Predicting the Significance of Genetic Variants in Parkinson's Disease

Authors: Priank Dasgupta[1*]& Abrar Choudhury[2]

Affiliations

[1] Eastlake High School
[2] University of California, San Francisco

* Corresponding author
E-mail: priank@outlook.com (PD)

Word count: 3682

Funding: The author(s) received no specific funding for this work.

Conflicts of interest: All authors declare no conflict of interest.

Abstract:

Objectives:
Different genetic variants in the human genome can give rise to distinct forms of Parkinson's Disease. This study aims to determine the significance of all variants in the human genome linked to Parkinson's Disease based on empirically validated pathogenicity data.
Methods:
We condensed the list of variants in the dbNSFP/ClinVar database to only those variants associated with Parkinson's Disease, used 2 interpretation criteria from the American College of Medical Genetics and Genomics/Association for Molecular Pathology's clinical guidelines to pair each empirically validated variant, with a variant with unknown significance, and finally analyzed for pathogenicity.
Results:
The analysis of variant pairs based on ACMG/AMP criteria revealed strong correlation (R-squared = 0.9943) between CADD scores for PS1 variants. However, for PM5 variants, the lower correlation (R-squared = 0.118) indicates poor predictive value. This indicates that the PS1 criteria is effective in predicting the significance of VUSs based on empirically validated data. Furthermore, we analyzed the COMT gene to predict how treatment of Parkinson's Disease can be affected.
Conclusion:
The results indicate that, for >1000 SNVs exome wide, we can accurately predict the significance of VUSs based off empirically validated data. On this basis, patients with currently unvalidated mutations can gain information about their likelihood of developing Parkinson's Disease. Further research is required to understand the clinical presentation of each variant.
Keywords:
Parkinson's Disease, PS1/PM5, Pharmacogenetics, VUS (Variant of Unknown Significance), ClinVar, American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)

Introduction:

The importance and frequency of genetic testing is vital for Parkinson's disease, and genetic testing is only expected to increase in this field. Although the amount of genetic testing is increasing for Parkinson's Disease, research on the differences between variants to create personalized treatments have not materialized. Much like Ductal Carcinoma, Ovarian Cancer, and Cystic Fibrosis, different mutations can cause varying severities of the same disorder(Bergeron & Cantin, 2019; Phillips, n.d.) . Despite extensive research done on the etiology and treatment of Parkinson's Disease, the role of genetics in disease onset and progression remains poorly understood.
Different genes harbor large variations contributing to human illness, meaning that identified genetic variability in patients allows doctors to treat the molecular causes of some diseases

directly (Bergeron & Cantin, 2019; Hamburg & Collins, 2010; Phillips, n.d.). This targeted approach improves treatment efficacy, reduces adverse reactions, and opens new possibilities for innovative therapies and prevention of genetic disorders The identification and targeting of genetic variability will continue to shape medical practice, leading to better patient care and outcomes (Bonifati, 2005; Cook, Schulze, Naito, et al., 2021). Timely and specific genetic identification in Parkinson's Disease is especially important as many patients develop symptoms after years of having the disease; there is no effective treatment at this stage of Parkinson's as irreversible neural damage has already occurred (Becker et al., 2002).

Pharmacogenetics is the relationship between a person's specific genome and how they respond to different medications. In this project, we are aiming to increase the understanding of different variant possibilities, and their impacts, in Parkinson's Disease so that a pharmacogenetic treatment approach can be taken. Current research on Parkinson treatment shows that different demographics respond differently to treatment. For example, some populations have a higher frequency of the Val158Met functional polymorphism in the COMT gene. This synonymous single nucleotide variant (sSNV) confers low enzymatic activity to Levodopa, the enzyme that produces dopamine (Corvol & Poewe, 2017; Hornykiewicz, 2010; Kalinderi et al., 2011; Meyer, 2000). This evidence suggests that different Parkinson's patients may benefit from personalized treatment plans after having conducted a genetic analysis to determine which specific variants are present.

Additionally, determining the significance of genetics in Parkinson's Disease can aid in family planning so that steps can be taken in order to reduce the risk of passing pathogenic variants on to future generations (Sellbach et al., 2006; Siderowf & Stern, 2008). The knowledge of possible disease carriers can push families to make informed decisions, for example, opting for pre-implantation genetic diagnosis in order to determine if a fetus has inherited a pathogenic variant. Furthermore, the growing trend of genetic testing among the general public (Ancestory.com, 23andMe) makes this study much more relevant in detecting genetic variants early (Grosse & Khoury, 2006; Tabarrok, 1994). If a variant is detected early, and its pathogenicity can be predicted, then the impact the variant can be better understood for the treatment of the disease (Becker et al., 2002; Bonifati, 2005; Cook, Schulze, Kopil, et al., 2021; Cook, Schulze, Naito, et al., 2021; Meyer, 2000). However, the shortcoming of current genetic testing for variants is the lack of research behind whether these mutations are meaningful in Parkinson's Disease. In other words, detecting variants without knowing the meaning of them is ineffective. As a result, this study's aim is to leverage existing information about variants that have been empirically verified in order to predict whether these variants of unknown significance (VUSs) have a meaningful impact on one's disease onset or progression.

