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The Differences in Significance of MDM2 Missense Mutations and TP53 Missense Mutations in Ovarian Epithelial Tumors

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ABSTRACT

TP53 is a tumor suppressor gene that encodes the p53 protein, which promotes apoptosis, triggers DNA repair, and prevents unregulated cell growth by acting as a transcription factor for genes. This study tests the difference in pathogenicity using driver mutations in MDM2 and TP53, specifically in ovarian epithelial tumors (OETs). To see which mutations affect tumor growth more than the other, specifically in OETs, missense mutation datapoints and information regarding the impact of the genetic variant in both MDM2 and p53 were collected. Datasets from these systems contained comprehensive data that was analyzed through three different tests: Mann-Whitney U Test, Bootstrapping Test, and Permutations Test. The results from this study help offer a new foundation of understanding on the level of pathogenicity in individuals who have both MDM2 mutations and TP53 mutations in OET. After all three tests were conducted, it was concluded that the results helped prove the hypothesis that missense mutations affecting p53 prove to be more pathogenic than missense mutations affecting MDM2.

INTRODUCTION

The feedback system that regulates p53, a protein that promotes apoptosis and prevents unregulated cell growth, relies heavily on the TP53 gene responsible for its translation, as well as its primary negative regulator, MDM2. TP53, located on chromosome 17, is transcribed into mRNA and subsequently translated to become the p53 protein. Because TP53 dictates the production of p53, mutations within this gene significantly impair the cell's ability to arrest the cell cycle or initiate apoptosis, which in turn allows pathogenic cells to thrive.

Similarly, mutations in MDM2 can be just as detrimental. As the protein regulator that acts as p53's primary inhibitor, an overabundance of MDM2, likely caused by gain-of-function mutations, leads to a stark decrease in p53 activity. As a consequence, missense mutations in both TP53 and MDM2 severely compromise cellular regulation, which would yield dangerous impacts that frequently drive oncogenesis.

Through this research, we aim to compare the oncogenic effects of missense mutations in MDM2 versus TP53, specifically focusing on their roles in the pathogenicity of ovarian epithelial tumors.

An important distinction to make within this study is the primary challenge faced in conducting a comparative analysis, given the heterogeneity of the genomic databases and their differing scaling systems used to quantify mutational pathogenicity. Because our methodology relies on predicting the impact of genetic variants across diverse computational models, the derived data yields inherently theoretical results.

However, despite the yielded theoretical results, they remain significant and applicable to clinical and research settings. Predictive databases like CADD, CancerVar, and PolyPhen-2 aggregate real-world genomic data, which allows us to accurately simulate and quantify the effects of these genetic variations without the need for testing.

This research paper seeks to evaluate such results by looking at the following question: What is the significance of MDM2 missense mutations and TP53 Missense Mutations in ovarian epithelial tumors and which has a stronger impact on pathogenicity? Firstly, the paper provides an overview of factors that play a role in this pathway. Then, the methodology details the robust framework that details the data collection process and isolates the comparative carcinogenicity of MDM2 and TP53 mutation through statistical analysis. Ultimately, the findings from the computational analysis are connected to tumor severity and are interpreted with note of study limitations.

BACKGROUND

Despite thorough published research on MDM2 and p53, there is limited comprehensive review of MDM2 and p53 in relation to other factors and one another. As in this section, it is critical to focus on providing a holistic overview of ovarian epithelial tumors, p53 and MDM2 activity, and their relation to tumor growth prior to delving into the experimental process.

TP53 and p53 Relationship, Functions, and Mutations

TP53 is a tumor suppressor gene on chromosome 17, specifically the 17p13.1 band. The gene is transcribed into mRNA, then translated into protein p53, which promotes apoptosis, triggering DNA repair, and preventing unregulated cell growth. p53 is a transcription factor (TF), which means that it binds to specific genes in the promoter region to recruit or block RNA polymerase. The recruitment of RNA polymerase promotes protein production of that gene.

A singular gene usually has multiple TFs; p53 induces gene transcription in cells in response to DNA damage, thus maintaining genomic stability. p53 is usually activated in the early S-phase with the synergy of RBP-Jκ and C/EBPβ-2, DNA binding factors that activate or repress p53 gene expression by binding, or not binding, to the same site p53 would attach to on the promoter (Reisman et al. 2012). If the mutation is severe, p53 may initiate apoptosis by upregulating Bcl-2 family members, such as BAX or PUMA,

pro-apoptotic effectors. In order to successfully conduct apoptosis, p53 must overcome the antiapoptotic threshold.

