conclusions. At the very least, differentiating N2a cells and confirming a neuronal phenotype could help to reduce the bias linked to their cancer origin.

- The rationale for using serum from animals exposed to exercise or a high-fat diet is also not fully explained. It would be helpful to clarify why serum is considered an appropriate tool to study TDP-43–related metabolic changes and how it is expected to connect systemic metabolism with cellular models.

- Finally, the use of post-mortem cortex as the human comparator for NSC-34 transcriptomes is not entirely convincing. Since NSC-34 are motor-neuron-like cells, spinal cord or motor cortex tissue would seem more directly relevant for ALS. Clarifying whether the aim was to capture general TDP-43–related signatures across brain regions, or specifically motor neuron mechanisms, would make this choice clearer.

These numerous but minor questions do not affect the overall very good quality of the Introduction.

The next part, study hypotheses, is presented in a very clear manner. However, the first hypothesis, as currently formulated, risks being too general, as numerous studies have already shown that TDP-43 dysfunction impacts glycolysis, oxidative phosphorylation, and

mitochondrial homeostasis. To avoid merely restating established findings, the authors should specify the novel aspect they intend to test — for instance, whether these effects differ across cell types, under particular metabolic challenges, or in combination with systemic metabolic cues.

The General Objective of the Study and the Specific Aims are clearly defined. The only potentially confusing aspect is the later division of the Results section, where the aims do not correspond directly to individual result parts (e.g., Aim I is explored in Parts I and II; Aim II in Part III; Aim III in Parts IV and V; Aim IV in Part VI; and Aim V in Part VII). This, however, is a minor observation and does not detract from the overall clarity of the work.

The methodology is described with sufficient detail to enable other researchers to reproduce the study and verify the results on independent material. The only few comments are mentioned below:

- there is an inconsistency in the description of the "Antibiotic-Free Medium (AFM)." The recipe includes 1% Pen/Strep, which contradicts the term "antibiotic-free." It also appears that the main difference from Complete Medium is the omission of FBS. The authors should clarify whether AFM is meant to be serum-free, antibiotic-free, or both.

- the manuscript does not specify the oxygen concentration used during cell culture. Since oxygen levels can significantly affect cellular metabolism and stress responses, this parameter should be clearly stated.

The most impressive part of the dissertation, however, is the presentation and interpretation of the results, which clearly demonstrate the doctoral candidate's fluency and expertise in this field. The results reflect a highly systematic approach to investigating activated metabolic pathways — progressing from gene-level analyses, through protein expression, functional studies, metabolic profiling, exploration of underlying mechanisms, validation of methods via reverse approaches, and finally an attempt at clinical translation. This meticulous, almost pedantic, scientific approach is truly impressive and highlights not only the candidate's diligence but also the exceptionally careful, insightful, and reliable guidance provided by his supervisors.

Expressed in a very simplified form, the doctoral candidate has obtained several important findings. The main results can be outlined as follows:

- NSC34 motor neurons display a hypermetabolic phenotype following TDP-43 knockdown, with increased glycolysis, enhanced mitochondrial respiration, ATP overproduction, and oxidative stress. Persistent AMPK activation indicates dysregulated energy sensing.

- BV2 microglia show a hyperglycolytic but adaptive response, characterized by transient oxidative stress that normalizes within 48 hours, reflecting cellular resilience.

- N2a neuroblastoma cells remain largely metabolically stable but exhibit a hypometabolic phenotype under knockdown, likely related to their cancer-derived glycolytic bias, raising concerns about their suitability as 'non-motor neuronal comparators.'

- Mutant TDP-43 expression produces only mild and selective changes across cell types, suggesting that knockdown more accurately models the predominant loss-of-function component of ALS pathology.

- Systemic factors modulate neuronal metabolic responses in a sex-specific manner, with high-fat diet and exercise serum exerting divergent effects in males and females.

- Patient comparisons confirm pathway-level overlap with ALS and FTLD tissue, reinforcing the relevance of metabolic disruption to human disease.

For the Results section, I would like to raise only few comments, organized according to its individual parts:

Part I - a minor point: In Figure 3.4, the axis labels are difficult to read due to the very small font size; enlarging the font would improve clarity.

