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**UTICA COLLEGE  
GRADUATE STUDIES**

**MASTER’S IN DATA SCIENCE**

THESIS

**Pan-Collagen Gene Copy Number Variation Survival Analysis of Ovarian Neoplasms from the TCGA Database**

by

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**Table of Contents** Page Number

Personal Acknowledgements………………………………………………………………. ii

Table of Contents………………………………………………………………………….. iv

Abstract ……………………………………………………………………………………. v

Introduction………………………………………………………………………………… 1

Literature Review…………………………………………………………………………... 4

Methods……………………………………………………………………………………... 6

Data…………………………………………………………………………………………. 14

Results………………………………………………………………………………………. 15

Discussion…………………………………………………………………………………... 19

References…………………………………………………………………………………... 23

Appendix A…………………………………………………………………………………. 29

Appendix B…………………………………………………………………………………. 29

Appendix C…………………………………………………………………………………. 31

Appendix D…………………………………………………………………………………. 34

Abbreviations……………………………………………………………………………….. 36

**Abstract**

The Cancer Genome Atlas (TCGA) is a large longitudinal database of cancer patients with varying cancer types which has led to many studies being published. This study, also based on TCGA, performed survival analysis, log-rank scoring, and Cox proportional hazard models comparing collagen gene copy number variation in ovarian cancer patients from the TCGA repository. Data cleaning was performed initially in Alteryx Designer and secondary data cleansing in RStudio version 1.4.1103. R-Studio was used for producing Kaplan-Meier curves and IBM SPSS version 25. Stratification was performed on copy number variation showing stratification alignments of deletion, normal, and duplication. A Kaplan-Meier survival curve was performed along with a log-rank test and Cox proportional hazard model finding 3 genes: COL12A1, COL4A3BP, and COL5A3 to have statistically significance relationships between decreased survival and copy number variation abnormalities. This study supports current literature and provides evidence that collagen gene copy number variations can have varying survival outcomes in ovarian neoplasm patients.

**Introduction**

The Cancer Genome Atlas (TCGA) has analyzed over 20,000 cancer observations across 33 different cancer types (Liu et al., 2018; *The Cancer Genome Atlas Program*, 2019). TCGA has been a colossal repository of genetic information pertaining to various neoplasm types with numerous publications emanating from this database alone, including novel discoveries concerning ovarian cancer (TCGA - Ovarian Serous Adenocarcinoma Study, 2018).

Ovarian cancer has a relative survival risk of 80% and is considered the most lethal gynecological cancer type (Cancer.org, 2014). One factor to be considered in ovarian cancer outcomes is the role of collagen, as collagen is a primary component in neoplasm fibrosis (Xu et al., 2019). Collagen can also affect neoplasm behavior through tyrosine kinase receptors, integrins, and various signaling pathways. Collagen has been studied to some extent as to these various mechanisms with regards to function in ovarian tumors; however, further research is needed (Ricciardelli & Rodgers, 2006).

Collagen is an extracellular matrix protein (ECM) that forms a barrier around organs and blood vessels (Dipiro et al., 2017, p. 917). Collagen is the most abundant type of ECM protein located in ovarian tissue (Cho et al., 2015). Multiple factors contribute to the growth of tumors including ECM remodeling enzymes and growth factors. Collagen is an essential of maintaining tissue homeostasis and ECM remodeling, such as ECM stiffness and elasticity, which are implicated in ovarian tumorigenesis (Cho et al., 2015).

Collagen is also essential in regulating ovarian cell morphology through various mechanisms including cellular communication and shape (Woodruff & Shea, 2007). For example, during tumorigenesis, collagen is remodeled into thick fibrils (Cho et al., 2015). This fibrillar structure is regulated by the COL12A1 gene, which is the collagen type XII alpha 1 chain in homo sapiens (NCBI – COL12A1, 2020).

Research has also focused on drug resistance mechanisms which have been linked to functional abnormalities rather than structural (Holohan et al., 2013). For example, the COL4A3BP gene, also known as the ceramide transfer protein (CERT) gene codes for a protein which is a regulator of ceramide transport and has been implicated in multidrug resistance in certain cancer treatments such as colorectal and breast cancer (Lee et al., 2011).

Understanding ECM organization and cell morphology is essential in understanding and gaining insight into the mechanisms of malignancy (Cho et al., 2015). Copy number variation (CNV) has been linked to complex and abnormal behaviors in diseases and drug resistance (Gamazon & Stranger, 2015). CNV is defined as structural variation which alters the number of copies of certain DNA regions (Thapar & Cooper, 2013). This is important as it has been estimated that 4.8% to 9.7% of the human genome is comprised of CNVs (Zarrei et al., 2015). CNVs are also important as it provides raw genetic material for gene divergence and expansion which has contributed to the evolution of humans (Perry, 2008). While CNV contributes such a large portion to the human genetic profile, it has been shown that roughly 100 genes can be removed from the genome in a human without phenotypical consequences (Zarrei et al., 2015). These structural variations affect base pairs with a duplication or deletion and have been identified as a facilitator for genomic disease states (Sharp et al., 2005). Thus, it is important to understand CNVs and their effects on humans.

There have been issues in the past regarding studying CNV. Next Generation sequencing (NGS) has replaced other methods as NGS has increased specificity in identifying CNVs and is also able to assess and identify pseudogene sequences (Kerkhof et al., 2017). NGS has had immense effects on genetic research by allowing us to expand our knowledge base and illustrating the importance of CNV effects (Zhao et al., 2020).

The research being conducted in this study evaluates the role of genetic abnormalities in collagen coding genes that may play a role in ECM organization and cell morphology by exploring heterogenous pan-collagen gene CNV and patient survival relationships in ovarian cancer.

A Kaplan-Meier survival analysis (KM), log-rank tests, and Cox-proportional hazard modeling were performed on ovarian cancer patient CNV mutations in 55 different collagen genes from the TCGA database. There are 46 genes that directly encode for collagen in the human genome, three collagen subfamily genes which code for proteins that are collagen-like (COLEC genes), two collagen beta(1-0)galactosyltransferase genes (COLGALT genes), a collagen like subunit of acetylcholinesterase (COLQ), and two pro-collagen enhancer genes (PCOLCE genes) (Gene Group, n.d.). The null hypothesis states no statistical differences in survival in ovarian cancer patients with any of the 55 collagen genes based on CNV stratification will be identified. The alternative hypothesis indicates there is a statistical difference in survival and death rates in ovarian cancer patients regarding CNV. For survival analysis the dependent variable is the time to event, death, or censored events while independent variables are gene CNVs.

Survival analysis is essential in CNV exploration as to potentially refine medical treatment for a more individualized approach in personalized medicine and target therapy and this importance has been emphasized in literature on genetics (Gamazon & Stranger, 2015).