There are many factors that play into p53 overcoming the antiapoptotic threshold in order for the protein to function properly. p53 expression levels are directly proportional to proarrest and proapoptotic genes, so factors that affect the proportionality of that could either cause p53 activation to cross the antiapoptotic threshold too much or too little (Kracikova et al. 2013). Factors that could do that include posttranslational modifications on p53, such as phosphorylation and acetylation (Chen 2016).

p53 is affected by UV radiation as it induces DNA lesions, which cause breaks in the double-stranded DNA, making p53 production much more difficult and disrupting genomic stability (Williams & Schumacher, 2016). Additionally, the DNA-binding factor, RBP-Jκ, is affected by various transforming proteins (Ansieau 2001), meaning that they have the potential to alter the way in which this binding factor interacts with p53, which can either increase or decrease p53 activation. C/EBPβ-2 is made up of protein isoforms: LAP1, LAP2, and LIP. The ratio in which these isoforms (LAP: LIP) interact must be maintained in order to properly regulate growth and development (Zahnow 2011). This means that factors affecting that ratio can create a domino effect and disrupt C/EBPβ-2 binding, causing p53 activation to be affected. Furthermore, p53 works with Bcl-2 family members to regulate apoptosis, but this pathway can be affected by cellular energy status or the activation of other signaling pathways (Qian et al. 2022), which can change the manner in which apoptosis is executed by p53.

Deleterious mutations on the TP53 gene will lead to (relative) inefficiency in the p53 protein. TP53 is one of the most commonly mutated genes in cancer patients, with up to 50% of individuals with cancer having a mutated TP53 gene (Olivier et al. 2010). Such mutations not only lead to the loss of tumor suppressive functions but also gain-of-function abilities that would promote oncogenesis, such as resistance to chemotherapy, deregulation of metabolic pathways, invasive capabilities, and increased metastatic potential. TP53 mutations are one of the most common in ovarian cancer, with about two-thirds being missense mutations. This is the first type of mutation we will be evaluating in terms of tumor growth and offering an empirical view of our results on ovarian cancer.

Our ongoing methodological research will have a focus on the specific mutations TP53 R248W and TP53 R273H. TP53 R248W is a missense mutation that alters a cytosine nucleotide to thymine; TP53 R273H is also a missense mutation that alters guanine to adenine. Respectively, both are found at positions 246 and 273, which are hotspot locations and classified as drivers. A driver mutation identifies these genetic alterations as cancer-causing. Investigating the relative pathogenicity of each mutation can help categorize patients, create personalized treatment methods, and improve prognostic measures. The simplified version of the overall effects of a mutation in MDM2 and p53 can be found in Figure 1.

Ovarian Epithelial Tumors

Ovarian Epithelial Tumors (OET) are the most common form in which ovarian cancer can manifest, as opposed to non-epithelial tumors such as germ cell or sex-cord stromal tumors. It's important to note that such cancers are a result of multiple mutated genes over time. However, having a singular mutation leads to a higher risk of cancer or may be an oncogene. OETs are found to be started in the epithelium of the ovaries and the fallopian tubes, which are the cells that line these parts of the female reproductive system. Within the OETs, there are five subtypes in which the tumors can be classified: high-grade serous, low-grade serous, clear cell, endometrioid, and mucinous ovarian cancer (Arora et al. 2024); this only includes malignant cancerous tumors.

Serous ovarian carcinomas are divided into two types. High-grade carcinomas are the most common type of OETs. These tumor cells differ from low-grade ovarian tumors as high-grade cancer cells are fast-growing and look abnormal, unlike the normal appearance and slow-growing nature of low-grade tumor cells. Low-grade serous ovarian cancer is much rarer than its high-grade counterpart and is typically diagnosed in later stages. Endometrioid ovarian tumors account for approximately 10% of OETs, much more than low-grade ovarian tumors, but similar to the prevalence of the clear cell type. High-grade serous carcinomas are the most common among the subtypes of the OETs, followed by endometrioid ovarian cancers (Kurian et al. 2005). The mutations we evaluate will be applied to OET, allowing us to gauge the impacts of the mutation on a specific disease.

p21 Regulation in Cell Cycle Control and Cancer

p53-directed growth regulation usually depends on the induction of p21. p21 is a cyclin-dependent kinase (CDK) inhibitor. CDKs are enzymes that regulate the cell cycle by phosphorylating target proteins at cell cycle checkpoints. Because these are "cyclin-dependent", cyclin, a protein, will attach itself to this enzyme to project its activation. Once the cyclin attaches to the CDK, the CDK's shape changes, creating a depression at the bottom that will later act as the active site for the new substrate. The CDK will attach a phosphate to the substrate via the ATP used during this process. Then, the substrate will leave, with its attached phosphate group. CDK drives the progression of the cell at each of the checkpoints by preventing proteins from inhibiting cell growth. For example, at the G1/S checkpoint, CDK phosphorylates the Retinoblastoma protein, which inhibits the cell cycle from progressing until phosphorylated (Topacio et al. 2019).