Part III raises several points for consideration. The data on BV2 microglia are presented clearly and highlight important differences between TDP-43 knockdown and mutation: knockdown induces a transient hyperglycolytic response with accompanying oxidative stress that subsequently normalizes, whereas mutant protein expression produces minimal effects. These findings emphasize the relative metabolic resilience of microglia. In contrast, N2a cells show overall metabolic stability with only subtle biochemical changes. This likely reflects their tumor-derived, glycolysis-oriented metabolism rather than genuine neuronal resilience. The authors should clarify whether they interpret these results as evidence of true resilience or rather as a limitation of the model.

Part VI. The experiments demonstrate that systemic metabolic factors strongly shape how motor neurons respond to TDP-43 knockdown, with clear sex-specific effects. Knockdown increased both glycolysis and mitochondrial respiration overall, but serum from high-fat diet animals suppressed glycolysis in both sexes. In males, it also reduced mitochondrial function, whereas in females it enhanced it — similar to the effect of serum from exercised animals. These findings highlight that systemic metabolism and sex-dependent factors modulate neuronal energy responses, an important consideration for understanding variability in ALS progression. The use of pooled serum is a valuable way to model systemic metabolic states, but because serum contains a complex mix of metabolites, hormones, and signaling molecules, it is difficult to identify which specific factors drive the observed effects. Follow-up studies using defined components (e.g., insulin, fatty acids, cytokines) would help clarify the underlying mechanisms.

In the Discussion, the emphasis is placed mainly on summarizing the results, while somewhat less attention is given to critically engaging with conflicting literature. The section could be further strengthened by integrating relevant clinical studies, including previous trials of metabolic modulators in ALS (e.g., CoQ10, creatine, dexpramipexole, TUDCA, ketogenic diets), to provide a more robust translational perspective.

In the Summary section, the following concerns arise:

Ad. 2 The conclusion that N2a cells display only a marginal hypometabolic response may be confounded by their tumor-derived origin. The observed profile could reflect intrinsic features of neuroblastoma metabolism rather than representative adaptations of non-motor neurons. This limitation should be explicitly acknowledged.

Ad. 4 The interpretation of serum effects remains somewhat vague. Although differences between HFD- and VE-derived serum are reported, the underlying mediators (e.g., lipids, hormones, cytokines, myokines) are not identified, which makes it difficult to attribute the observed changes to specific systemic signals. The authors should either propose a mechanistic framework or explicitly acknowledge that the serum experiments are primarily phenotypic and exploratory rather than mechanistically resolved.

The section on Future Research Directions is a particularly interesting part of the dissertation, as it opens the space for constructive suggestions and the consideration of potential solutions:

- the use of iPSC-derived motor neurons provides valuable patient-specific insights; however, organotypic spinal cord slice cultures from TDP-43 transgenic animals could represent an equally, if not more, informative model for validating metabolic findings. Such ex vivo preparations preserve the native neuronal–glial microenvironment and regional architecture, thereby enabling the study of TDP-43–related metabolic alterations in a context closer to in vivo physiology. The authors may wish to consider this as a complementary approach.

- several of the proposed 'future directions' (e.g., AMPK modulation, mitochondrial enhancers, dietary interventions) have already been tested in ALS clinical trials, with mostly disappointing or inconclusive results (e.g., CoQ10, creatine, dexpramipexole, olesoxime, high-calorie/ketogenic diets). In addition, TUDCA and the TUDCA + sodium phenylbutyrate combination (AMX0035) have shown some positive but ultimately mixed clinical outcomes. It would therefore be important for the authors to acknowledge this prior work and clarify how their suggested strategies provide novel insights or directions beyond these earlier efforts

Overall, the thesis is well-structured and scientifically ambitious. It integrates transcriptomics, metabolic testing, functional validation, and translational comparisons with human material. The candidate shows strong technical skills and independence in scientific reasoning, and demonstrates a solid theoretical knowledge base. He has also proven his ability to carry out scientific research.

The subject matter of Ismail Gbadamosi's doctoral dissertation is original, cognitively important, and the content of the work corresponds to the topic specified in the title. He has prepared an ambitious, original, and scientifically valuable dissertation. It convincingly demonstrates novel cell-type-specific vulnerabilities to TDP-43 dysfunction and integrates both mechanistic and systemic perspectives. The candidate shows mastery of advanced experimental techniques, independence in reasoning, and the ability to interpret complex datasets critically.