Hopefully, by increasing the knowledge base of CNV effects on patient survivability, a considerable impact on patient outcomes with increased survivability may be achieved. The goal of this research is to increase the knowledge base of CNV effects in hopes for eventual targeted collagen or targeted gene therapies in ovarian neoplasm treatment.

**Literature Review**

TCGA has produced multiple studies that contribute to human understanding of genetics (The Cancer Genome Atlas – Publications, 2019). Ovarian cancer has been studied with regards to collagen in the past; however, little has been investigated regarding CNV. A PubMed literature search was performed using medical subject heading (MeSH) terms for “Ovarian Neoplasms” and “Collagen”. The literature review produced 293 results involving genetic studies involving collagen and ovarian neoplasms.

Gene expression is generally the most commonly researched topic with regards to neoplasms as normalization techniques in high-throughput RNA sequencing are more available and widely used (Dillies et al., 2012). For example, gene-drug interactions in ovarian cancer have been investigated but with respects to gene expression instead of CNV (Teng et al., 2013). High-throughput RNA sequencing advances may be the reason why CNV has not been studied as often as gene expression, as the increase in RNA sequencing technology has been the focus in the scientific community. This is surprising considering that a systemic study of the human population has shown noticeable effects from CNV mutations (Shaikh, 2017).

Collagen has been shown to be involved in multiple aspects of tumorigenesis (Cho et al., 2015). One study linked collagen gene expression to metastasis promotion through the transforming growth factor (TGF-β1) signaling pathway (Cheon et al., 2013). Another linked the collagen gene COL2A1 and higher gene expression with delayed tumor relapse in high-grade ovarian cancer patients (Ganapathi et al., 2015). Drug resistance due to collagen gene expression by inhibiting molecular penetration and in turn decreasing tumor apoptosis has been studied as well in ovarian neoplasms and was suggested to involve gene expression of COL5A3 (Januchowski et al., 2016). Another study found decreased gene expression levels of XI alpha 1 collagen gene COL11A1 with decreased ovarian tumor invasiveness and oncogenic potential (Wu et al., 2013).

Some investigation has been done regarding CNV and TCGA. One study investigated gene-drug interactions with regards to CNV in glioblastoma multiforme and lower grade brain glioma (Spainhour & Qiu, 2016). Another study by the same author investigated CNV with drug exposure and survival data which allowed inference to drug-gene interactions which effect patient survival which was then put into a portal called the gene-drug interaction for survival in cancer (GDISC) (Spainhour et al., 2017).

As an important bias to mention from TCGA literature, sequence homology can create technical artifacts, which in turn affects downstream analysis and mapping which potentially also cause concerns (Webster et al., 2019). These sequence homologies are created from shared evolutionary roots where DNA regions share high similarity or can even be pseudogenes. This phenomenon has also been found in TCGA data (Buckley et al., 2017). This can possibly create bias in the genetic database where an estimated 5-10% of cancer patients may be more predisposed to certain cancer types (Garber & Offit, 2005). This is important as cancer is caused by inherited genetics and genetic changes which can be passed to offspring in which these progenitors may work synergistically (Buckley et al., 2017).

One other bias worth mentioning is that TCGA is originally built upon the Genome Reference Consortium Human Build 37 (GRCh37) or more commonly known as the HG19 build (Gao et al., 2019). This is the refence genome that is used for comparison in the TCGA, and any further research based upon this work should keep this in mind (GRCh37 – hg19 – Genome, 2009).

**Methods**

This study uses a non-parametric Kaplan-Meier survival plots, log-rank tests, and semi-parametric Cox proportional hazard modeling. This study does not assume distributions of events as it is likely not accurate to assume that hazard rates or probability of an event is constant; therefore, only non-parametric and semi-parametric methods are used.

Figure 1 illustrates the process of the data pipeline in this study.

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**Figure 1. Pipeline of TCGA Data**

The Kaplan-Meier survival curve (KM) is used in this study as it can account for censored data. Censoring is defined as the cutoff of survival time when the endpoint of interest has not been studied due to loss of follow-up (Kaplan & Meier, 1958). The Kaplan-Meier survival curve makes a couple of assumptions. The first is that all survival patients are independent of each other, or rather one patient surviving does not affect another patient surviving or having an event, in this case death. The second assumption is that censoring does not lead to an increased or decreased likelihood of events. Censoring occurs independently. The requirements to plot a Kaplan-Meier survival curve are status of last observation and time to event. If one is to compare Kaplan-Meier curves, then data regarding what characteristic is being studied must be assigned to each group (Rich et al., 2010). The Kaplan-Meier curve is considered to be the “gold standard” in survival distribution estimation and is also used instead of the Nelson-Aalen estimator in this study. This is due to the Nelson-Aalen estimator is consistently large in estimates when compared to the Kaplan-Meier estimation method (Bohoris, 1994). It is more appropriate to move into statistical details and other tests after the creation of Kaplan-Meier curves, involving hazard and survival functions, because the Kaplan-Meier curve itself cannot account for differences in covariates, such as CNV, as the Kaplan-Meier curve is considered as a visual illustration only.

The hazard function and survival functions are both integral parts of survival analysis modeling. The survival function explains via probability of a subject surviving beyond a specific point in time (Kleinbaum & Klein, 2012, p. 54). The point in time can be considered a device failure, for example, end of a study period, or in this case, death. The hazard function, otherwise known as a failure rate, is the rate of occurrence of a certain event during the given time interval. The hazard rate is also known as the Cox proportional hazard model. The survival function and hazard function are related and can be converted to each other (Schober & Vetter, 2018). For example, when the survival function is high, then the hazard rate is lower and there is increased survival, or less events take place which means they are inversely proportional.

To obtain the survival function equation, one must first look at distribution function of survival time of an individual, also known as the cumulative distribution function of *T*, notated in Figure 2 (Collet, 2015, p. 10).

*F(t) = P(T < t) = ∫0 t f(u) du*

**Figure 2: Cumulative Distribution Function.**

*T* will always be a positive number and is defined as a random variable associated with survival time. Figure 2 represents that survival time is less than some value of the variable *t,* which is defined as a specific point in time. *∫0 t f(u) du* is defined as the integral of the probability density function since any value of *t* can be a positive value. Therefore, this equation can be transformed into the survivor function shown in Figure 3 (Cox, 1972).

*S(t) = P(T > t) = 1 − F(t)*

**Figure 3: Survival Function.**

The survival function is then defined as the probability that survival time, *T*, is greater than a specific time, *t*; or that an individual survives beyond a specific time. Inversely, one can also define the survival function as the probability that one or more events take place after time *t* (Collet, 2015, p. 13; Cox, 1972). Once probabilities have been obtained, statistical significance needs to be examined through log-rank testing.