We quantified the pathogenicity of over 1000 variants by conducting a comparative analysis between pathogenic/likely pathogenic (P/LP) classified variants, benign/likely benign (B/LB), and VUS based on the American College of Medical Genetics/Association for Molecular Pathology's ACMG/AMP criteria. Specifically, we focused on two criteria: PS1, which denotes the presence of the same amino acid substitution, and PM5, which indicates the occurrence of the same

codon substitution. By employing these criteria, we established a robust assessment of variant pathogenicity (Bhat et al., 2023). Through our comprehensive analysis, we intended to contribute valuable insights into the functional impact and significance of these variants, further enhancing our understanding of genetic diseases and facilitating more accurate diagnosis and treatment decisions. We determined the pathogenicity of unknown variants in Parkinson's Disease in order to further the pharmacogenetic understanding of this disease. It is our goal to prove that it is possible to predict the significance of variants in Parkinson's Disease by comparing them to empirically validated data on a molecular basis.

Materials and Methods:

Variant Subsets and Annotations:

We parsed through all SNVs and possible nsSNVs in databases ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and dbNSFP (http://database.liulab.science/dbNSFP) respectively. Each variant was assigned one of 2 classifications based on clinical studies: P/LP or B/LB. For variants that had no empirically validated data, VUS was assigned to them, indicating that their significance on health outcomes was uncertain or undetermined. These variants assigned with VUS were the focus of our study. We condensed the list to only 1070 variants which were related to Parkinson's Disease then annotated them based off their classification (Figure 1). Variants were classified as Parkinson's related by looking at their phenotype list and gene symbol.

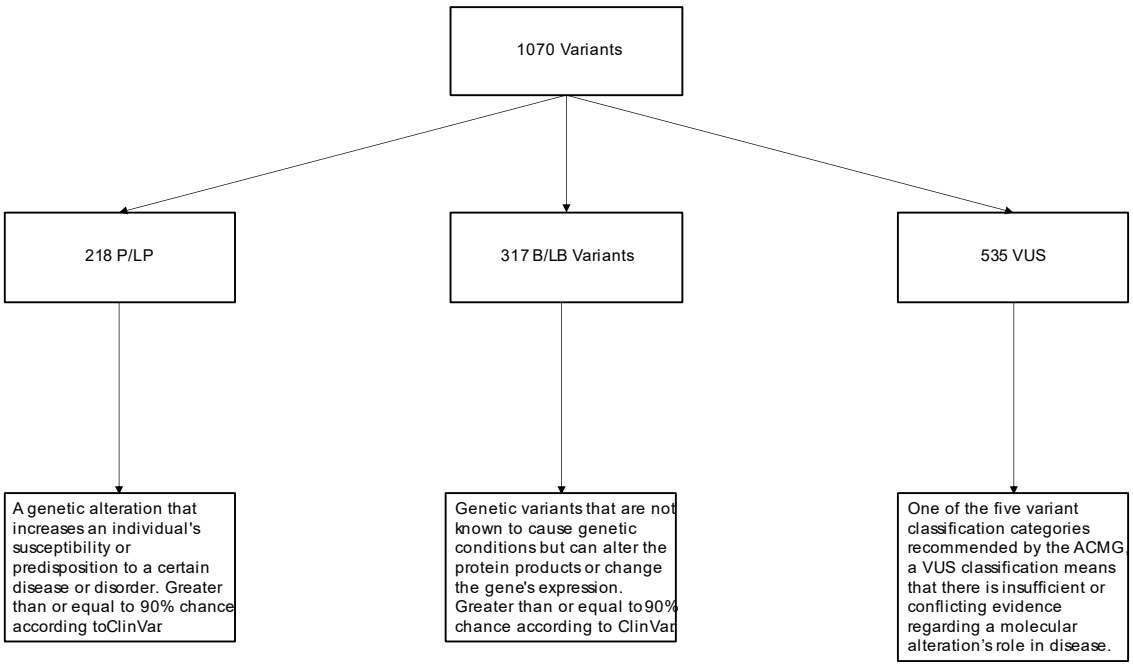


Figure 1. 1070 variants were annotated into three different categories.

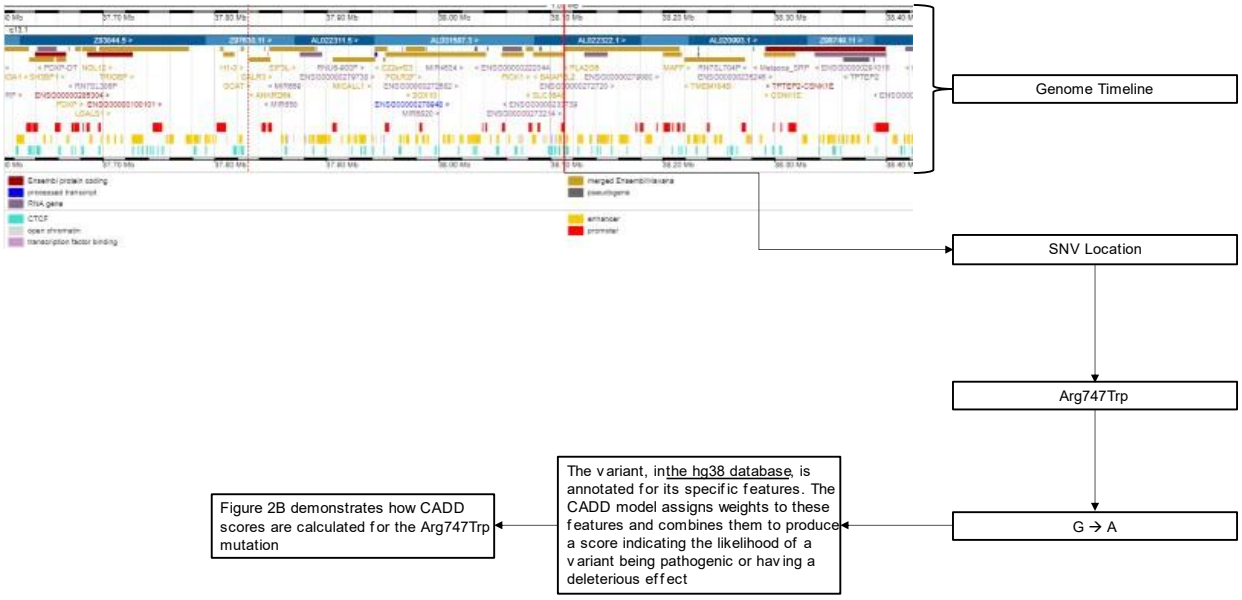
ACMG/AMP Criteria Interpretation for Variant Pairing:
We utilized two evidence models from the ACMG/AMP criteria in order to pair a VUS with a variant of empirically validated significance based on molecular variant information. We used PS1, same amino acid substitution, and PM5, same codon substitution (Table 1).