While some limitations regarding model systems, interpretive caution, and translational extrapolation remain, these do not diminish the overall merit of the work. On the contrary, they highlight promising avenues for future refinement and extension.

In summary, I conclude that the submitted doctoral dissertation meets the conditions specified in Article 187 of the Act of 20 July 2018 on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). In view of the above, I hereby request the Scientific Council of the Institute of Experimental Biology to admit Ismail Gbadamosi to the further stages of the procedure for awarding a doctoral degree.

Due to the very high substantive value of the work, verified by the publication of its results in peer-reviewed scientific journals included in the JRC list, highly rated in the assessment structure of the Ministry of Science and Higher Education, and taking into account the doctoral student's scientific activity, I request that the dissertation submitted to me for review be awarded a distinction.

Prof. dr hab. n. med. Anna Sarnowska

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Poznań, 14.09.25

REVIEW OF THE DOCTORAL DISSERTATION

The dissertation submitted by Ismail Gbadamosi, MSc., titled: *"TDP-43-Metabolism Interplay in Neurodegenerative Disorders"*

The dissertation was prepared at the Laboratory for Translational Research in Neuropsychiatric Disorders, BRAINCITY—Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences in Warsaw. Professor Leszek Jan Kaczmarek and Professor Ali Jawaid supervised the work.

Clarification of dissertation authorship. The acknowledgements section includes the following statement: "My heartfelt thanks go to Dorota Dymkowska for metabolic flux analysis, Bartłomiej Gielniewski for RNA sequencing, Sandra Binias for transcriptomic analysis, Natalia Nowak and Artur Wolny for confocal microscopy, Debadeep Chaudhury for splicing analysis, and Łukasz Bijoch for image analysis. I am also grateful to my external collaborators—Blanca Aldana (Denmark), who provided valuable lab support and expertise in dynamic metabolic mapping, and Isabel Duarte and Ramiro Magno (Portugal), whose guidance in transcriptomic analysis strengthened this work immensely." In relation to this statement by Mr. Gbadamosi, my question is about clarifying the potential contributions of other authors to each experiment in the thesis. During the dissertation presentation, please include a detailed list of "who did what" for each slide, including results and methodology.

The structure of the doctoral dissertation was organized as a manuscript with the main sections, including the introduction, aims of the study, materials and methods, results, discussion, and references. Unfortunately, it lacks section numbering, which would improve navigation in longer scientific documents like a dissertation.

The introduction explores the connections between metabolic disorders and two neurodegenerative diseases: amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), both characterized by the pathological mutations of TDP-43 and degeneration of neurons in the CNS. Mr. Gbadamosi cites several epidemiological cohort studies indicating that conditions such as high BMI, diabetes, and dyslipidemia, along with moderate physical activity, are associated with protective effects and improved survival rates in ALS and FTD. Interestingly, the literature shows an opposite trend for individuals who engage in very high levels of physical activity. The discussion refers to various studies, including works by the promoter. The reference section lists Jawaid et al., 2018a, while within the dissertation, there are citations for Jawaid et al., 2018; Jawaid et al., 2018a; Jawaid et al., 2018b. Is this the same review? The later sections cover the structure and functions of TDP-43, which regulates RNA metabolism, DNA repair, and mitochondrial function. Pathological changes involve TDP-43 shuttling between the nucleus and cytoplasm, aggregation, and resulting toxic effects. Overall, the introduction provides a comprehensive overview of the topic.

However, the main criticism concerns the dissertation's structure. The sections following the “introduction” are confusing, especially the division into hypotheses and aims, which spans nearly two pages and is overelaborate. The section includes “Hypotheses,” a brief paragraph about the “objectives of the study,” and five detailed aims that correspond to the hypotheses but are worded differently and contain more detail. The final paragraph, where Mr. Gbadamosi explains which hypotheses and aims are addressed in each of the eight parts of the results, adds further confusion. Because of the complicated division into hypotheses, aims, and parts, the “Results” section becomes difficult to follow. The subdivision into PARTS I to VIII is unfortunate, as these sections often present interconnected results that are better shown together rather than in separate segments. This issue is especially evident with the four main hypotheses and five aims, which do not match the structure of the parts. For example, parts I to III should be combined. Another issue is that the results span over 100 and contain many sections of unnecessary text that could be part of the discussion or introduction, and many could be safely removed. The writing is often overly long and repetitive, which dilutes the scientific message and makes the findings hard to understand. A more concise and organized approach would improve both accuracy and clarity. Additionally, the section