A non-parametric log-rank test is constructed by separating each event time of the groups being studied and will help show the differences between the two groups in the Kaplan-Meier survival curve. A table is created showing the number of deaths (event), *d*, number of subjects alive, and total number of subjects. This is completed at every death event and the observations are treated as independent events. This procedure is known as the Mantel-Haenszel time-stratified procedure (Collet, 2015, p. 47; Mantel & Haenszel, 1959). This procedure gives the (relative) risk ratio (RR). The RR is defined by the equation in Figure 4.

*RR = ai / na  
 bi / nb*

**Figure 4: Risk Ratio Equation**

The variable ai is defined as the number of events for stratified group *a* while the variable bi is defined as the number events for stratified group *b*. The variable *n* is the total population of each group as defined by the subscript. This equation gives the relative risk of one outcome group compared to another. For example, if group *a* was calculated with an *ai / na =* 0.1 as the numerator and *ab / nb =* 0.07 then the RR would equal 1.43. This translates to group *a* having a 43% higher chance of the event. The next step is to make sure this ratio is statistically significant.

The log-rank test will also give a probability value (p-value) for the difference between the two groups plotted on the Kaplan-Meier survival curve while assuming the null hypothesis of no difference between the two groups. The greater the gap in survival on the Kaplan-Meier survival curve between the two groups, the lower the p-value. The p-value is taken from a chi-square test where an alpha level has been set, generally 0.05, along with degrees of freedom (Pearson, 1900). Degrees of freedom is defined as the number of comparison groups minus one (Gosset, 1908; LaMorte, 2016). The log-rank chi square test is used instead of an analysis of variance (ANOVA) test in survival analysis because categorical data is being used. However, the log-rank chi square test can be considered a type of one-way ANOVA for survival analysis (Fisher, 1925; Grace-Martin, 2018). The chi square equation is shown in Figure 5.

*X2 = Σ(Ojt – Ejt)2 Ejt*

**Figure 5: Chi Square Equation.**

Ojt represents the observed number of events for the observed *jth* group over time while Ejt represents the expected number of events in the *jth* group over time (LaMorte, 2016). The chi square number for each group are the sums for the observed and expected events computed in the chi square equation, at each event time. Expected events are calculated from the proportion of events occurring at each time with data from both groups totaled. This is better defined as total number of events divided by the total number at risk. The obtained value is then multiplied by number at risk in each group. The sum of this number is Ejt. The p-value is then determined from the chi square table of critical values where a statistically significant finding is where the chi square value is greater than the corresponding critical value on the table (Fischer, 1925). Generally, the hypothesis is considered statistically significant if the chi square value is greater than 3.84 which corresponds to being less than the alpha value of 0.05. This is the same alpha value for this investigation. Once events have been plotted, and differences have been shown to be statistically significant, one should model the hazard or risk through the Cox proportional hazard model.

The Cox proportional hazard model is a regression and is considered the most utilized regression model in survival analysis (Chilamkurthy, 2020; Cox, 1972). This modeling technique allows researchers to investigate relationships between covariates and survival time. The model will allow a hypothesis about survival being equal or different to the data to be tested and is considered a natural extension of the log-rank test (Tibshirani, 1982). The Cox proportional hazard model allows the hazard to change over time but assumes that the hazard ratio is proportional or constant. For example, if the data presented a hazard ratio of male to female with males being twice as likely of an event than a female, it would assume this ratio is constant over time, or that the risk for the male is the same compared to a female at any point in time. Therefore, the Cox proportional hazard model is considered a semi-parametric model. However, assuming a constant ratio for the hazard model is considered unrealistic in the health sciences but allows for easier interpretation of the data as this research hasn’t assumed any distributions (Kennedy, 2019; Zweiner et al., 2011).

The Cox proportional hazard model also makes other assumptions (Cox, 1972). Much like the Kaplan-Meier survival curve, the first assumption is that censoring does not lead to an increased or decreased likelihood of events, or rather that the censoring is non-informative. It also assumes that survival times are independent which is defined here as a patient surviving is not dependent on another patient surviving or having an event. Censoring generally will affect survival curve shape primarily when many censored observations happen at a specific point in time leading to large flat lines in the survival model (Prinja et al., 2010). The Cox proportional hazard model also assumes that the baseline hazard is unspecified and that the treatment variables do not change over time. The most important assumption is that the survival curves do not cross each other (Kennedy, 2019; Zweiner et al., 2011). This assumption can be met by using the Kaplan-Meier survival curve and investigating to see if survival curves cross (Zweiner et al., 2011). If they do not, then this assumption has been met. The last assumption is that the log hazard rate is a linear function of the variables, much like logistic regression, where the log odds are the linear function of the variables.

The Cox proportional hazard model is expressed via the hazard function which is defined as the cumulative risk of an event occurring by time, *t*. The simple form of the hazard function equation is shown in Figure 6 (Collet, 2015, p. 13).

*H(t) = − log S(t)*

**Figure 6: Hazard Function.**

The hazard function also illustrates the cumulative number of expected events that occur from time zero until a specified time, *t*, and encapsulates the risk of death up until time *t* and is communicated through the hazard ratio.

The hazard ratio is the exponential parameter estimate of proportional hazard models, or two groups with a hazard functions, which may then be used to approximate the ratio of hazard rates between a comparison or control group and a treatment group (Bradburn et al., 2003). The hazard ratio is similar to the relative risk ratio as discussed earlier, although not the same (Sutradhar & Austin, 2008). Relative risk ratio does not factor the timing of the event like hazard ratio. The hazard ratio is evaluated by looking at the values which signifies if the hazard ratio is higher or lower than the comparison or control group.

Another way of defining the hazard ratio is through the equation in Figure 7 (Bradburn et al., 2003).

*H(t) = H0(t) \* exp( b1X1 + b2 X2+…bi Xi )*

**Figure 7: Hazard Function Expanded**

H(t) is defined as the hazard function, which is determined through variables *X 1, X 2,… Xi.* H0(t) is defined as the baseline hazard which means that this is the hazard if all covariates of *X1, X2,… Xi* are equal to zero. The variable *t* is the specified survival time and *b1, b2,…bi* are notated as the coefficients, or hazard ratios, which explain the impact of covariates *X1, X2,… Xi.* Thus, the Cox proportional hazards model may be written as a multiple linear regression of the log of hazard with the variables *X1, X2,… Xi*. H0(t), which is the baseline hazard, can be roughly interpreted as an “intercept”. However, this “intercept” will vary over time, thus, it is not considered to be the same type of intercept seen in standard linear regressions. When coefficients *b1, b2,…bi* are greater than zero, as *X1, X2,… Xi* increase in value, the survival likelihood decreases, or the likelihood of an event increases. If a hazard ratio is greater than one, then covariates are positively correlated with an event happening and thus, hazard ratios greater than one are negatively correlated with survival time (Bradburn et al., 2003).