When the cell is damaged, p21 expression is upregulated via the activation of p53. p21 will then bind to the CDK complex (with cyclin), preventing it from binding to the Retinoblastoma protein, and due to the lack of CDK activity, the protein will then fail to activate the genes required for the cell to progress. p53 requires p21 to release Bax in order to promote cell death; the p53/p21 complex is what regulates cell death (Kim et al. 2017). BAX and PUMA act as pro-apoptotic effectors downstream to the p53/p21 complex. Loss of p53 function would lead to reduced PUMA and BAX expression, leading to cells evading apoptosis, even when p21 levels are high. Moreover, p21 is primarily necessary for stress

responses, not unstressed processes. In non-cancerous cells, p21 is effective when there is DNA damage. However, if p53 is mutated, it cannot communicate as effectively with p21. In ovarian epithelial cells, there are detectable p21 expression levels, whereas in cells with a mutant or null p53, it was found that p21 expression was diminished (Elbendary et al. 1996). Essentially, this means that DNA damage recovery is made more strenuous when p53 is mutated, as a result of a mutation in TP53, which would lead to loss of apoptotic cell function.

MDM2 Regulation and its Role in Cancer: Structure, Mutations and p53 and MDM4 Interaction

MDM2 is a protein regulator produced from a gene located on chromosome 12, specifically at 12q13-14. The main function of MDM2 is to act as a p53 inhibitor, meaning that it manages p53 through negative regulation. Decreased levels of p53 activity are related to increased MDM2 activity, as it causes an inactivation of the apoptotic functions of p53 as well as the cell cycle arrest functions of the protein (Iwakuma & Lozano, 2003); this would be considered a loss-of-function (LOF) for the protein. Increased levels of the protein regulator are comparable to the effects of a mutation of the p53 protein, a common cause of many tumors.

Additionally, MDM2 functions as an E3 ubiquitin ligase for the mono-ubiquitination of p53, which can lead to protein degradation as MDM2 can transfer ubiquitin to p53 directly through a thiol-ester linkage. Though MDM2 is thought to be the main regulator for the p53 protein, there are other factors, such as p300, a protein that, if added to create an MDM2:p53:p300 complex, can stimulate poly-ubiquitin chains (Wu & Maki 2013), or ARF, another tumor suppressor that can induce the p53 pathway through the suppression of MDM2 activity (Brooks & Gu 2006). To add on, MDMX (also named MDM4), is another protein regulator that, though it is structurally similar to MDM2, does not have the ability to ubiquitinate p53 directly. Instead, MDM4 can help stabilize p53 if it were to experience poly-ubiquitination, but that help is dependent on the ratio in which MDM2 and MDM4 are present. If that ratio is low, both protein regulators may work together to support p53 degradation. Aside from the interaction between MDM2 and MDM4 and their collective effect on p53, MDM4 can also interact with the p300 protein to affect the tumor-suppressing function of p53 through acetylation (Brooks & Gu 2006). These additional factors play a role in the interaction between MDM2 and p53 and influence the function of p53 through either a loss-of-function (LOF) or gain-of-function (GOF).

Though p53 is impacted by MDM2, the protein also responds to cellular stress and transitions within the cell cycle, among other factors. In the case that p53 was to be influenced by such factors, the conditional responses of post-translational modifications (PTMs) may also occur. The two main PTMs that impact the MDM2: p53 interaction include phosphorylation and ubiquitination.

In the case that DNA damage occurs, the agents that cause it to signal the beginning of the phosphorylation cascade of p53. The areas where phosphorylation occurs are dependent on the type of damage that is done; for example, DNA strand breaks would most likely relate to phosphorylation of p53 at Ser15. These cascades are necessary in order to stabilize the protein and activate it through blocking the

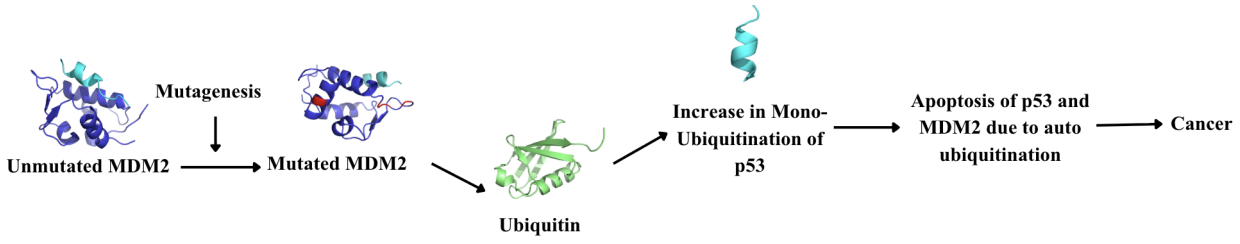
interaction between MDM2 and p53 or recruiting other proteins, such as p300; the way that p53 is stabilized is also dependent on where in p53 the phosphorylation occurs (Wu & Maki, 2013). Depending on the severity of the DNA damage, phosphorylation of p53 may not be enough, and phosphorylation of MDM2 may also have to occur to decrease p53 degradation induced by the E3 ligase activity of MDM2 (16).

