Vol 11 No 3 Year 2026

E-ISSN: 2528-410X

RESEARCH ARTICLES

Characterisation of the Impact of Genetic Variation of the Beclin1 Gene on Functionality of Autophagy Proteins

Fadhilla Ika Herliana[1], Tegar Adriansyah Putra Siregar[2]

[1]Faculty of medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No. 53, Medan 20217, Indonesia

[2]Department of Microbiology and Immunology, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No. 53, Medan 20217, Indonesia

Corresponding Email: fadhilaikaaa@gmail.com
tegaradriansyah@umsu.ac.id

Abstract: Autophagy (from the Greek words auto, meaning alone, and phagi, meaning to eat) is an important pathway that regulates the homeostasis of an organism. When this balance is disturbed, pathological conditions can develop. Autophagy is divided into three types: (1) macroautophagy, (2) microautophagy, and (3) Chaperone-mediated autophagy (CMA). Modulator of the autophagy stage, (1) initiation, when starvation occurs, mTORC1 is inhibited and phosphorylated by AMPK, which results in activation of ULK1. Under normal conditions, cells use autophagy to recycle metabolites and nutrients from damaged organelles and misfolded proteins. The discovery of BECLIN1 was caused by the anti-apoptotic protein Bcl-2. Genetic variation is the difference in DNA sequence between individuals in a population. Autophagy works by removing damaged proteins and organelles during stress and ageing, playing an important role in regulating organism development, collaborating with the adaptive immune system, maintaining energy homeostasis, and maintaining protein and organelle quality control. This study aims to determine the impact of genetic variations in the BECLIN1 gene on the autophagy process. Methodology with a bioinformatics analysis approach using SIFT and PolyPhen-2 software. The results of the BECLIN1 gene prediction using SIFT software for Missense mutations showed that 31% of samples were Deleterious and 69% of samples were tolerated. Frameshift mutations found that 42.85% of samples experienced Deleterious and 57.14% of samples experienced Tolerated.

Keywords: BECLIN 1, autophagy, missense, frameshift, sift, polyphen-2

INTRODUCTION

Autophagy is a survival mechanism that helps maintain cell homeostasis because it plays a role in removing misfolded or aggregated proteins, clearing out damaged organelles, such as mitochondria, endoplasmic reticulum, and peroxisomes.[1,2] Autophagy helps maintain

BULETIN FARMATERA
E-ISSN: 2528-410X

cellular homeostasis under different conditions; for example, during nutritional stress, it allows the formation of energy or macromolecules. In cell differentiation, it provides a precursor metabolite for morphogenesis, and when an organelle is no longer needed or does not perform its function properly, it is degraded to be replaced by a new synthesis.[1,3]

Autophagy is divided into three types: (1) macro autophagy, (2) micro autophagy, and (3) Chaperone-mediated autophagy (CMA).[1,3] Proteins involved in the process of autophagy are: (1) ULK kinase core complex, (2) class III PI3K I complex, (3) ATG9A/ATG2-WIPI1/2 trafficking system, (4) ATG12 conjugation system, (5) LC3 conjugation system. [1,4]

Autophagy stage modulators, (1) initiation, when mTORC1 starvation occurs, is inhibited and phosphorylated by AMPK, resulting in ULK1 activation.[2,4] Furthermore, the ULK1 complex phosphorylates the Ambra1 protein, which allows the attraction of the BECLIN-1-VPS34 complex (BECLIN1-VPS34-VPS15-ATG14L).[2,4] VPS34 produces phosphatidylinositol-3-phosphate (PIP3) and engages DFCP1 to promote nucleation.[2,4] Through clathrin-mediated endocytosis produces phagofor,2 (2) elongation, pro-LC3 is cleaved by ATG4B and produces LC3-I.[2,4] Then ATG7 and ATG3 process LC3-I to be conjugated to phosphatidylethanolamine (PE) and ATG12-ATG5-ATG16L system to produce LC3-II.2 The formation of this complex is necessary for the elongation of the phagophore.[2,4] (3) Autophagosome degradation and recycling: Adult autophagosomes fuse with lysosomes to form autolysosomes.[2,4] In lysosomes, degradation occurs with hydrolytic enzymes that are active at acidic pH.2 Vacuolar ATPase (vATPase) regulates this pH.2 Finally, the degraded product is released into the cytosol for recycling. [2,4]