**Data**

Patient clinical and survival data was obtained from TCGA database under the TCGA-OV project (ovarian cancer). CNV data was obtained from The Broad Institute of MIT and Harvard, which is part of TCGA, from the ovarian cancer archives in the form of comma separated value (CSV) files (*Broad GDAC Firehose*, 2016). The original files were not modified for data integrity purposes. Data was initially cleaned and joined using Alteryx Designer (Version 2019.4.8.22007) and data cleaning workflow can be referenced in Appendix A (Alteryx, 2006). Additional data cleaning and exploratory data analysis (EDA) was performed on the new data frame with the DataExplorer and SurvMiner packages in RStudio (Version 1.2.5033) (RStudioTeam, 2021). Survival analysis methods were completed in IBM’s SPSS Statistics (Version 25) (IBM Corp, 2017).

55 collagen gene CNV columns were analyzed in EDA. Varying distributions of each gene were found and can be seen in Appendix B. CNV range for each gene spanned from negative two through positive two. Table 1 lists the reference to each value and stratified groupings into smaller groups for ease of analysis as well as increasing power of the study.

**Table 1. Copy Number Variation (CNV) Description and Stratified Groupings**

|  |  |  |
| --- | --- | --- |
| Value | Description | Stratified Groupings |
| -2 | Complete Deletion | Deletion |
| -1 | Partial Deletion |
| 0 | Normal | Normal |
| 1 | Partial Duplication | Duplication |
| 2 | Complete Duplication |

The timeframe column titled “Days.to.Death” displayed a skewed right distribution with a high spike at the beginning of the timeframe. Maximum days to death was 5,481 days and minimum was eight days with a median of 864 days and a mean of 989 days. There were 564 observations with the status column displaying 291 events of death and 273 censored non-events. Censoring is defined as where the event, death, did not occur during the observation (Prinja et al., 2010). Censoring is either due to loss of patient follow-up data or non-occurrence of death in this study. Table 2 shows the summary of the censored data for each stratification where N is defined as the population or observations. 48.4% of the data had censored events.

**Table 2. Summary of Censored Data**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Censored Observations Summary** | | | | |
| **COL12A1** | Total N | N of Events | Censored | |
| N | Percent |
| A-Norm | 234 | 113 | 121 | 51.7% |
| B-Del | 196 | 118 | 78 | 39.8% |
| C-Dup | 134 | 60 | 74 | 55.2% |
| Overall | 564 | 291 | 273 | 48.4% |
| **COL5A3** |  |  |  |  |
| A-Norm | 154 | 76 | 78 | 50.6% |
| B-Del | 193 | 110 | 83 | 43.0% |
| C-Dup | 217 | 105 | 112 | 51.6% |
| Overall | 564 | 291 | 273 | 48.4% |
| **COL4A3BP** |  |  |  |  |
| A-Norm | 133 | 72 | 61 | 45.9% |
| B-Del | 410 | 207 | 203 | 49.5% |
| C-Dup | 21 | 12 | 9 | 42.9% |
| Overall | 564 | 291 | 273 | 48.4% |

Kaplan-Meier (KM) survival curves were applied to all independent variables. Genes COL12A1, COL4A3BP, COL5A3 were found statistically significant with a p-value threshold of < 0.05. Log-rank tests and Cox-proportional hazard models were applied to the three significant findings.

It is important to mention that the log-rank test is used instead of the Prentice modified Wilcoxon test, which is more sensitive and may make this study more susceptible to type-1 error bias (Peto & Peto, 1972; Kalbfleisch & Prentice, 1980/2002). The Wilcoxon test is more sensitive as it compares weights of each event by the number of population at risk at event time, rather than weighing all events equally like the log-rank test (Visintainer, 2016). The use of the log-rank test helps control for this type-1 error bias.

Another topic worth mentioning is there are roughly five primary equations that can be used within the Cox-proportional hazard model, to which the Efron and Breslow seem to be the most common (Xu, 2019). The Efron method is generally seen as more accurate than the Breslow method but more difficult to use (Breheny, 2019; Li, 2019). This issue is generally brought up when two groups have a tie in events (Breslow, 1972). The Breslow method is used in this study within the Cox-proportional hazards model as it is the baseline estimation method used in SPSS software, used in this study, and can’t be modified (Singer & Willett, 2003, p. 525). The use of this estimation method may potentially control for type-1 error bias although may decrease accuracy when there are ties in events, to which this data has a relatively small amount of cases as time is measured in days in this study.

**Results**

Log-rank tests and Cox-proportional hazard model primary results are displayed in Table 3.

**Table 3. Log-rank Results and Hazard Ratios**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Coefficient** | **P-value** | **Hazard Ratio** | **Lower 95% CI** | **Upper 95% CI** |
| **COL5A3 Normal** |  | 0.015\* |  |  |  |
| **COL5A3 Deletion** | 0.299 | 0.045\* | 1.349 | 1.006 | 1.808 |
| **COL5A3 Duplication** | -0.083 | 0.586 | 0.921 | 0.684 | 1.240 |
| **COL4A3BP Normal** |  | 0.026\* |  |  |  |
| **COL4A3BP Deletion** | -0.075 | 0.586 | 0.928 | 0.709 | 1.214 |
| **COL4A3BP Duplication** | 0.731 | 0.019\* | 2.077 | 1.125 | 3.835 |
| **COL12A1 Normal** |  | 0.047\* |  |  |  |
| **COL12A1 Deletion** | 0.245 | 0.063 | 1.277 | 0.987 | 1.654 |
| **COL12A1 Duplication** | -0.115 | 0.474 | 0.891 | 0.650 | 1.221 |

**\*Statistical significance with p-value < 0.05.**

All three gene survival models were statistically significant overall as defined by the p-value in Table 3 under the normal stratification, however, not all CNV stratifications were significant.

In Table 3, the COL5A3 coefficients were negative for duplication CNV and positive for deletion CNV when compared to normal CNV. This translates to an increased survival for duplication and a significant decrease in survival for deletion mutations. However, the duplication stratification was not statistically significant.

Hazard ratios for COL4A3BP were only significant between the normal CNV and duplication mutations, showing that the duplication mutation has significant decreased survival. The large coefficient value of 0.731 corroborates the decrease in survival for duplication mutations.

The coefficients for the COL12A1 gene with deletion and duplication mutations were not statistically significant while the overall model was statistically significant. COL4A3BP had positive coefficients which imply lower probability of survival with duplicated and normal CNV, however, only duplication and deletion CNV was statistically significant.