p53 degradation by MDM2 occurs through the proteasome as MDM2 recruits other ubiquitin-dependent factors to deactivate p53. Additionally, MDM2 can poly-ubiquitinate p53, which marks the protein for proteasomal degradation (Hu et al. 2012). MDM4 acts as a regulator for the interactions between MDM2 and p53, as it has the power to either positively or negatively influence the ubiquitination process of p53 through the formation of heterodimers with MDM2 (Hu et al. 2012). Poly-ubiquitination of p53 can be seen as dangerous to the protein as it becomes the target of degradation, but mono-ubiquitination of the protein does not have that same effect. p53's interaction with MDM2 can lead to either process, simply depending on the circumstances and environment in which the interaction is occurring (Brooks & Gu 2006). This is the second mutation we will be evaluating and comparing to TP53 missense mutations.

Expression of Autoregulatory Feedback Loop including p53, p21, and MDM2

As established, p53 is activated in response to DNA damage. p53 is phosphorylated as a signal for protein stabilization and tumor-suppressor function activation. If not phosphorylated, MDM2 then binds to p53 and ubiquitinates it for degradation. p53 ubiquitination occurs during unstressed conditions. If p53 is not ubiquitinated and is attempting to rectify DNA damage, it will then go on, as phosphorylated, and activate p21, which will then inhibit the cyclin CDK complex from allowing the cell to further progress.
If there is a TP53 missense mutation that leads to a loss of function in p53, the p53 may not be able to effectively communicate with p21, thus the p21 would not be able to effectively inhibit cell progression. Essentially, if p53 expression is low, p21 expression will also be typically low because its stimulus is ineffective. On the other hand, a gain-of-function mutation in MDM2 is more likely to be cancerous. This is because an increase in MDM2 expression leads to a decrease in p53 expression; MDM2 tags p53 with ubiquitin, which leads to the degradation of p53 by the proteasome (Lukashchuk et al. 2007).

A



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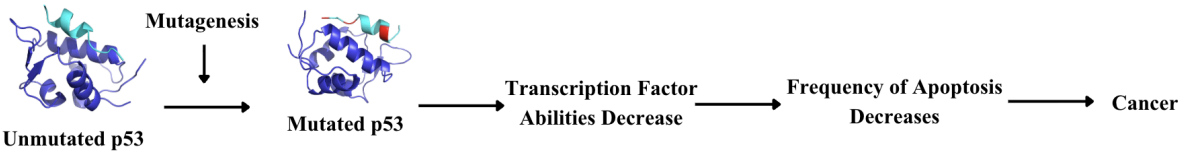

Figure 1. (A) Simplified cascade effects of a mutated MDM2 protein. MDM2 also plays a vital role in p53 activity as it regulates the protein through a negative feedback loop. (B) Simplified cascade effects of a mutated p53 protein. A mutated p53 gene leads to decrease in transcription, leading to a decrease in apoptosis and continued cell growth.

METHODS

Data Collection through Intogen

The study tests the difference between MDM2 and TP53 missense mutations. In order to generalize the results, we randomly collected 32 MDM2 and 30 TP53 missense mutation datapoints from Intogen. Some data points had a nucleotide base pair change, at the same position, that were slightly adjusted in order to successfully extract further data about those mutations from other databases. These adjustments were made transparently and consistently, ensuring the integrity of our dataset remained preserved. Our mutation sampling was based on the statistical power they hold; in other words, the identification of clinically significant mutations (which was later cross-checked through CancerVar and PolyPhen-2) and were balanced with the practical constraints of data availability.

Intogen, or Integrative OncoGenomics, specifically, is a database that looks at mutational cancer drivers and how they are involved in specific mechanisms across various tumor types. These samples are analyzed and pre-processed to ensure accurate results and use seven different methods to identify cancer drivers.

IntOGen uses a pre-process to look through various cancer samples, utilizes different methods to properly identify the cancer drivers, and then looks at the output of all methods to create a database of driver genes and their mechanisms of action.

Our study tests the differences in pathogenicity between the two populations: MDM2 and TP53 missense mutations. These mutations are driver mutations, mutations that are directly contributing to cancer development by causing uncontrolled cell growth and survival. To test the difference in pathogenicity in

these two mutations, three different systems were used to predict the impact of the genetic variants: CADD, CancerVar, and Poly-Phen 2.

Measuring Tools

Our study tests the differences in pathogenicity between the two populations: MDM2 and TP53 missense mutations. This was accomplished by using three different systems that predict the impact of the genetic variants: CADD, CancerVar, and PolyPhen-2. Each datapoint was entered into the measuring tools, allowing us to obtain a numerical score that scales the carcinogenicity of that specific mutation.