Under normal conditions, cells use autophagy to recycle metabolites and nutrients from damaged organelles and misfolded proteins.[5,6] During this autophagic cell death controlled scenario, the anti-apoptosis protein Bcl-2 (the main regulator of apoptosis) binds to the BH3 domain of the BECLIN1.[5,6]

The discovery of BECLIN1 due to the anti-apoptosis protein Bcl-2.[7,8] The BECLIN1 gene is located on the human chromosome 17q21 and is highly homologous to the yeast autophagy gene Atg6, which plays a central role in autophagy via the PI3KC3 or Bcl-2 complex.9 Genetic analysis revealed BECLIN1 to be a tumour suppressor candidate, monoallele removed in 40-75% of sporadic breast and ovarian cancers.[10,11]

Genetic variation is a difference in the DNA sequence between individuals in a population.12 Variations occur in germ cells, namely sperm and ovum, and also in somatic cells.[12] Mutations and recombination are the main sources of variation.[12]

Autophagy works by removing proteins and organelles damaged during stress and ageing, plays an important role in regulating the development of organisms, cooperating with the adaptive immune system, maintaining energy homeostasis,

BULETIN FARMATERA
E-ISSN: 2528-410X

and maintaining quality control of proteins and organelles.[4]

METHOD

The research was observational and descriptive with a bioinformatics approach to determine the genetic variation of the BECLIN1 genes in autophagy proteins. The population in this study is data on genetic variation of the BECLIN1 genes in the NCBI dbSNP database https://www.ncbi.nlm.nih.gov/snp/.

The sample of this study is data on the genetic variation of the BECLIN1 genes in the NCBI dbSNP database that meet the inclusion and exclusion criteria. The sampling technique used in this study uses purposive sampling with pengambilan sampel non-randomised. After calculating the sample size using the Slovin formula, a minimum of 98 samples were obtained and used in the study.

In this study, 100 samples of Missense variations taken from dbSNP were taken in order, starting from sample number 1 to 100, and there were 2 samples, namely in numbers 37 and 67, when entered into the software, no results were obtained; they were replaced with samples numbers 101 and 102. The Frameshift variation was found in 28 samples, but 5 samples were not from the BECLIN1 gene, and 2 samples could not be used due to the same amino acid substitution, so the prediction results were not obtained in either software; the final sample used was 21 samples of the Frameshift variation.

The data analysis used in this study is quantitative data analysis with descriptive statistics. The data obtained is then presented in the form of a narrative and a frequency distribution table.

After being analyzed using software, the score of the fatality of a genetic mutation will be determined, then the results of the score will be processed into the Microsoft excel program and adjusted to descriptive statistics to be interpreted into the form of percentages, then it will be presented with a narrative, a frequency distribution table, and a pie chart as a visualization of the table.

RESULT

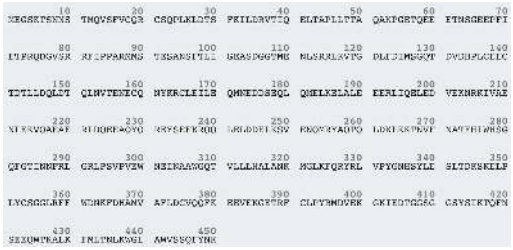

Figure 1. Protein Sequence of the BECLIN1 Gene

The protein sequence of BECLIN1 in Homo sapiens (human) organisms was obtained from the UNIPROT database with a length of 450 amino acids. The gathering of amino acids resulting from mRNA translation will form proteins. If there is a mutation that causes the amino acid sequence to change, the protein formed is not suitable and will affect the functionality of the protein.