Hazard ratios for statistically significant findings include gene COL4A3BP, for duplicated CNV, there is a hazard ratio of 2.077. This translates to just over a 2:1 ratio for increased chance of death which means that there is a 67.5% probability or odds that a patient with this gene duplication will have an event before a patient with the normal stratification. Hazard ratios for COL5A3 were statistically significant for deletion mutations at 1.349. These translate roughly to a ratio of 1.35:1 ratio for chances of death; or a 57.4% probability or odds of death for a patient with the COL5A3 deletion mutation before a patient with the normal stratification. A hazard ratio of one for each stratification would be equivalent to each stratification having a 50% probability that a patient with this mutation will have an event before someone who doesn't have a mutation. These odds are calculated from the equation Probability = Hazard Ratio / (1 + Hazard Ratio). A forest plot shown in Figure 8 displays a graphical representation of the hazard ratios. Note that the normal stratification is not displayed as it is located at the vertical line on the x-axis at the 1 marker for baseline comparison to the other stratified CNV mutations.

**Figure 8. Forest Plot of Hazard Ratio.**

Figure 8 illustrates that the hazard ratio for COL4A3BP duplication stratification confidence interval is quite wide. This is due primarily to lack of data yet was statistically significant. Statistically significant stratifications have markers highlighted in red in Figure 8. All markers right of the line marked at hazard ratio of 1 are considered to have an increased chance of death, and markers less than 1 are considered to have increased survival compared to the baseline of no mutations of respective collagen genes. The x-axis value of 1 is the baseline and is the normal stratification.

Figure 9 displays a bar chart of coefficients which are correlated directly to the hazard ratios. Highlighted in blue are the statistically significant findings for each stratified gene. Values to the right of baseline, at value zero, translate to an increased change of event, and negative values to the left of the baseline represent stratifications with a decreased chance of event. The zero marker is baseline and is the normal stratification.

Chart

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**Figure 9. Coefficient Bar Chart**

Figures 10a, b, and c show Kaplan Meier survival plots for each collagen gene.

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**Figure 10a. COL5A3 Kaplan-Meier Curve.**

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**Figure 10b. COL12A1 Kaplan-Meier Curve.**

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**Figure 10c. COL4A3BP Kaplan-Meier Curve.**

**\*** **Shown in Appendix C are additional Kaplan-Meier curves with confidence intervals shown (created in R-Studio) and Appendix D which are hazard function models.**

Gene COL5A3 in Figure 9a has a very distinct and visible deletion mutation survival difference between roughly 300 days and 1,700 days then converges again to where all three stratifications do not show considerable differentiation at day 2,000, then splits off again. It is important to note that the deletion curve crosses the normal CNV curve at around 2400 days.

Gene COL12A1 shown in Figure 9b illustrates deletion of CNV with a lower survival probability starting around 1,000 days, however, these mutation stratifications were not statistically significant. This figure also has survival curves crossing with the duplication stratification crossing both deletion and normal CNV lines.

Collagen gene COL4A3BP in Figure 9c is only significant with regards to duplication when compared to normal CNV. However, the data for this stratification was sparse as can be seen by the number of events as compared to the other stratifications. This model does not display curve overlap with the statistically significant duplication stratification but does show curve overlap between normal and deletion CNV stratifications.

Table 4 displays observed against expected outcomes from the log-rank test of each gene with asterisks marking statistical significance.

**Table 4. Observed and Expected Scores of Each Stratified Gene.**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | N | Observed | Expected |
| COL12A1 = Deletion | 196 | 118 | 98.8 |
| COL12A1 = Duplication | 134 | 60 | 71.6 |
| COL12A1 = Normal\* | 234 | 113 | 120.7 |
| COL4A3BP = Deletion | 410 | 207 | 215.64 |
| COL4A3BP = Duplication\* | 21 | 12 | 5.63 |
| COL4A3BP = Normal\* | 133 | 72 | 69.73 |
| COL5A3 = Deletion\* | 193 | 110 | 87.5 |
| COL5A3 = Duplication | 217 | 105 | 121.9 |
| COL5A3 = Normal\* | 154 | 76 | 81.5 |

**\*Statistical significance with p-value < 0.05**.

Log-rank scores show the numerical differences in events that are expected versus observed. Highlighted in red are observed events which are greater than expected events, showing a decreased probability of survival.

Table 5 lists median days of survival with upper and lower limits of confidence intervals.

**Table 5. Mean and Median Days of Survival with Confidence Interval Limits**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Mean\* | | | | Median | | | |
| **Estimate** | **Std. Error** | **95% Confidence Interval** | | **Estimate** | **Std. Error** | **95% Confidence Interval** | |
| **Lower Bound** | **Upper Bound** | **Lower Bound** | **Upper Bound** |
| COL4A3BP-Norm\*\* | 1514.131 | 105.792 | 1306.779 | 1721.484 | 1384.000 | 53.393 | 1279.351 | 1488.649 |
| COL4A3BP-Del | 1733.717 | 102.339 | 1533.132 | 1934.302 | 1336.000 | 74.888 | 1189.219 | 1482.781 |
| COL4A3BP-Dup\*\* | 857.090 | 180.283 | 503.736 | 1210.445 | 457.000 | 604.318 | 0.000 | 1641.463 |
| COL4A3BP Overall | 1657.578 | 82.768 | 1495.352 | 1819.804 | 1354.000 | 60.357 | 1235.700 | 1472.300 |
| COL12A1-Norm\*\* | 1724.716 | 141.697 | 1446.990 | 2002.442 | 1448.000 | 90.526 | 1270.569 | 1625.431 |
| COL12A1-Del | 1392.603 | 77.250 | 1241.193 | 1544.013 | 1259.000 | 95.189 | 1072.429 | 1445.571 |
| COL12A1-Dup | 1921.353 | 189.715 | 1549.512 | 2293.194 | 1451.000 | 184.249 | 1089.872 | 1812.128 |
| COL12A1 Overall | 1657.578 | 82.768 | 1495.352 | 1819.804 | 1354.000 | 60.357 | 1235.700 | 1472.300 |
| COL5A3-Norm\*\* | 1550.938 | 85.389 | 1383.576 | 1718.300 | 1516.000 | 115.276 | 1290.059 | 1741.941 |
| COL5A3-Del\*\* | 1363.360 | 88.693 | 1189.522 | 1537.198 | 1102.000 | 43.327 | 1017.078 | 1186.922 |
| COL5A3-Dup | 1938.087 | 157.527 | 1629.334 | 2246.840 | 1446.000 | 68.071 | 1312.581 | 1579.419 |
| COL5A3 Overall | 1657.578 | 82.768 | 1495.352 | 1819.804 | 1354.000 | 60.357 | 1235.700 | 1472.300 |

**\*Estimation limited to largest survival time if censored.  
\*\*Statistically significant**

COL5A3 has overlap in the mean limits of confidence intervals between normal and deletion. There are other overlaps, however, only statistically significant stratifications are mentioned.