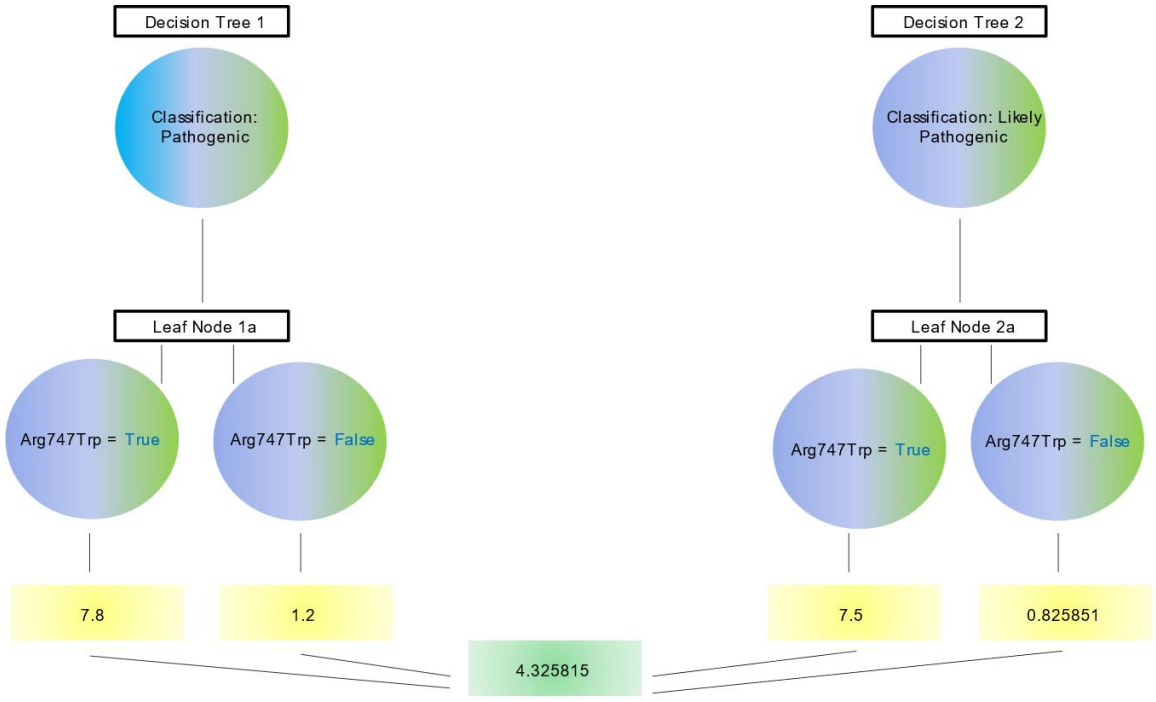
PS1: Strong Evidence	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val->Leu caused by either G>C or G>T in the same codon
PM5: Moderate Evidence	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys

Table 1. Examples of PS1 and PM5 criteria
Looking at both missense and nonsense variants, we parsed through ClinVar two times in order to pair all the variants. We paired 2 variants together if they both caused the same amino acid substitution or if they caused a different amino acid substitution, but in the same codon. By doing this, we could predict the significance of previously unknown variants.

Quantifying Significance:
After predicting the significance of all the variants associated with Parkinson's disease by pairing them with an empirically validated P/LP or B/LB variant, we wanted to quantify the pathogenicity of every variant in order to understand the impact that form of Parkinson's Disease may have. We utilized score predictors that were based on the hg38 reference genome data (Pan et al., 2019). Specifically, we employed two widely used score predictors, namely CADD (Combined Annotation Dependent Depletion) and REVEL (Rare Exome Variant Ensemble Learner) (Ioannidis et al., 2016; Rentzsch et al., 2019; van der Velde et al., 2015). These predictors leverage comprehensive genomic annotations and machine learning algorithms to estimate the pathogenicity or functional impact of genetic variants (Figure 2).

A


Genome Timeline
SNV Location
Arg747Trp
G → A
The variant, inthe hg38 database, is annotated for its specific features. The CADD model assigns weights to these features and combines them to produce a score indicating the likelihood of a variant being pathogenic or having a deleterious effect
Figure 2B demonstrates how CADD scores are calculated for the Arg747Trp mutation


Decision Tree 1
Classification: Pathogenic
Leaf Node 1a
Arg747Trp = True
Arg747Trp = False
7.8
1.2
Decision Tree 2
Classification: Likely Pathogenic
Leaf Node 2a
Arg747Trp = True
Arg747Trp = False
7.5
0.825851
4.325815

B

Figure 2. The CADD algorithm locates a variant and calculating significance. A. Identifying variant locus and type. B. Random Forest model visualization.