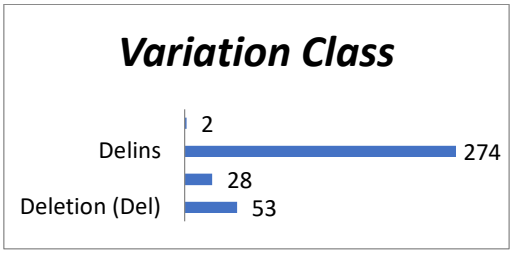

Figure 2. Distribution of genetic variation of the BECLIN1

E-ISSN: 2528-410X

gene in *the NCBI* database

Genetic variation is a change in the sequence of genes due to changes at the DNA level. Based on Figure 2, it is obtained that there are 4 genetic variations of the BECLIN1 gene in the NCBI database. It is known that there are 2 *variations of Multiple Nucleotide Variants* (MNV), *274* variations of Delins, 28 *variations of Insertions*, and *53* variations of Deletions.

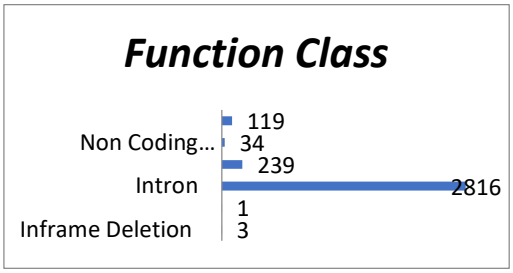

Figure 3. Distribution of the sub-categories of genetic variation of the BECLIN1 genes in *the NCBI* database

The occurrence of a change in the sequence of sequences causes genetic variation in a gene. Genetic variations in genes can cause functional impairment that can be fatal or non-fatal. Based on the function class in Figure 3, *Synonymous* is 119, Non-Coding Transcript Variant is 34, *Missense* is 239, *Inton* is 2816, *Inframe Insertion* is 1, and *Inframe Deletion* is 3.

Table 1. Results of Missense *Variation Prediction Analysis* with SIFT Software

Skor	Mutation Effect	Number of samples	Percentage (%)
0.00 – 0.04	*Deleterious*	31	31
0.00 – 1.00	*Tolerated*	69	69
	Total	**100**	**100**

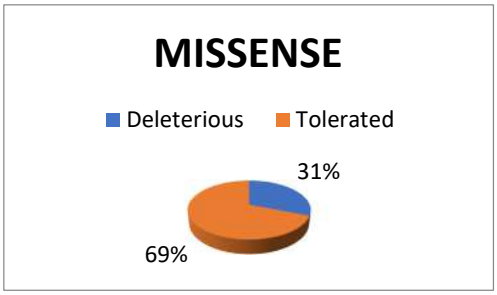

Figure 4. Percentage Missense Variation Analysis with SIFT Software

Based on the results of the research presented in Table 1, *Missense variations* that have been analysed using SIFT showed that 31% of the samples were *Deleterious* and 69% of the samples were *Tolerated*. If an amino acid in a protein sequence is replaced by another amino acid, the protein sequence will change and will affect the function of that protein. *Deleterious* samples are thought to affect protein function so that it cannot function normally. Meanwhile, samples whose results *were tolerated* were not expected to affect protein function. The fatality score of the SIFT software can be seen in Table 1.

Table 2. Results of Analysis of *Predictive Frameshift* Variation with SIFT Software

Skor	Mutation Effect	Number of Samples	Percentage (%)
0.00 – 0.04	*Deleterious*	9	42,85
0.05 – 1.00	*Tolerated*	12	57,14
	Total	**21**	**100**