COL4A3BP does not have a mean overlap in confidence intervals between normal and duplication, which are also statistically significant.

Figure 11 illustrates the mean survival time measured in days for each stratification. Markers colored in red indicate the statistically significant results while the vertical line at 1,657 indicates the overall mean survival for each stratified group as a baseline.

Chart

Description automatically generated

**Figure 11. Mean Days of Survival Forest Plot**

**Discussion**

Potential mechanisms of action are also important to keep in mind with these findings as they may help establish causality in the future or lead to more research. The COL12A1 gene is a protein coding gene which is associated with fibrils and type-I collagen interactions between the fibrils and the protein matrix (GeneCards - COL12A1, 2021). This roughly translates to tensile strength of the ECM. This could potentially have affects on tumorigenesis or drug resistance mechanisms.

The COL4A3BP gene has a different coding mechanism than the other two statistically significant genes and doesn’t directly code for a protein. This gene encodes for phosphorylation of a terminal on a chain of type-IV collagen and also affects the ceramide transporter (CERT-I) protein I (GeneCards – COL4A3BP, 2021). CERT proteins are known for interacting with ECM components such as collagen type-IV as well as having affect on the clearance of apoptotic cells (Bode et al., 2014). This may affect the signaling in cell death which might have a direct effect on tumorigenesis (Scheffer et al., 2011). It is possible this is a mechanism behind why mutations in this CNV affect survival of ovarian cancer patients. However, this mechanism with CNV mutations should be researched more.

The COL5A3 gene is a protein encoding gene which encodes for a type of less abundant fibrillar collagen (GeneCards – COL5A3, 2021). Mutations in this gene cause connective tissue abnormalities, which could potentially affect the ovaries, but are more commonly seen in the disease grouping called Ehlers-Danlos Syndrome (MalaCards, 2021).

These are novel results and have not been previously published. However, these findings should be further investigated based on the possibility of false discovery previously mentioned in other literature as this is a risk with genetic research (Efron, 2005). The reasoning for this is having an alpha level set at 0.05. There are an estimated 30,000 genes in the human body (Human Genome Project FAQ, 2013). If one multiplies 30,000 genes by 5% then there are 1,500 genes that could potentially have a finding that is random with an alpha set at 0.05 (Genetics Home Reference, 2019). However, very few genetic studies seem to use any controls for false discovery, such as a Bonferroni p-value correction for example and are generally due to lack of quality and quantity of data (Dahiru, 2011). The Bonferroni correction, while quite popular, may not be a valid method to use in complex or complicated situations such as rare interactions or low-frequency events which can be found in genetic research. To remedy this, there are researchers trying to develop more robust by decreasing type-1 error bias while not decreasing the study power (Yi et al., 2014).

There are other methods of survival analysis which may contribute to controlling for type-1 error. Bayesian survival analysis (BSA) has been mentioned as a potential model and has been found to control for type-1 error more than frequentist survival analysis but has increased type-2 error bias (Kelter, 2020). It should also be noted that while BSA may be less prone to type-1 error bias it generally requires subjective data analysis and expert knowledge of parameters and potential prior estimation to perform as well as frequentist survival analysis (Omurlu et al., 2009).

Type-1 error bias should be kept in mind as the primary limitation to this study is quality and quantity of data. Obtaining genetic data is quite difficult and expensive as the cost of TCGA as of 2015 is $375 million (“The Future of Cancer Genomics”, 2015). Although gene sequencing is becoming cheaper and more accurate, a strong argument can be made for keeping alpha at 0.05 with confidence intervals and not strongly controlling for false discovery (Ulrich, 2016).

Other methods were applied as well to help control for type-1 errors such as the log-rank test being used over the Wilcoxon test and potentially the Breslow method of the Cox-proportional hazard. While objectivity is a strong goal to maintain in any study and can be difficult to achieve, finding appropriate data in genetics is also difficult, and for this reason, I argue that using a 95% confidence interval is acceptable, stipulating that said research is further explored or reproduced with other data and statistical methods are applied appropriately.

An argument for not controlling for false discovery are the underlying mechanisms of collagen which have multiple and various effects on ovarian neoplasms (Xu et al., 2019). Selecting a subset of specific genes with known effects on a specific neoplastic tissue seems appropriate. One would not randomly pick a set of genes to investigate where the gene is not expressed in the tissue being studied. Thus, this strengthens the argument for using alpha at 0.05 and not using a Bonferroni p-value correction.

The methods for CNV detection with high-throughput sequencing also contribute to false discovery rates (Jiang et al., 2018). CNV detection is due to read coverage of the genome and relies on multiple variables which include mappability, local characteristics of gene sequence, and having CNV itself (Sims et al., 2014). An increase in observations would be appropriate to help control for this as well as appropriate normalization of data. It is also possible that a combination of TCGA repository and future cancer databases could be utilized and combined to increase observation numbers, such as the Genotype Tissue Expression Project (GTEx) which has been combined with the TCGA recently (Wang et al., 2018). It is possible that this future research structure may not be feasible with technology advancing in genomics at a fast rate as sequencing methods have been evolving rather quickly (Davis, 2015). However, TCGA is an enormous longitudinal archive of genetic information, and more CNV survival analysis studies should be performed on other cancer types to help create a baseline knowledge of CNV and collagen effects on neoplasms.

Because there are evolutionary artifacts in the TCGA germline based on natural selection, future CNV research should possibly be performed on a new cancer repository (Webster et al., 2019). Evolutionary artifacts are characterized as when X and Y chromosomes share a common evolutionary source with high resemblance. Pseudogenes can be considered a type of evolutionary artifact and are defined as a DNA sequence that resembles a gene but is inactive as it has mutated over time, generally due to evolution (Ostrander, n.d.). It is most likely that the evolutionary artifacts and pseudogenes have not introduced much bias into this study as they are generally controlled for in sequencing. However, one should be aware that they exist and that they are not able to be controlled for in this research, as quantity and quality of data are limiting factors. Evolutionary artifacts would most likely not make a difference in treatment options that may arise from research such as this.

Another bias worth mentioning is that data collection was done by multiple people at different locations, as this is secondary data. It is difficult to analyze the integrity of the data in TCGA. However, care has been taken in the data collection process for use in quantitative polymerase chain reaction (qPCR) and uses a genetic reference to allow qPCR normalization which has allowed greater consistency when performing research from the TCGA database (Krasnov et al., 2019). As previously stated, there has even been recent work where the GTEx was combined with the TCGA into a pipeline which unifies RNA sequencing data and therefore allows greater normalization (Wang et al., 2018).