The database for CADD is a way of scoring the deleteriousness of genetic variants within the human genome. The score is meant to show an estimate for the variant to potentially become pathogenic by integrating diverse annotations into a single measure. This measure is helpful for the study due to the usage of diverse genome annotations that are scored with any possible human single-nucleotide variant (SNV), allowing researchers to gain an understanding of how different missense mutations in TP53 can affect ovarian cancer risk. CADD runs on a scale of 1-99, with a score over 20 being considered "pathogenic", and in this case, that would be cancerous.

Secondly, CancerVar is a software tool that interprets different somatic variants of tumors to help address inconsistencies between existing databases. The scoring for CancerVar is based on a four-tier system: strong clinical significance (Tier I), potential clinical significance (Tier II), uncertain significance (Tier III), and benign/likely benign (Tier IV). Next, the evidence is assigned a score based on the classification of the evidence: clinical significance, neutral, or benign. All of these evidence types are then weighted by importance and combined to obtain a final score, which is used to classify variants into one of the four tiers. CancerVar also assigns a numerical value running from 0 to 11, with 11 being the most pathogenic score. CancerVar is more specific, as it looks at the oncogenicity of the mutant within ovarian cancer specifically.

Lastly, PolyPhen-2 is a tool to help predict the impact of amino acid substitution on a human protein based on the sequence, phylogenetic, and structural information characterizing the substitution. PolyPhen-2 specifically focuses on nonsynonymous single-nucleotide polymorphisms (SNPs) located in coding regions that result in amino acid variation in protein products of genes. This runs on a scale of 0-1, with 1 being more deleterious, and predicts the impact on the protein of the gene due to an amino acid substitution.

Data Analysis

To analyze the information collected, we conducted three tests: a Mann-Whitney U Test, a Bootstrapping Test, and a Permutations Test. The tests were conducted for CADD, CancerVar, and PolyPhen-2.
The Mann-Whitney U Test indicates if the populations, MDM2 and TP53, have a significant difference. The U (Mann-Whitney's U statistic) measures the degree of overlap between the two distributions. A

large U- value indicates less overlap and a greater difference between the two groups. In the context of the Mann-Whitney U test, a small p-value suggests that the observed difference between the two groups is statistically significant. It means it's unlikely that such a difference would occur by random chance if the groups were actually the same. A large p-value suggests that the observed difference could easily happen by random chance, and thus, we don't have enough evidence to conclude that the groups are different.

Additionally, in order to ensure we weren't limited to the 62 collected data points, we ran Bootstrapping Tests, which is a test that took our sample distributions and sampled them with replacement to simulate our data as a population of 10,000, to view the difference between these populations on a greater scale. Bootstrapping stimulates resampling from the existing data, and with replacement, the study received variability, helping eliminate effects of the limited dataset.

Lastly, the Permutations Test randomized the variables and simulated a random distribution of the mean difference between the population to confirm that the results received in bootstrapping weren't due to random effects. This test uses an empirical null distribution to confirm or reject the findings of bootstrapping. The permutation test constructs the null distribution empirically rather than mathematically; it asks: if the gene labels were assigned randomly, what would the distribution of mean differences look like?

RESULTS

Our study showed us that p53 missense mutations had a greater effect on the pathogenicity of ovarian epithelial tumors when compared to missense mutations in MDM2. This was found through a series of tests. Starting with the results from the Mann-Whitney U Test for CADD scores, we proved that the two populations we considered, the missense mutations in TP53 and MDM2, based on CADD scores, are significantly different.

The Mann-Whitney U Test for CancerVar and Polyphen-2 also proved that the p53 missense mutations are more influential in pathogenicity when compared to MDM2 missense mutations. This test serves as the first step of our data analysis, proving that the results we get are actually significant. A larger U value indicates less overlap and a greater difference between the ranks of the observations in both groups. Given the high U value of 718 for CADD scores, we reject the null hypothesis that the distribution of missense mutations of TP53 is the same as that of missense mutations for MDM2. This allows us to say that TP53 CADD scores are greater than MDM2 CADD scores. The U value for Cancervar is 960, and the U value for Polyphen-2 is 806. Both of these values are high, which allows us to conclude that TP53 Cancervar scores are greater than MDM2 Cancervar scores and TP53 Polyphen-2 scores are greater than MDM2 Polyphen-2 scores. This further proves that these results are significant to 3 different measures of pathogenicity in ovarian tumors.