For the initial round of quantification, we employed a rigorous procedure to assess the functional consequences of genetic variants using the CADD scoring framework. The CADD ensembl program uses relevant genomic features, such as conservation scores, protein domain information, and evolutionary conservation data to annotate variants. Subsequently, these annotated features were fed into the CADD algorithm, which leverages machine learning techniques to calculate variant-specific CADD scores. The CADD scores provided a quantitative measure of the potential pathogenicity or functional impact of each variant. Higher CADD scores indicated a greater likelihood of a variant being deleterious or significantly affecting protein function.

An example using the clinically classified P/LP variant 22-38112541-G-A shows, in a much simpler form, how each node contributes to a sub score which are all combined to create a single prediction (Figure 2A). After the locus is identified, the CADD algorithm uses a Random Forest model to quantify pathogenicity.

Random forests are an ensemble learning technique that combines multiple decision trees to enhance prediction accuracy. The model estimates variant pathogenicity, providing valuable insights into the functional impact of genetic variants in various contexts. For example, for the clinically classified P/LP variant 22-38112541-G-A, two decision trees, pathogenic and likely pathogenic, are classified twice using a Boolean value in the Leaf Node. The algorithm then quantifies a score based on numerical data associated with the variant.

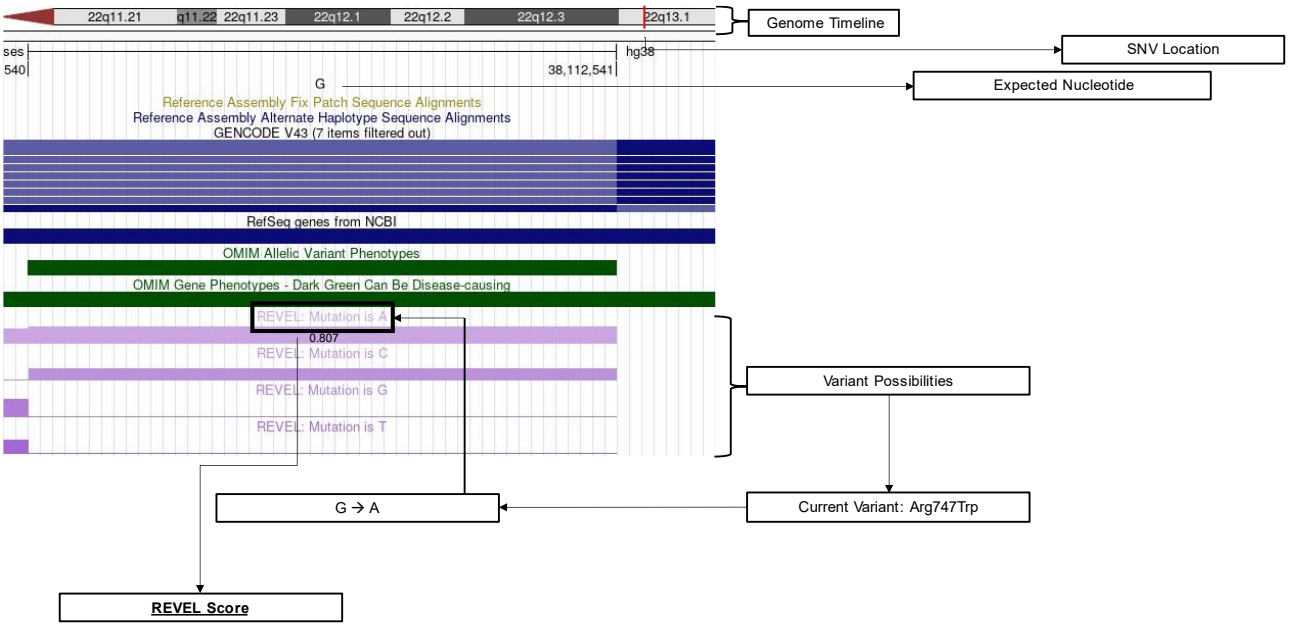


Figure 3: The REVEL program demonstrates the different variant possibilities for one locus

In addition, we also used the REVEL (Rare Exome Variant Ensemble Learner) scoring methodology to evaluate the potential impact of genetic variants on protein function. REVEL is a well-established algorithm that integrates multiple features, including conservation scores, protein-specific annotations, and variant-level functional predictions, to generate variant-specific scores. The REVEL scoring algorithm utilizes an ensemble learning approach, leveraging a machine learning model trained on a large dataset of pathogenic and benign variants. By incorporating diverse genomic features and training on a comprehensive dataset, REVEL can provide a more comprehensive assessment of variant pathogenicity. The resulting REVEL scores provide a quantitative measure of the likelihood of a variant being deleterious or affecting protein function. Higher REVEL scores indicate a higher probability of functional impact or pathogenicity.

REVEL combines 13 other predictive algorithms to deduce the impact for several different versions of one SNV. Specifically for the variant 22-38112541-G-A, the SNV is a G→A substitution according to hg38 information, and the cumulative REVEL score is 0.807, indicating high chances of pathogenicity. Using the 2 scoring methods, we were able to predict and quantify the significance for >500 VUS with 2 layers of confidence because of the ACMG/AMP criteria pairing (Figure 3).

The incorporation of REVEL scores in addition to CADD scores provides valuable benefits in variant analysis. While CADD scores focus primarily on functional impact, REVEL scores offer a more comprehensive evaluation by considering additional features such as conservation, allele frequency, and disease-specific information. This multi-faceted approach enhances the assessment of variant pathogenicity, enables cross-validation of findings, and prioritizes disease-relevant variants.

Results:

CADD Score Comparison:

Table 2

	P/LP variants	B/LB variants	**Total**
PS1 sites	28 **(5.24%)**	13 **(2.43%)**	41 **(7.68%)**
PM5 sites	190 **(35.6%)**	303 **(56.7%)**	493 **(92.3%)**
Total:	218	316	534

Table 2. Distribution for variants among the CADD score calculations.