Studies such as this can be considered foundational to future personalized cancer treatments and target therapy. However, most antineoplastic molecules are of a narrow therapeutic index which means that small dose alterations may lead to toxicity; thus, chemotherapy dosing changes based on genetics may be unlikely from CNV studies at this time, but this may change in the future (Eaton & Lyman, 2019). There is potential for focused or targeted gene therapy which may be able to use information from studies such as this; especially as targeted gene therapy becomes advanced and viable (Zaimy et al., 2017).

A two to three gene CNV signature test could possibly be implemented from studies such as this to present information about probability for survival to the patient and physician. This potential CNV signature could also be assimilated into a gene expression and CNV panel for a more complete study in predictive power of genetics and proteomics in patient survival.

It is also important to consider various social responsibilities when doing genetic research. Federal and state privacy laws should be accounted for when collecting data and performed appropriately so as not to release private health information without consent. Fortunately, this is not an issue within TCGA, although researchers should be aware of the social and ethic responsibilities and complications that arise with medical data.

Another social responsibility worth keeping in mind, is that studies such as survival analysis performed on genetic data will most likely have biases; and medical products, treatments, or tests designed from said research should have biases explicitly stated to the product, treatment, or test’s respective creators. It would be highly unethical, and could cause harm to patients, if faulty and biased research are used to produce treatments or tests which may lead to incorrect conclusions or decisions about a patient’s health. Thus, explicitly stating biases in said research helps contribute to this process or preventing unethical and biased conclusions. It would be prudent to have findings replicated in another genetic database when bias may be a major complication.

More studies on collagen in patient genetic profiles for all types of cancer regarding CNV should be conducted to further our base knowledge which may lead to better treatments in the future.

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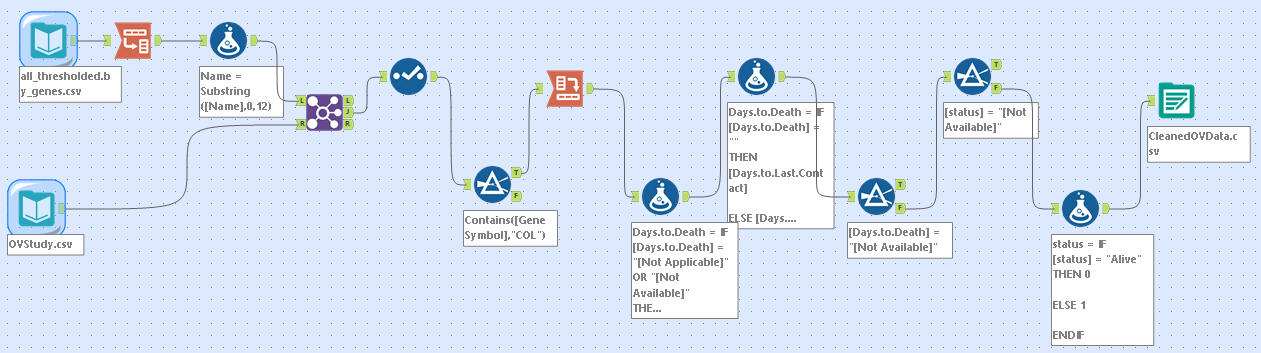
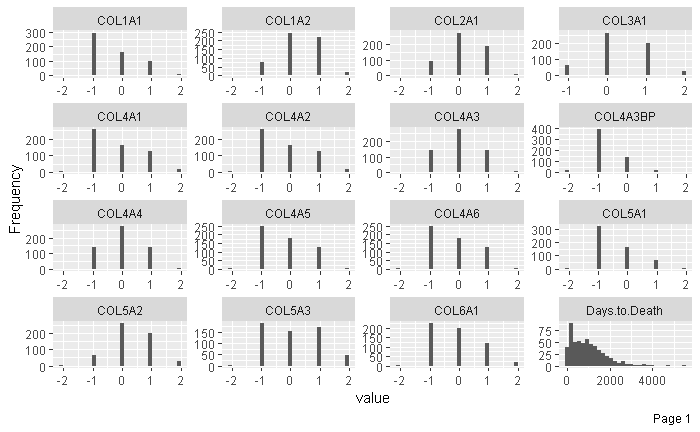
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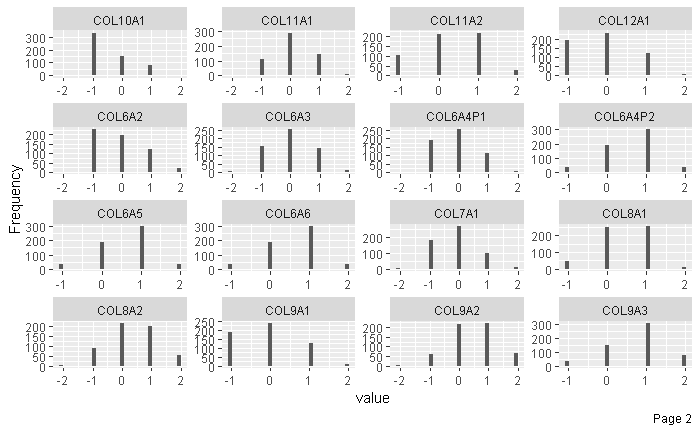
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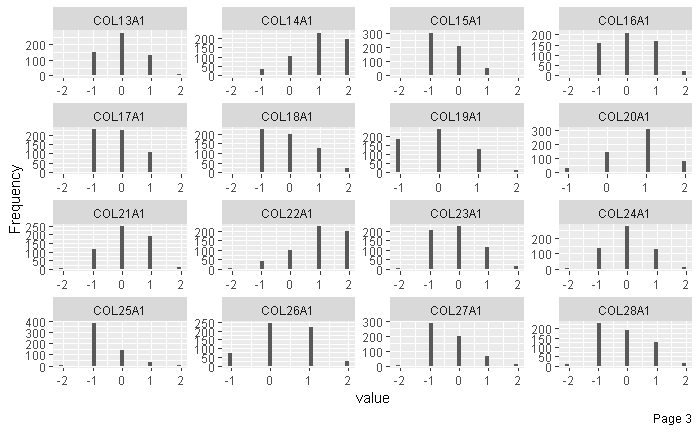
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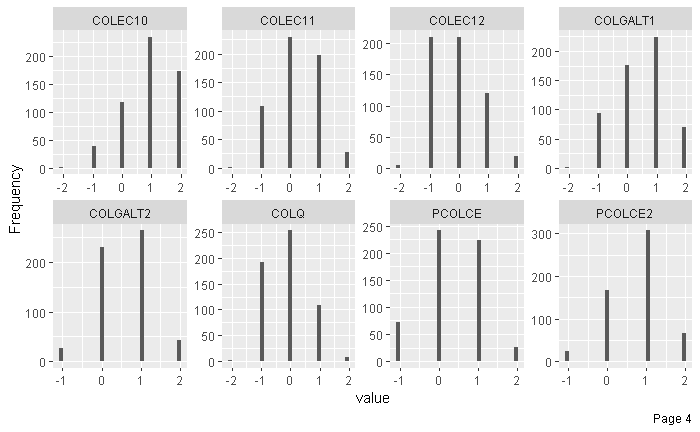
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**Appendix A**