To further confirm validity, the bootstrapping test for the CADD scores of TP53 and MDM2 was run by doing 10,000 iterations. The difference between the data for the respective missense mutations had a value of 4, with the mean CADD score for TP53 being greater at 26.86, whereas the mean CADD score for MDM2 is 22.86. The 95% confidence interval (CI) for missense mutations in TP53 is [25.86, 27.84], differing from the CI for missense mutations in MDM2, which is [20.88, 24.72]. This means that the 95% CI of the difference for missense mutations in TP53 and MDM2 is [1.89, 6.26], making the overall bootstrap p-value 0.0001. Figure 2 serves as a visual representation of the difference in the CADD scores for the variables tested, showing a clearly greater value for TP53. Figure 3 is a visualization of the mean difference between TP53; it's important to note that 0 doesn't fall into the 95% confidence interval, which allows us to observe the trend of the difference between the scores. In other words, 0 is not a noted difference, meaning there is an observed difference between the two groups.

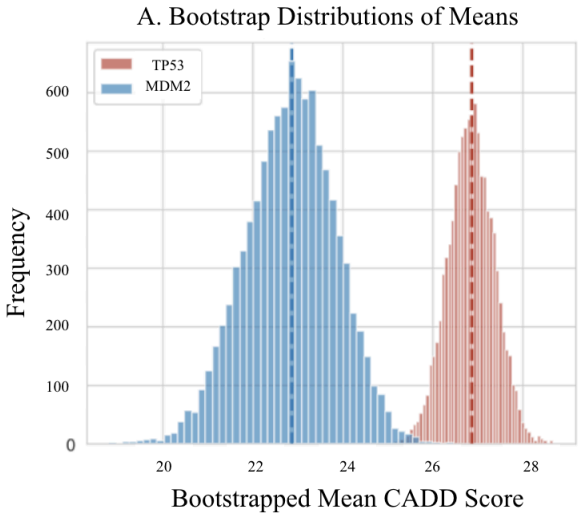

Figure 2. Bar diagram visualization of the mean CADD score for missense mutations in TP53 and MDM2, indicating higher pathogenic score for TP53.

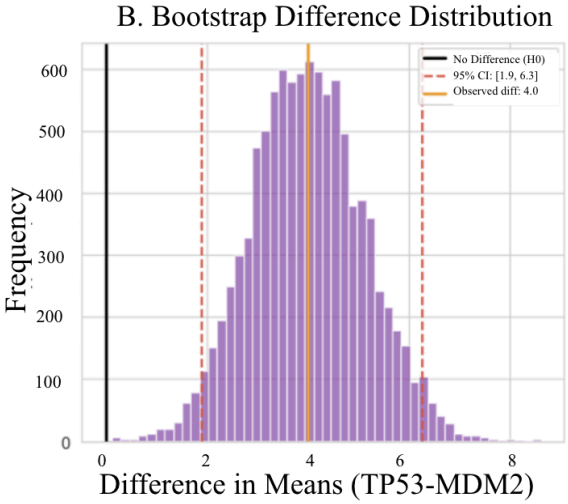

Figure 3. Bar diagram visualization of the convergent distribution of mean CADD scores for missense mutations in TP53 and MDM2, indicating a mean difference of 4.0.

The bootstrapping test for the CancerVar scores of TP53 and MDM2 was also run by doing 10,000 iterations. The mean Cancervar score for TP53 is 10.667, whereas the mean Cancervar score for MDM2 is 3.938, which is much lower than the score for TP53. The difference between the data for the respective missense mutations is 6.729. The 95% confidence interval (CI) for missense mutations in TP53 is [10.33, 10.90], which is much higher than the CI for missense mutations in MDM2, which is [3.44, 4.44]. This means that the 95% CI of the difference for missense mutations in TP53 and MDM2 is [6.14, 7.28], which resulted in the overall bootstrap p-value smaller than 10^-4. Figure 4 shows the difference in the CancerVar scores for the variables tested, clearly showing a much greater value for TP53. Whereas Figure 5 is a visualization of the mean difference between TP53 and MDM2. 0 does not fall within the 95% confidence interval, making the trend of higher pathogenicity scores for TP53 from CancerVar clear.

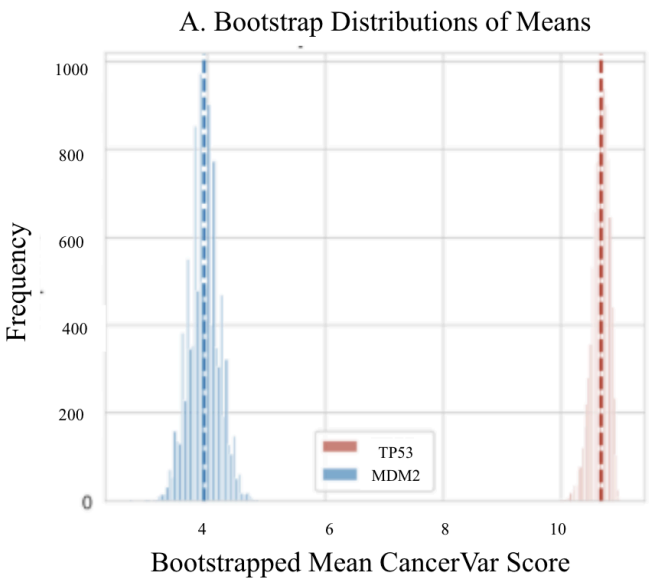

Figure 4. Bar diagram visualization of the mean CancerVar score for missense mutations in TP53 and MDM2, indicating higher pathogenic score for TP53.