BULETIN FARMATERA
E-ISSN: 2528-410X

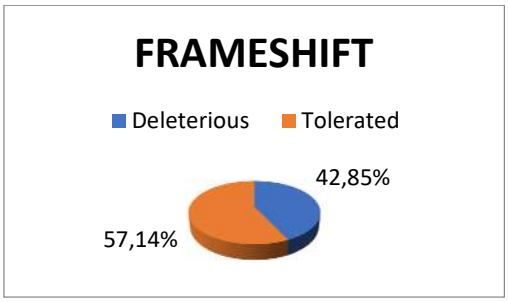

Figure 5. Percentage Analysis of *Frameshift* Variation with SIFT Software

Based on the results of the study presented in Table 2, it turns out that the *Frameshift* variation that has been analysed using SIFT obtained 42.85% of the sample is *Deleterious* and 57.14% of the sample is *Tolerated*. If an amino acid in a protein sequence is replaced by another amino acid, the protein sequence will change and will affect the function of that protein. Therefore, according to the results of the analysis above, Deleterious protein*s* are estimated to be unable to function normally, while proteins that are tolerated *are* estimated not to affect the function of these proteins. The fatality score of the SIFT software can be seen in Table 2.

Table 3. Results of *Missense Variation Prediction* Analysis with Polyphen-2 Software

Skor	Mutation Effect	Number of Samples	Percentage (%)
0,000 - 0,367	*Benign*	45	45
0,493 - 0,894	*Possibly Damaging*	14	14
0,827 - 1,000	*Probably Damaging*	41	41
	Total	**100**	**100**

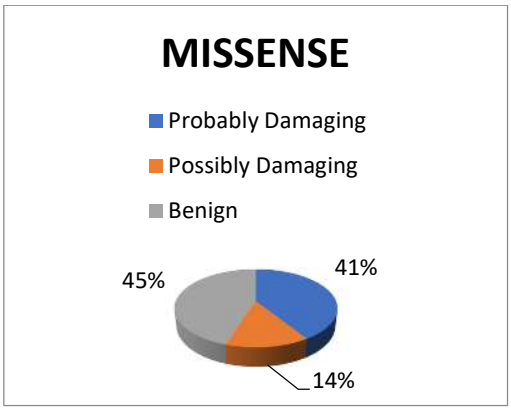

Figure 6. Percentage Missensee Variation *Analysis with Software*

Based on the results of the study presented in Table 3, it shows that *the Missense* variation that has been analysed using the PolyPhen-2 software obtained results of 45% for *Benign*, 14% for *Possibly Damaging*, and 45% for *Probably Damaging*. So according to the results of the analysis above, the sample that experienced *Benign* did not affect the function of the protein, *the result of Possibly Damaging* is estimated to affect the function of the protein and may or may not affect the function of the protein, and the result *of Probably Damaging* can affect the function of the protein so that it cannot function normally.

Table 4. Results of Analysis of *Predictive Frameshift* Variation with Polyphen-2 Software

Skor	Efek Mutasi	Jumlah Sampel	Persentase (%)
0,000 - 0,218	*Benign*	6	28,57
0,559 - 0,865	*Possibly Damaging*	4	19,04

BULETIN FARMATERA
E-ISSN: 2528-410X

0,962 - 1,000	*Probably Damaging*	11	52,38
	Total	**21**	**100**

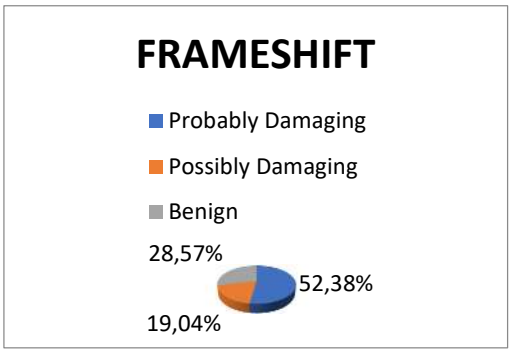

Figure 7. Percentage Analysis of *Frameshift* Variation with Polyphen-2 Software

Based on the results of the study presented in Table 4, it shows that the *Frameshift* variation that has been analysed using the PolyPhen-2 software obtained results of 28.57% for *Benign*, 19.04% for *Possibly Damaging*, and 52.38% for *Probably Damaging*. So according to the results of the analysis above, the sample that experienced *Benign* did not affect the function of the protein, *the result of Possibly Damaging* is estimated to affect the function of the protein and may or may not affect the function of the protein, and the result *of Probably Damaging* can affect the function of the protein so that it cannot function normally.