contains material and methods details, such as unnecessary descriptions of blood centrifugation. The figures and tables also have problems: Figure 2 is missing, as after Figure 1, the sequence jumps directly to Figure 3. The numbering and inclusion of tables are inconsistent - Tables 1 and 6 are missing and not listed, while Tables 3, 4, 5, 7, and 8 are present and listed, and Table 2 is included but not listed.

The methodology of the dissertations, including advanced and numerous wet lab procedures such as molecular biology, in vitro cell culture, in vivo procedures, animal tissue collection, and statistical analytics, aligns with the requirements for an outstanding doctoral thesis. The statistical analyses were described and performed correctly. The data are tested for normality with the Shapiro-Wilk test. The thesis uses unpaired t-tests with Welch's correction, which are suitable for comparing two groups when variances may differ, Mann–Whitney U tests, which are correctly used for non-parametric data, and two-way repeated measures ANOVA with Sidak's post hoc test applied for time-course (media substrate levels). The p-values are indicated in the figure descriptions. The sample size was indicated; however, the number of samples required for experiments was not always sufficient (for human samples).

The results. The structure, figures, and tables are confusing, making it difficult to follow the findings. The aims specifically inquire about the effects of TDP-43 knockdown or mutations on cellular dysfunction and how they impact different neural cells based on models resembling motor neurons (NSC-34), microglia (BV 2), and non-motor neural cells (neuroblastoma N2A). The transcriptomics data upon TDP-43 knockdown count genes dysregulated beyond + 1.5 fold change. Is it not too optimistic to perform GSEA and conclude that metabolic genes are mostly affected? Regarding the most dysregulated genes with high fold changes: upregulated include AK 7, Samsn1, Ceacam18, Arhgef18, and Isma1 (Ism1 ?), and downregulated are Snx10, Jazf1, Tas2r137, and DPY1911. None of these genes is further discussed, even though JAZF 1 and Ism 1 are heavily involved in metabolism. No protein-level validation has been conducted for these genes. The transcriptomic dysregulation of the selected metabolic genes tested later (Figure 3.5. 5) is unconvincing. Additionally, none are examined at the protein level, which is essential to draw valid conclusions. A similar issue applies to the mutation experiments, which show many strongly downregulated genes with a log fold change of 5. Are these genes connected to metabolism? They are not validated by real- time PCR. Moreover,

the expression results of the selected metabolic genes are not particularly informative, nor are they tested at the protein level. Based on these experiments, the most severe mutation, MVT, has virtually no effect on the transcriptome of metabolic genes. These transcriptomic data should be addressed during the thesis defense. Notably, TDP- 43 knockdown or mutation in NSC- 34 results in altered metabolism and physiology, while other cell types appear unresponsive. Microglia adapt through transient glycolysis activation, and neuroblastoma cells seem resistant, exhibiting hypometabolism. Overall, the findings are valuable but need consolidation; dividing into parts I to III dilutes the scientific content and narrative.

Similarly, the entire AMPA division is unnecessarily divided into PART IV and V. It shows that knockdown of TDP-43 causes transient AMPK activation, while mutation leads to prolonged AMPK activation after glucose stress. This suggests impaired energy sensing involving AMPA as a potential cellular stress mechanism in ALS and FTD. This is a significant finding as it helps elucidate the mechanisms of these diseases in the brain. Parts IV and V of the dissertation contain unnecessary content that should be reorganized, either moved to the introduction or primarily to the discussion: Part IV includes the first two and last two chapters; Part V comprises four chapters in “Effects of Glucose Manipulation on AMPK…”. Overall, about 3 to 4 pages of misplaced and overly detailed text from a total of 10 pages should be removed. Within these 10 pages, only 9 references are made to figures, leading to a high dilution of results with extra text. However, references are only made to Figure 6, with no references to Figure 7 in the results. Since the disturbances in AMPA in response to TDP-43 dysregulation provide key mechanistic insights into the thesis, several statements emphasize the background of these findings, notably that AMPA is an important sensor of energy and metabolism. Unfortunately, these claims lack supporting references or citations.