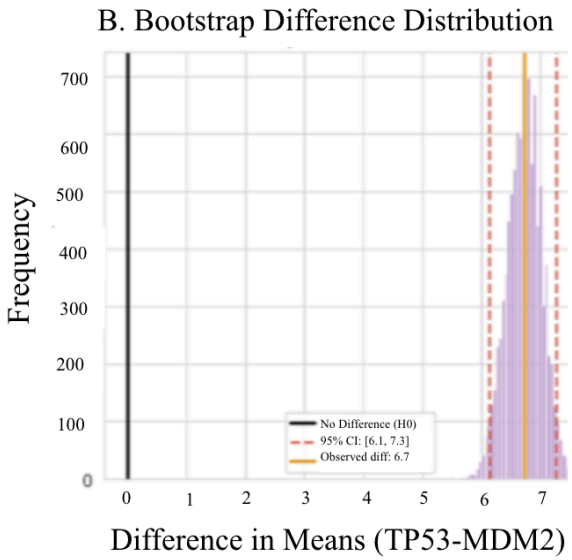

Figure 5. Bar diagram visualization of the convergent distribution of mean CancerVar scores for missense mutations in TP53 and MDM2, indicating a mean difference of 6.7.

Lastly, the bootstrapping test for the PolyPhen-2 scores of TP53 and MDM2 was also run by doing 10,000 iterations, with the mean PolyPhen-2 score for TP53 being 0.99 and the mean score for MDM2 being 0.77. This means that the difference between the data for the respective missense mutations is 0.22, and the 95% CI for missense mutations in TP53 and MDM2 is [0.97, 1.00] and [0.64, 0.89], respectively, resulting in the difference for missense mutations as [0.10, 0.35]. Similar to the overall bootstrap p-value of CancerVar, the overall bootstrap p-value is smaller than 10^-4. Figures 6 and 7 show the difference in the PolyPhen-2 scores for the variables tested and the mean difference between those variables. Given that 0 does not fall within the 95% confidence interval, the same as the other two datasets, the trend of higher pathogenicity scores for TP53 remains strong.

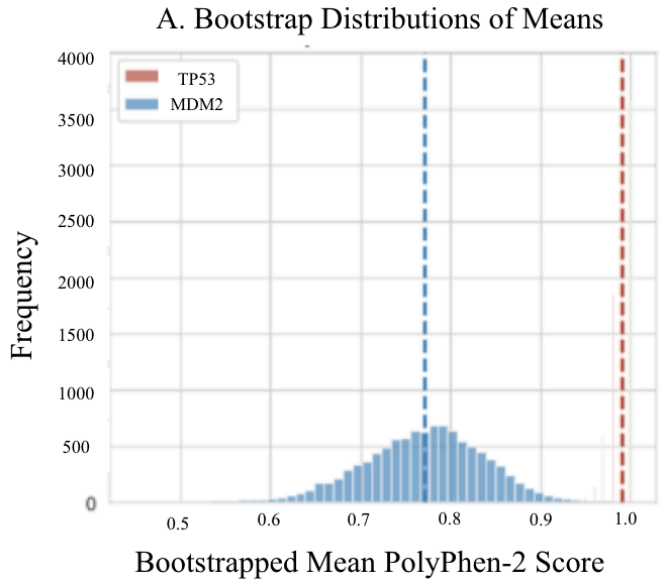

Figure 6. Bar diagram visualization of the mean PolyPhen-2 score for missense mutations in TP53 and MDM2, indicating higher pathogenic score for TP53.

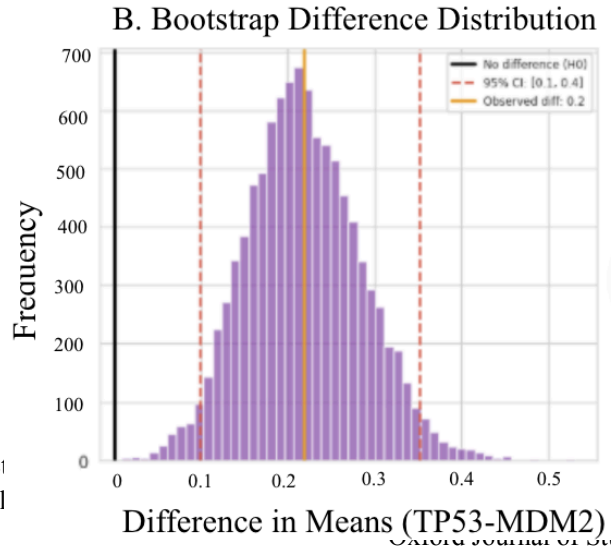

Figure 7. Bar diagram visualization of the convergent distribution of mean PolyPhen-2 scores for missense mutations in TP53 and MDM2, , indicating a mean difference of 0.2.