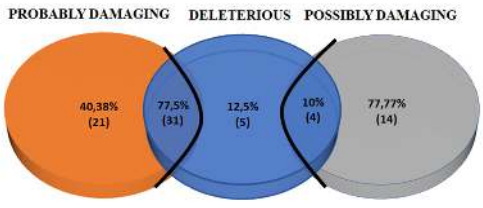

Figure 8. Results of *Missense* and *Frameshift* Gene BECLIN1 Fatal Sample Analysis Based on Crossover of SIFT and Polyphen-2 Software

Based on the SIFT software, the fatality prediction is *Deleterious*, while PolyPhen-2 predicts fatality as *Probably Damaging* and *Possibly Damaging*. Based on the results of the research presented in Figure 8, the results of cross-analysis were obtained from a sample of fatal genetic variation based on the two software used, which means that the sample from the cross had a fatality rate included in the score of the two software. As seen in the picture, it turns out that only 77.5% (31 samples) of *the Deleterious* in SIFT are *Probably Damaging* in PolyPhen-2, while 10% (4 samples) of *the Deleterious* in SIFT are *Possibly Damaging* in PolyPhen-2. So it can be concluded that a sample that is fatal in the SIFT software is not necessarily fatal in the PolyPhen-2 software. Cross data from the genetic variation sample based on both software is shown in Appendix 3.

DISCUSSION

From the results of the above study, it is shown that the protein BECLIN1 with a length of 450 amino acids (aa) and a molecular mass of 60KDa, where this BECLIN1 protein encodes *the BECN1* gene located on chromosome 17q21 with a length of 14151 bp.

The results of this study are supported by previous research conducted by Sargeet Kaur and Harish Changotra on BECLIN1 interactions with modifications and pathologies related to autophagic diseases, showing that the BECLIN1 protein has 450 amino acids (aa) with a molecular mass of 60KDa encoding the

E-ISSN: 2528-410X

BECN1 gene.[13] Protein BECLIN1 has 3 domains, namely: (1) *Bcl-2-Homology-3* (BH3) domain (aa105-130), (2) *Coiled-Coil domain* (CCD) (aa 175-264), (3) *Evolutionary Conserved Domain* (ECD) (aa 248-450).[13,14]

Based on data from NCBI dbSNP, the BECLIN1 gene has 3,831 SNP variations with various types of mutations. The genetic variation analysed in this study was focused on *Missense* and *Frameshift mutations* according to the inclusion criteria, so that 100 and 21 samples were determined, respectively, based on the calculation of the minimum sample size using the Slovin formula.

Previous research conducted by Silvia Vega-Rubín-de-Celis and Saurabh V. Laddha showed that mutations in BECLIN1, particularly *Missense*, are often found in several types of cancer, such as colon, ovarian, and skin cancers.[15,16] Due to its proximity to BRCA1 and its role as a tumour suppressor gene, the BECLIN1 mutation has the potential to trigger cancer.[15,16] These mutations are generally destructive and can cause changes in protein structure and function.[16]

Bioinformatics studies using mutation prediction software, such as SIFT and PolyPhen-2, have consistently reported that most *Missense* mutations in BECLIN1 are predicted to be Deleterious or Probably Damaging.[17] Similar findings also appear in other genes, such as MC4R, CHRNA5, and AKT1, suggesting that Missense mutations with high prediction scores correlate with decreased or loss of protein function.[17]

Based on Imane Douiyeh's research, et al used the MC4R gene as a research sample that analysed fatality predictions using seven software and one of them was SIFT and PolyPhen-2.[18]. The samples used were 16 with *Missense* variations. From the results of the study using SIFT software, 16 *samples were obtained as Deleterious* and using PolyPhen-2, 16 samples were obtained as probably damaging.[18]

Based on other research conducted by Feyeza Sadia Laskar et al, the results of *the Deleterious* prediction cause the gene protein to not function normally, and the prediction results of *Probably Damaging* from PolyPhen-2 are also the same, namely, causing the protein to not function normally.[19] Proteins cannot function because there is damage to the protein caused by mutations in amino acids, so that the sequence of proteins changes.[19,20]