The experiments in part VII investigate extrinsic systemic metabolic factors that might contribute to TDP-43-induced metabolic dysregulation in motor neuron-like cells. To do this, he fed mice a high-fat diet or exposed them to voluntary exercise. Afterwards, he collected serum from the mice to treat NSC-34 motor neurons and then measured the glycolytic and mitochondrial responses in these cells. The setup, as described, seems somewhat unusual because the neuronal cells are exposed to HFD serum as a stimulant, whereas in real life, they do not interact with serum; instead, they are exposed to cerebrospinal fluid. Therefore, my question is, why wasn't HFD cerebrospinal fluid from the HFD or VE mice used to stimulate

the cells? If obtaining it was too complicated, why wasn't the proteinless brain homogenate used instead? This could have been considered in addition to the serum experiments. Another question concerns the medium used to stimulate cells with serum. The sex differences in responses to stimulation by female and male serum suggest some thought about the origin of the NSC-34 cells. I checked this issue and found that the genotype of both lines used to generate the hybrid is XY, hence the hybrid is also XY. Therefore, it is very likely that the sex differences observed are due to this issue and the hormones present in serum. The estrogens promote the shift from glycolysis towards oxidative phosphorylation, which Mr. Gbadamosi observed with female serum. Therefore, in my opinion, using females in these experiments may not be sound enough.

Part VII is exploratory, and its analysis, as reviewed in the thesis, does not add significantly new knowledge; it mainly provides extra silhouette data and text. It does not include any example gene or other interesting details. For example, in table 9.1, there are FTD vs. control comparisons, with 139 upregulated and 449 downregulated genes, which is reasonable. Meanwhile, for two ALS vs. control samples, there are 1292 upregulated and 1051 downregulated genes, showing high variability among samples. Because the study used valuable postmortem tissue with only two ALS patients in the experimental design, this experiment should not be performed until more ALS brains are collected, let alone included in the dissertation.

My last question concerns the therapeutic implications of the new knowledge gained in the thesis. Is it possible to identify strategies based on the findings that can be immediately implemented? Are there any novel blood metabolites useful for diagnostics? Are there drugs that can be quickly tested off-label? If possible, please suggest potential substances and mechanisms related to the thesis results.

Final thesis assessment, including publication achievements: Mr. Ismail Gbadamosi's academic record is generally above average. His doctoral dissertation features a section listing numerous research articles, which is impressive for an early-career PhD student. These articles were peer-reviewed, revised, and published in scientific journals indexed in Journal Citation Reports. His scientific activities include both first-author papers and collaborative publications. Additionally, he has coauthored books and conference materials. Mr. Gbadamosi presents strong evidence regarding how TDP-43 levels or mutations influence disturbed

metabolic phenotypes. Moreover, the thesis emphasizes that the AMPA receptor mediates metabolism sensing in motor neurons. These findings have clear implications for ALS and FTD diagnosis, disease progression monitoring, and therapy. The data in the thesis are significant and advance current knowledge, reflecting substantial effort. However, many hypotheses and the conclusions are speculative, such as the fitness-dependent risk factor or patient-specific trajectories, without support in experimental evidence. Writing the thesis also required considerable effort. Nonetheless, the data presentation is weak, making it difficult to follow. The hypotheses and aims are verbose and awkwardly expressed. Many sections of the text are redundant, wordy, and should be relocated to the discussion and introduction. Additionally, there are missing citations, figures, and tables.

Conclusions

Mr. Ismail Gbadamosi addressed a significant scientific problem and conducted original research with substantial effort. The research is crucial to understanding the pathogenesis of ALS and FDA. I declare that Mr. Gbadamosi's doctoral dissertation meets the conditions outlined in art. 187 sec. 1-4 of the Act on Higher Education and Science of July 20, 2018 (Journal of Laws of 2018, item 1668, as amended). Based on my positive assessment of his research in his thesis, I believe Mr. Gbadamosi should be admitted to the final stages of the doctoral process.

Professor Maciej Figiel