The Permutations test, which confirms the bootstrapping results, yielded a 'highly significant' result, which solidifies the observed trend from the bootstrapping tests for all the different databases. For this test, we used p-values of 0.0002, 0.0000, and 0.007 for CADD, CancerVar, and Polyphen-2, respectively. These numbers were derived from the permutations test and went through 10,000 permutations when analyzing the scores, which yielded highly significant results as the observed differences were all larger than 99.9% of permuted differences. This further proves that TP53 missense mutations have a greater impact than MDM2 missense mutations on overall ovarian pathogenicity, all of which can be seen in Table 8.

Score	TP53 Mean	MDM2 Mean	Difference	Boot 95% CI	Significance
CADD	26.860	22.861	3.999	[1.88, 6.25]	Yes
CancerVar	10.667	3.938	6.729	[6.14,7.28]	Yes
PolyPhen-2	0.990	0.771	0.219	[0.10, 0.36]	Yes

Table 8. Shows the significance of missense mutations in TP53 and MDM2 from various databases through a bootstrapping and permutations test. Each database had varying ranges of defining pathogenicity and was confirmed as significant.

The mutations that we analyzed as a part of this study ranged from different positions within their genomic sequences, but they were all viewed independently as a nucleic acid substitution. When we discuss the results, different factors such as those related to the positioning of the mutation may affect its severity. Something additional that we might consider is how mutations may interact with one another if there were multiple at a time. Due to these confounding variables and the limited number of mutations used in this study, our results of TP53 mutations having a greater impact on ovarian tumor pathogenicity may not apply to all scenarios. Additionally, our study doesn't favor hotspot locations in which it has been previously found for mutations to have greater pathogenicity, but it does allow for the hypothesis that similar positions in mutations can mean similar outcomes. Though we used key qualifications for generalizability, such as a large sample size per group and including a representative sample of mutations from different positions, other confounding variables make it hard to generalize, though our research suggests a very clear message on which types of mutations have a greater effect.

DISCUSSION

The p53 missense mutation leads to a loss of function in p53, in which case p53 may not be able to effectively communicate with p21, resulting in p21 not being able to effectively inhibit cell progression. On the contrary, a gain-of-function missense mutation in MDM2 is more likely to be cancerous. This is because an increase in MDM2 expression leads to a decrease in p53 expression; MDM2 tags p53 with ubiquitin, which leads to the degradation of p53 by the proteasome. These missense mutations have direct effects on ubiquitination, which results in different p53 mutations affecting the cell cycle differently, and the same goes for mutated MDM2.

The three datasets that we used for this study were sourced from Cancervar, Polyphen-2, and CADD. This study serves as a first step in recreating databases such as the ones mentioned previously, which are all consistent in their records and show a more accurate reading of the mutations in terms of their carcinogenicity. This would have to be done through understanding the root cause, in this case, the mutation that had caused the ovarian cancer to take hold and grow. Doing this would ensure that mutations are accurately rated in their effects on a patient. Though this seems to be a direct path to understanding the effects of these missense mutations, other confounding variables can determine how these mutations can form and how they are expressed in a person, as it won't always be the same. These mutations may be passed down from parent to offspring, making the mutations and resulting cancer hereditary. But many mutations also occur naturally, and there may be other genetic factors that affect how those mutations are expressed, such as possible mutations in both MDM2 and p53, or a mutation in p21. Additionally, the effect of these mutations on ubiquitination can play a role in how mutated MDM2 and p53 interact with cellular processes that inhibit cell progression. With these factors to consider, measuring the effects of such missense mutations in MDM2 and p53 can become difficult.

CONCLUSION

Our data analysis shows that there is a heavier involvement in p53 mutations when testing for deleterious activity. The dataset is comprehensive of data from ovarian cancer mutants, depicting the harmful effects on ovarian cancer patients. Our study shows that TP53 missense mutations were deemed more pathogenic than MDM2 missense mutations.

Despite limitations, this data further supports the investigation regarding ovarian tumor severity and spotlights certain hotspot mutations; it is valuable in predicting the significance of certain mutations on ovarian tumors. Our data refines our understanding of mutation-driven carcinogenicity and may supplement, but does not directly offer, the patient-based consequences of these mutations in an experimentally-analyzed clinical setting. Identifying mutations with higher predicted carcinogenic potential allows for increased accuracy in genetic testing and enables precision treatments, tailored to individual mutation profiles, contributing to better prognostic assessments.

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