**  
  
Appendix B**







**Appendix C**

COL12A1

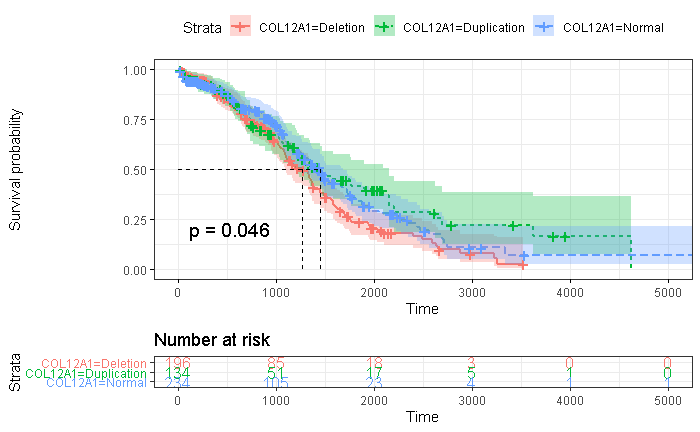


Figure COL12A1 survival has stratified curve of CNV changes (n = 564). Time is measured in days. Green illustrates duplicated copy number variation showing increased survival probability while red illustrates deletion of copy number variation displaying lower survival probability. Blue displays normal copy number variation. The model’s p-value of 0.046 demonstrates a statistically significant model when stratifying between COL12A1 duplication and deletion with regards to survival time. The dotted lines display the 50% survival probability between duplication, deletion, and normal copy number variation.

COL4A3BP

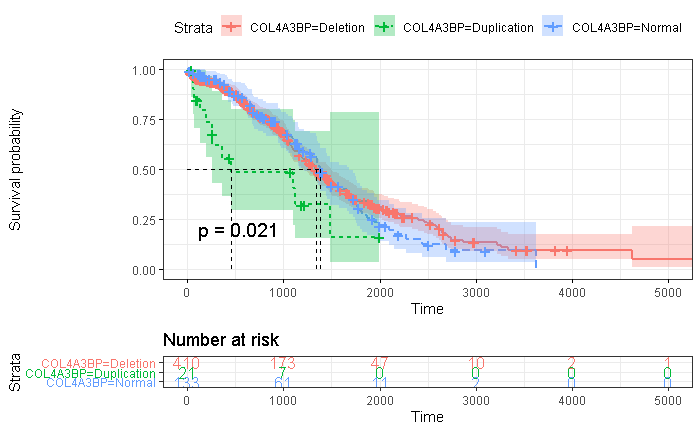


Figure COL4A3BP survival has stratified curve of CNV changes (n = 564). Time is measured in days. Green illustrates duplicated copy number variation displaying a decreased survival probability while red illustrates deletion of copy number variation. Blue displays normal copy number variation illustrating an increased survival probability compared to copy number variation duplication. The model’s p-value of 0.021 demonstrates a statistically significant model when stratifying between COL12A1 duplication vs normal and deletion copy number variation with regards to survival time. The dotted lines display the 50% survival probability between duplication, deletion, and normal copy number variation.

COL5A3

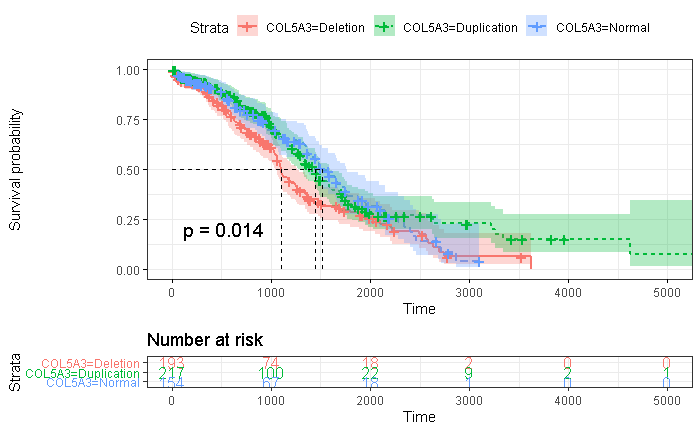


Figure COL5A3 survival has stratified curve of CNV changes (n = 564). Time is measured in days. Green illustrates duplicated copy number variation displaying an increased survival probability while red illustrates deletion of copy number variation illustrating a lower survival probability when compared to normal and duplicated copy number variation. Blue displays normal copy number variation. The model’s p-value of 0.014 demonstrates a statistically significant model when stratifying between COL5A3 deletion vs normal and duplicated copy number variation with regards to survival time. The dotted lines display the 50% survival probability between duplication, deletion, and normal copy number variation.

**Appendix D**

COL12A1

Chart

Description automatically generated

COL4A3BPChart

Description automatically generated

COL5A3

Chart, line chart

Description automatically generated

**Table of Abbreviations**

Analysis of Variance (ANOVA)

Bayesian Survival Analysis (BSA)

Beta(1-0)galactosyltransferase Gene (COLGALT)

Ceramide Transfer (Protein) (CERT)

Collagen-like Gene (COLEC)

Collagen-like subunit of acetylcholinesterase (COLQ)

Collagen Gene 12A1 (COL12A1)

Collagen Gene 4A3BP (COL4A3BP)

Collagen Gene 5A3 (COL5A3)

Comma Separated Value (CSV)

Copy Number Variation (CNV)

Deoxyribonucleic Acid (DNA)

Exploratory Data Analysis (EDA)

Extra-cellular Matrix (ECM)

Genome Reference Consortium Human Build 37 (GRCh37) (HG19)

Genotype Tissue Expression Project (GTEx)

Kaplan-Meier (KM)

Medical Subject Heading (MeSH)

Next Generation Sequencing (NGS)

Not Applicable (NA)

Ovarian Cancer (OV)

Probability Value (p-value)

Pro-collagen enhancer gene (PCOLCE)

Quantitative Polymerase Chain Reaction (qPCR).

Ribonucleic Acid (RNA)

Risk Ratio (RR)

The Cancer Genome Atlas (TCGA)

The Office of The National Coordinator for Health Information Technology (ONC)

Transforming Growth Factor (TGF)