The vast difference in proportion between PM5 sites (92.3%) and PS1 sites (7.68%) within the genome is notable in our study (Table 2). PM5 sites encompass a significantly larger portion, indicating that variations affecting the same codon substitution occur more frequently compared to variants resulting in the exact same amino acid substitution. This disparity may arise from the inherent genetic variability present in different codons, allowing for more diversity in codon-level variations. In contrast, PS1 variants, representing specific amino acid changes with established pathogenic effects, are comparatively rarer but offer valuable insights into well-documented and clinically significant variants. Despite their lower prevalence, the deliberate inclusion of PS1 variants enables focused investigations into their functional consequences and disease implications, contributing to a comprehensive understanding of the genetic landscape under study.

We plotted our CADD analysis of PM5 and PS1 criteria based on both P/LP and B/LB to gain insights into the potential functional impact and pathogenicity of the variants. Specifically, we aimed to determine whether variants meeting PM5 and PS1 criteria, in the context of P/LP and B/LB classifications, exhibit similar CADD scores and thus share comparable functional effects. This analysis allowed us to explore the potential concordance between the predicted functional impact of P/LP and B/LB variants with PM5 and PS1 evidence, shedding light on the potential pathogenicity of the variants in question.

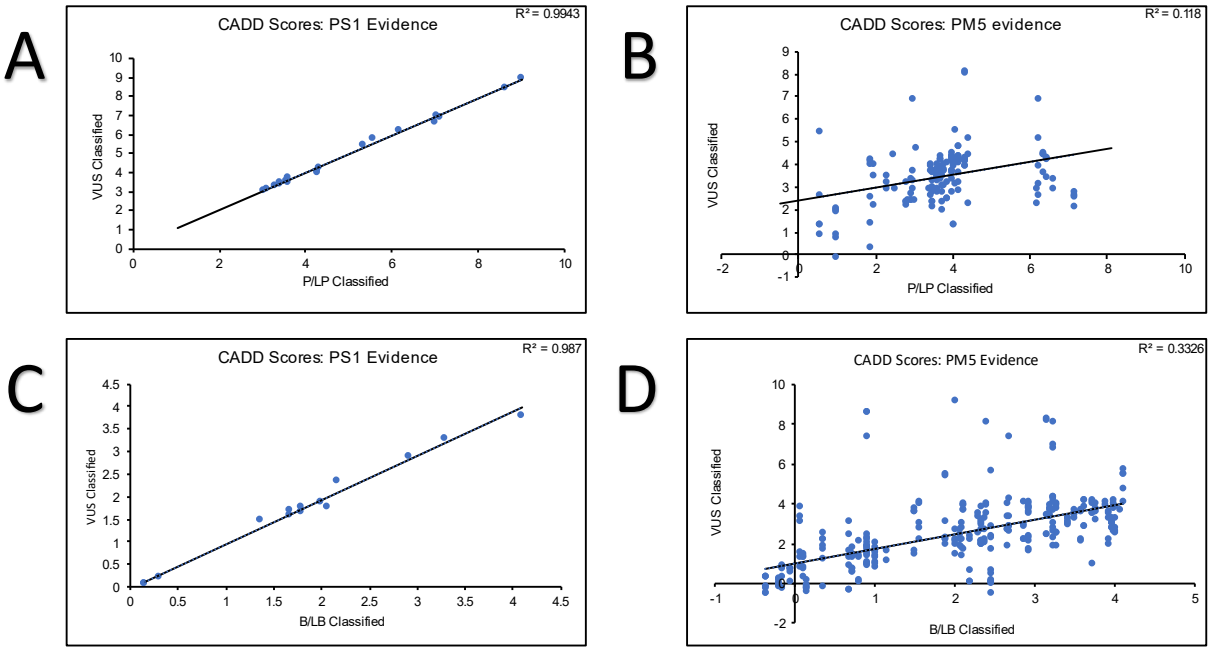


Figure 4. Correlation between CADD scores of classified and VUSs. A. PS1 evidence for P/LP variants. B. PM5 evidence for P/LP variants. C. PS1 evidence for B/LB variants. D. PM5 evidence for B/LB variants.

Our analysis revealed a high R-squared value of 0.9943, indicating a strong relationship between the P/LP and VUS classified variants and their significance in Parkinson's disease based on PS1 guidelines (Figure 4). The high R-squared value indicates that PS1 criteria is effective in identifying variants that have a significant association with Parkinson's disease.

The CADD scores between the P/LP variants and the classified variants correlate closely to each other, suggesting that the VUS variants share similar functional effects and contribute to nearly identical Parkinson's Disease impacts. Overall, the correlation between the points indicates strong evidence for pathogenicity and significance among VUSs that have been annotated to match with P/LP classified variants. PS1 criteria, based on its evidence for predicting pathogenicity in Parkinson's disease, provides strong evidence.

We found that correlation for the PM5 evidence was significantly lower, having an R-squared value of 0.118, compared to the PS1 variant pairs (Figure 4B).

The lower R-squared value in the graph indicates a weaker correlation between the CADD scores of the P/LP variants and their corresponding VUS variants when connected through PM5 evidence. This suggests a higher level of variability in the functional effects and potential disease impacts of the VUS variants in relation to the P/LP variants. The spread of CADD scores for the VUS variants indicates a wider range of potential functional consequences, highlighting the complexity and diverse nature of these variants within the context of PM5 evidence.

Comparing the PM5 data to the PS1 data, we observe a substantial difference in the R-squared values. The difference in R-squared values between PS1 and PM5 evidence was 0.8761, an incredible 88.1% difference in predicted accuracy.