Based on another study conducted by Mahalakshmi Kumaraguru et al., the CHRNA5 gene was used to identify pathogenic mutations in the gene.[21] Genes were analysed for predictions of genetic variation using three software (SIFT, PolyPhen-2, and PROVEAN).[21] After analysis, the results of the distribution percentage of the three software were obtained, namely 83% (134) *damaging* and 17% (27) *no damage* [21]

In this study, in silico SNP analysis that damages the oncogene of the human AKT1 gene used software from PolyPhen-2, SIFT, PROVEAN, SNAP-2, Mutation Assessor, PANTHER, PON-P2, and P-Mut.[22] In the study, we searched for *missense SNPs* by finding 12 very dangerous SNPs that could affect the structure and function of the AKT1

BULETIN FARMATERA
E-ISSN: 2528-410X

protein.[22]

Then, to differentiate this SNP study from other SNPs, this study used the BECLIN1 genes for *Missense* and *Frameshift* SNPs, which were analysed using two software to investigate the mutation effects of *Missense* and *Frameshift*.

In the SIFT software, the scores used to determine the Deleterious and Tolerated samples are taken from the information in the SIFT software. It is stated that if the score is <0.05, it is predicted to be Deleterious.

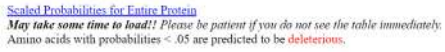
Scaled Probabilities for Entire Protein
May take some time to load!! Please be patient if you do not see the table immediately.
Amino acids with probabilities < .05 are predicted to be deleterious.

Figure 9. SIFT software prediction score

Based on previous research conducted by Imane Douiyeh et al. also stated that the score on the SIFT software of ≤0.05 is predicted to be deleterious and >0.05 is predicted to be Tolerated [16]

The results of the analysis using SIFT software were obtained on the BECLIN1 genes that experienced *Missense Deleterious* mutations of 31%, *Tolerated* 69%, and *Frameshift* mutations of the BECLIN1 gene *obtained Deleterious* 42.85%, *Tolerated* 57.14%.

The results of the analysis of the PolyPhen-2 software on BECLIN1 genes that experienced *Missense* mutations were *obtained as Benign* 45%, *Possibly Damaging* 14%, and *Probably Damaging* 41%, while the *Frameshift* mutation of the BECLIN1 gene was obtained *as Benign* 28.57%, *Possibly Damaging* 19.04%, and *Probably Damaging* 52.38%.

From the results of the analysis that has been carried out by both SIFT and PolyPhen-2 software on BECLIN1 genes in *the Missense* and *Frameshift* SNPs, it can be estimated that mutations caused by BECLIN1 genes can be damaging, so that they affect the functionality of proteins.

CONCLUSION

1. BECLIN1 genes that undergo *Missense* and *Frameshift mutations* can affect the functionality of autophagy proteins.
2. The use of PolyPhen-2 and SIFT software can provide mutation prediction based on scores.

REFERENCES

1. Gómez-Virgilio L, Silva-Lucero MDC, Flores-Morelos DS, et al. Autophagy: A Key Regulator of Homeostasis and Disease: An Overview of Molecular Mechanisms and Modulators. *Cells*. 2022;11(15):1-40. doi:10.3390/cells11152262

2. Cao W, Li J, Yang K, Cao D. An overview of autophagy: Mechanism, regulation and research progress. *Bull Cancer*. 2021;108(3):304-322. doi:10.1016/j.bulcan.2020.11.004

3. Ichimiya T, Yamakawa T, Hirano T, et al. Autophagy and autophagy-related diseases: A review. *Int J Mol Sci*. 2020;21(23):1-21. doi:10.3390/ijms21238974

4. Li X, He S, Ma B. Autophagy and autophagy-related proteins in cancer. *Mol Cancer*. 2020;19(1):1-16.

E-ISSN: 2528-410X

doi:10.1186/s12943-020-1138-4

5. Prerna K, Dubey VK. Beclin1-mediated interplay between autophagy and apoptosis: New understanding. *Int J Biol Macromol*. 2022;204(December 2021):258-273. doi:10.1016/j.ijbiomac.2022.02.005