The correlation coefficient (r) for the PS1 graph was found to be 0.9971, indicating an extremely strong positive correlation. Conversely, the correlation coefficient for the PM5 graph was 0.343, reflecting a weaker positive correlation.

Additionally, the coefficient of determination (R^2) can be interpreted as the proportion of variance in the dependent variable (VUS CADD scores) that can be explained by the independent variable (P/LP CADD scores). For the PS1 graph, the high R-squared value of 0.9943 indicates that approximately 99.43% of the variance in VUS CADD scores can be accounted for by P/LP CADD scores. In contrast, the lower R-squared value of 0.118 for the PM5 graph suggests that only approximately 11.8% of the variance in VUS CADD scores can be explained by P/LP CADD scores.

These calculations reinforce the considerable disparity between the PM5 and PS1 data. The high R-squared and correlation coefficient values in the PS1 graph demonstrate a strong relationship and predictability, while the lower R-squared and correlation coefficient values in the PM5 graph signify a more diverse and less predictable functional impact among the VUS variants.

In conclusion, the contrasting R-squared values (PS1: 0.9943 and PM5: 0.118) indicate a significant difference in the strength of the correlation and predictability of the functional effects between the two variant groups. These findings highlight the importance of considering different criteria and evidence when interpreting variant significance.

In the context of Parkinson's disease, the lower R-squared value observed in the graph comparing the PM5 variants' CADD scores with the corresponding VUS variants raises interesting implications. The wider range of CADD scores and higher variability among the VUS variants connected through PM5 evidence suggest a diverse spectrum of potential functional consequences in relation to the disease. This variability may reflect the complex genetic landscape and multifactorial nature of Parkinson's disease. While the PS1 variants exhibit a stronger correlation and potentially more predictable functional impact, the diversity of CADD scores among the VUS variants associated with PM5 evidence highlights the need for further exploration and research to elucidate their specific contributions to Parkinson's disease. Overall, genetic variants, like those that occur in the COMT gene, are significant to different outcomes of Parkinson’s Disease, and with the amount of possible SNVs in the gene, it’s vital to predict if a SNV occurring in a gene will have any significant impact on the development of Parkinson’s Disease.

REVEL Score Comparison:

Table 3:

	P/LP variants
PS1 sites	17 **(44.7%)**
PM5 sites	21 **(55.3%)**
Total sites	38

Table 3. Amount of REVEL scores

Due to lack of comprehensive REVEL scores for all variants, we only looked at a subset of REVEL score in analysis.

Exactly like the CADD scores, we plotted the REVEL scores for P/LP variants against their VUS counterparts for both PS1 and PM5 evidence. Since REVEL incorporates 12 scoring algorithms other than CADD, these numbers give a different angle on the evidence for pathogenicity for different variants.

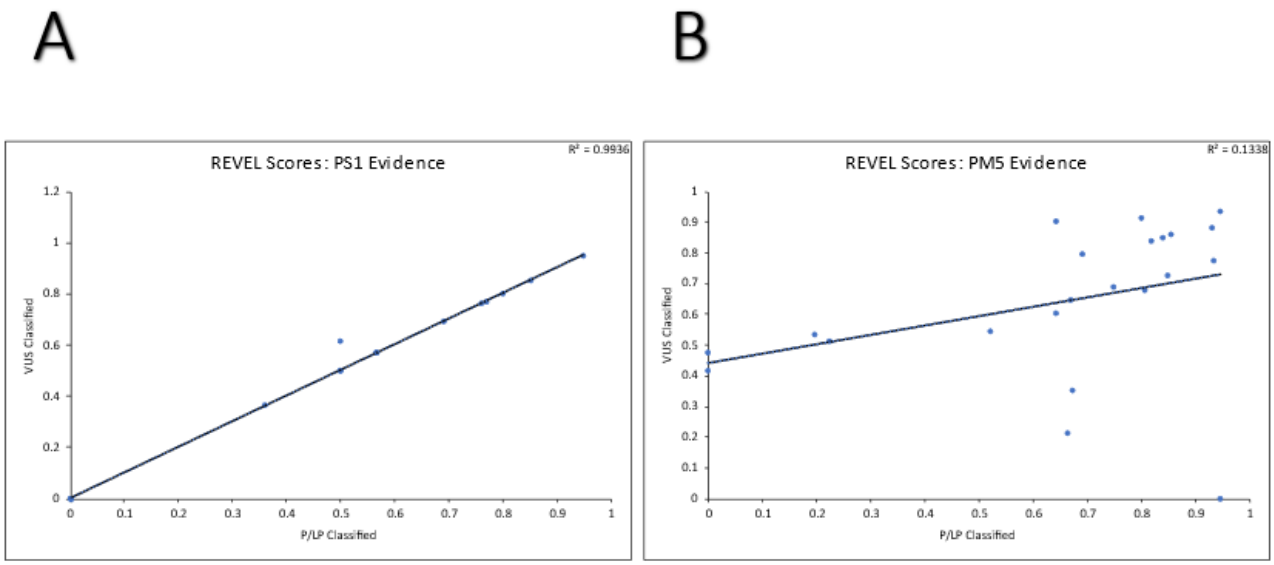


Figure 5. Correlation between REVEL scores of classified and VUSs of P/LP variants. A. PS1 evidence. B. PM5 evidence.

Analyzing the REVEL scores graph, we can delve into the calculations to gain deeper insights into the correlation between P/LP and VUS variants.

The calculated correlation coefficient is 0.9968 for the PS1 variants. This high correlation coefficient indicates a strong positive correlation between the REVEL scores of the P/LP variants and their corresponding VUS variants within the PS1 criteria.

Similarly, for the PM5 variants, with an R-squared value of 0.1338, the correlation coefficient is 0.3659. This lower correlation coefficient reflects a weaker positive correlation between the REVEL scores of the P/LP variants and the VUS variants within the PM5 criteria.

These calculated correlation coefficients reinforce the observation that the PS1 variants exhibit a stronger relationship and higher predictability in terms of their functional impact, as indicated by the REVEL scores. In contrast, the PM5 variants show a lower correlation and greater variability, suggesting a wider range of potential functional consequences among the VUS variants.

We then isolated one important gene in determining the progression of Parkinson's Disease, COMT, and examined the significance of different SNVs to further prove the relevance of determining what specific variants a person has in order to personalize their treatment.

COMT Gene Variant Analysis:

The COMT gene and its polymorphism, specifically the COMT Val158Leu variant (19-35039140-G-C), have significant impacts when it comes to Parkinson's Disease. As a key player in dopamine metabolism, the COMT enzyme is responsible for the degradation of dopamine in the brain. Although the impact of the Val158Leu variant has been studied, its counterpart, Val158Met's (19-35039140-G-A) significance is unknown. We leveraged the procedures described above to predict the significance that the Val158Met variant has on Parkinson's Disease and its impact on treatments such as levodopa.

In the context of Parkinson's disease, the impact of the COMT polymorphisms on levodopa treatment is particularly relevant. REVEL scores were run on both variants, since they were related based on ACMG/AMP criteria, to predict if the significance of variant 19-35039140-G-A Val158Met could be predicted based on the empirically validated 19-35039140-G-C Val158Leu.

	Variant Name	REVEL Scores
P/LP Classified	19-35039140-G-C **Val158Leu**	0.598
VUS Classified	19-35039140-G-A **Val158Met**	0.663

Table 3. REVEL Scores for variant pair in COMT gene.

The P/LP classified Val158Leu variant's REVEL score of 0.598 places it within the moderate to high confidence range for pathogenicity, corroborating its classification as pathogenic or likely pathogenic. The VUS variant Val158Met exhibits a similar REVEL score, further validating the predictive capacity of using classified variants to assess VUS significance. The comparable REVEL scores between the known pathogenic variant and the VUS variant in the same tier range highlight the potential utility of utilizing established variant classifications to infer the functional impact of VUS. This finding strengthens the notion that the analysis of classified variants can serve as a valuable tool in predicting the pathogenicity of VUS, facilitating more precise and personalized genetic evaluations in clinical settings.

The Val158Met VUS variant's REVEL score fell within the same tier range as the known pathogenic Val158Leu variant. By leveraging established variant classifications, clinicians can gain insights into the potential pathogenicity of VUS variants and tailor treatment approaches accordingly. Understanding the genetic basis of levodopa response and the interplay between

COMT polymorphisms and treatment outcomes can pave the way for precision medicine strategies in Parkinson's disease.

Val158Met and Val158 Leu both lead to more rapid dopamine degradation. This could result in fluctuations in dopamine levels after levodopa intake, causing variations in motor response and potentially leading to the development of motor complications, such as dyskinesias. Understanding the impact of the COMT polymorphisms on dopamine metabolism and levodopa response is essential for personalized treatment strategies in Parkinson's disease. It highlights the significance of genetic factors in individual variations of drug responses and emphasizes the need for tailored therapies to optimize the management of Parkinson's patients based on their specific genotypes.

Conclusion:

This research demonstrates that empirically validated SNVs can be used to accurately predict the pathogenicity of VUSs. This research also clearly illustrates the importance of identifying specific SNVs in one's human genome, but also raises the question of the pharmacogenetic approach that can be taken to treat a Parkinson patient based on their specific circumstances.

A limitation to consider as part of our project is the heavy reliance on CADD and REVEL. CADD is entirely based and trained upon currently known pathogenic variants and their annotations, leading the algorithm to sometimes be influenced by the representation and coverage of pathogenic variants in the dataset. Similarly, REVEL relies on selective accuracy weighting which can introduce biases and uncertainties. Additionally, REVEL's, unlike CADD's, algorithm will not incorporate every variant in its calculation because it incorporates 13 different algorithms, all with different requirements.

Our research suggests additional research into the pharmacogenetic field of Parkinson's Disease. We recommend practitioners to increase the amount of genetic testing and quantifiability.

Considering these calculations, we can conclude that comparing the REVEL scores of P/LP and VUS variants provides insights into the potential significance of the variants. The higher R-squared and correlation coefficient values in the PS1 group demonstrate a stronger correlation and more predictable functional impact. In contrast, the lower R-squared and correlation coefficient values in the PM5 group signify greater heterogeneity and variability within the VUS

variants. These calculations highlight the differences between the two criteria in terms of their ability to detect and predict the functional significance of variants.

In summary, incorporating the calculated correlation coefficients for REVEL scores enhances our understanding of the relationship between P/LP and VUS variants. The analysis reveals that PS1 variants demonstrate a stronger correlation and higher predictability, while PM5 variants exhibit greater variability and heterogeneity. These findings underscore the significance of considering multiple criteria and evidence when assessing variant significance. By leveraging established variant classifications, such as the Val158Met VUS variant, we gain insights into the potential functional impact of variants in Parkinson’s Disease. This comprehensive approach contributes to the growing field of precision medicine, enabling tailored treatment strategies based on individual genetic profiles, ultimately advancing our understanding of variant significance and guiding personalized management of Parkinson's disease.

Acknowledgments

We appreciate the support from Abrar Choudhury, MD, PhD for the mentorship throughout this paper.