6. Xie Q, Liu Y, Li X. The interaction mechanism between autophagy and apoptosis in colon cancer. *Transl Oncol*. 2020;13(12):100871. doi:10.1016/j.tranon.2020.100871

7. Xu HD, Qin ZH. Beclin 1, Bcl-2 and Autophagy. *Adv Exp Med Biol*. 2019;1206:109-126. doi:10.1007/978-981-15-0602-4_5

8. Tran S, Fairlie WD, Lee EF. Beclin1: Protein structure, function and regulation. *Cells*. 2021;10(6). doi:10.3390/cells10061522

9. Liu R, Zhang S, Wan R, Deng J, Fang W. Effect of Beclin-1 gene silencing on autophagy and apoptosis of the prostatic hyperplasia epithelial cells. *Clinics*. 2022;77(January):1-8. doi:10.1016/j.clinsp.2022.100076

10. Vega-Rubín-de-celis S, Kinch L, Peña-Llopis S. Regulation of beclin 1-mediated autophagy by oncogenic tyrosine kinases. *Int J Mol Sci*. 2020;21(23):1-15. doi:10.3390/ijms21239210

11. Yang Y, Klionsky DJ. Autophagy and disease: unanswered questions. *Cell Death Differ*. 2020;27(3):858-871. doi:10.1038/s41418-019-0480-9

12. Pritchard, Dorian J., Korf, BR. *Medical Genetics at a Glance*. 3rd ed. WILEY-BLACKWELL; 2013.

13. Kaur S, Changotra H. The beclin 1 interactome: Modification and roles in the pathology of autophagy-related disorders. *Biochimie*. 2020;175:34-49. doi:10.1016/j.biochi.2020.04.025

14. Ye J, Zhang J, Zhu Y, et al. Targeting autophagy and beyond: Deconvoluting the complexity of Beclin-1 from biological function to cancer therapy. *Acta Pharm Sin B*. 2023;13(12):4688-4714. doi:10.1016/j.apsb.2023.08.008

15. Vega-Rubín-de-celis S. The role of Beclin 1-dependent autophagy in cancer. *Biology (Basel)*. 2020;9(1):1-13. doi:10.3390/biology9010004

16. Laddha S V, Ganesan S, Chan CS, White E. Mutational Landscape of the Essential Autophagy Gene BECN1 in Human Cancers. (10):485-490. doi:10.1158/1541-7786.MCR-13-0614

17. Kaur S, Vashistt J, Changotra H. Identification of molecular signatures and molecular dynamics simulation of highly deleterious missense variants of key autophagy regulator beclin 1: a computational

E-ISSN: 2528-410X

based approach. *J Biomol Struct Dyn*. 2023;0(0):1-14. doi:10.1080/07391102.2023.2252097

18. Douiyeh I, Khamlich J, Al E. Computational analysis of missense variants of human MC4R and childhood obesity Imane. *Cell Mol Biol*. 2023;51(1):1.

19. Laskar FS, Bappy MNI, Hossain MS, et al. An in silico Approach towards Finding the Cancer-Causing Mutations in Human MET Gene. *Int J Genomics*. 2023;2023. doi:10.1155/2023/9705159

20. Syed NA, Bhatti A, John P. Molecular dynamics simulations and bioinformatics' analysis of deleterious missense single nucleotide polymorphisms in Glyoxalase-1 gene. *J Biomol Struct Dyn*. 2023;41(23):13707-13717. doi:10.1080/07391102.2023.2181654

21. Kumaraguru M, L L, Priyadharsini VJ, I MA, S R. Identification of Pathogenic Missense Mutations in the CHRNA5 Gene: A Computational Approach. *Cureus*. 2023;15(10):1-18. doi:10.7759/cureus.47519

22. Zhang R, Akhtar N, Wani AK, Raza K, Kaushik V. Discovering Deleterious Single Nucleotide Polymorphisms of Human AKT1 Oncogene: An In Silico Study. *Life*. 2023;13(7). doi:10.3390/life13071532