References

1.Becker, G., Müller, A., Braune, S., Büttner, T., Benecke, R., Greulich, W., Klein, W., Mark, G., Rieke, J., & Thümler, R. (2002). Early diagnosis of Parkinson's disease. *Journal of Neurology*, *249*(3), iii40–iii48. https://doi.org/10.1007/s00415-002-1309-9

2.Bergeron, C., & Cantin, A. M. (2019). Cystic Fibrosis: Pathophysiology of Lung Disease. *Seminars in Respiratory and Critical Care Medicine*, *40*(6), 715–726. https://doi.org/10.1055/s-0039-1694021

3.Bhat, V., Adzhubei, I. A., Fife, J. D., Lebo, M., & Cassa, C. A. (2023). Informing variant assessment using structured evidence from prior classifications (PS1, PM5, and PVS1 sequence variant interpretation criteria). *Genetics in Medicine*, *25*(1), 16–26. https://doi.org/10.1016/j.gim.2022.09.009

4.Bonifati, V. (2005). Genetics of Parkinson's disease. *Minerva Medica*, *96*(3), 175–186.

5.Cook, L., Schulze, J., Kopil, C., Hastings, T., Naito, A., Wojcieszek, J., Payne, K., Alcalay, R. N., Klein, C., Saunders-Pullman, R., Simuni, T., & Foroud, T. (2021). Genetic Testing for Parkinson Disease: Are We Ready? *Neurology: Clinical Practice*, *11*(1), 69–77. https://doi.org/10.1212/CPJ.0000000000000831

6.Cook, L., Schulze, J., Naito, A., & Alcalay, R. N. (2021). The Role of Genetic Testing for Parkinson's Disease. *Current Neurology and Neuroscience Reports*, *21*(4), 17. https://doi.org/10.1007/s11910-021-01100-7

7.Corvol, J.-C., & Poewe, W. (2017). Pharmacogenetics of Parkinson's Disease in Clinical Practice. *Movement Disorders Clinical Practice*, *4*(2), 173–180. https://doi.org/10.1002/mdc3.12444

8.Grosse, S. D., & Khoury, M. J. (2006). What is the clinical utility of genetic testing? *Genetics in Medicine*, *8*(7), Article 7. https://doi.org/10.1097/01.gim.0000227935.26763.c6

9.Hamburg, M. A., & Collins, F. S. (2010). The Path to Personalized Medicine. *New England Journal of Medicine*, *363*(4), 301–304. https://doi.org/10.1056/NEJMp1006304

10.Hornykiewicz, O. (2010). A brief history of levodopa. *Journal of Neurology*, *257*(2), 249–252. https://doi.org/10.1007/s00415-010-5741-y

11.Ioannidis, N. M., Rothstein, J. H., Pejaver, V., Middha, S., McDonnell, S. K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., Cannon-Albright, L. A., Teerlink, C. C., Stanford, J. L., Isaacs, W. B., Xu, J., Cooney, K. A., Lange, E. M., Schleutker, J., Carpten, J. D., … Sieh, W. (2016). REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *The American Journal of Human Genetics*, *99*(4), 877–885. https://doi.org/10.1016/j.ajhg.2016.08.016

12.Kalinderi, K., Fidani, L., Katsarou, Z., & Bostantjopoulou, S. (2011). Pharmacological treatment and the prospect of pharmacogenetics in Parkinson's disease. *International Journal of Clinical Practice*, *65*(12), 1289–1294. https://doi.org/10.1111/j.1742-1241.2011.02793.x

13.Meyer, U. A. (2000). Pharmacogenetics and adverse drug reactions. *The Lancet*, *356*(9242), 1667–1671. https://doi.org/10.1016/S0140-6736(00)03167-6

14.Pan, B., Kusko, R., Xiao, W., Zheng, Y., Liu, Z., Xiao, C., Sakkiah, S., Guo, W., Gong, P., Zhang, C., Ge, W., Shi, L., Tong, W., & Hong, H. (2019). Similarities and differences between variants called with human reference genome HG19 or HG38. *BMC Bioinformatics*, *20*(2), 101. https://doi.org/10.1186/s12859-019-2620-0

15.Phillips, K.-A. (n.d.). *Immunophenotypic and Pathologic Differences Between*.

16.Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., & Kircher, M. (2019). CADD: Predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Research*, *47*(D1), D886–D894. https://doi.org/10.1093/nar/gky1016

17.Sellbach, A. N., Boyle, R. S., Silburn, P. A., & Mellick, G. D. (2006). Parkinson's disease and family history. *Parkinsonism & Related Disorders*, *12*(7), 399–409. https://doi.org/10.1016/j.parkreldis.2006.03.002

18.Siderowf, A., & Stern, M. B. (2008). Premotor Parkinson's disease: Clinical features, detection, and prospects for treatment. *Annals of Neurology*, *64*(S2), S139–S147. https://doi.org/10.1002/ana.21462

19.Tabarrok, A. (1994). Genetic testing: An economic and contractarian analysis. *Journal of Health Economics*, *13*(1), 75–91. https://doi.org/10.1016/0167-6296(94)90005-1

20.van der Velde, K. J., Kuiper, J., Thompson, B. A., Plazzer, J., van Valkenhoef, G., de Haan, M., Jongbloed, J. D. H., Wijmenga, C., de Koning, T. J., Abbott, K. M., Sinke, R., Spurdle, A. B., Macrae, F., Genuardi, M., Sijmons, R. H., & Swertz, M. A. (2015). Evaluation of CADD Scores in Curated Mismatch Repair Gene Variants Yields a Model for Clinical Validation and Prioritization. *Human Mutation*, *36*(7), 712–719. https://doi.org/10.1002/humu.22798