Role of *ATG7* gene in Vitiligo

Project report submitted in partial fulfilment of the requirement of the major project for the Degree of

Bachelor of Technology

in

BIOTECHNOLOGY

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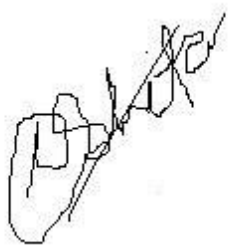
CANDIDATE'S DECLARATION

We hereby declare that work presented in this report entitled "*Role of ATG7 gene in Vitiligo*" in partial fulfilment of the requirements for the award of degree in *Bachelor of Technology* in the *Department of Biotechnology and Bioinformatics* [BT & BI] from Jaypee University of Information Technology Waknaghat, Solan, H.P. is an authentic record of my own work carried out under the supervision of Dr. Udaybanu, Associate Professor in Department of BT & BI.



Ishita Kawar (171815) Ekta Choudhary (171816)

This is to certify that the above statements made is true to best of my knowledge. Dr. Udaybanu M

(Associate Professor in the Department of Biotechnology and Bioinformatics)

SUPERVISORE'S CERTIFICATE

This is to certify that the work titled "Role of ATG7 gene in Vitiligo" carried out by Ekta Choudhary and Ishita Kawar during the 4th year (2020-2021) in requirement for the award of degree of Bachelor of Technology in Biotechnology of Jaypee University of Information Technology, Solan has been carried out under my supervision. The work done has not been submitted to any other university or college to obtain a degree or recognition.

Signature of supervisor

Name of supervisor: Dr. Udayabanu M

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INTRODUCTION

Vitiligo is an acquired immune disorder which is characterized by depigmentation of skin occuring in patches due to lack of melanocytes in the dermis or inability to produce melanin. It can affect any part of the body including hair as well as inside of the mouth. Vitiligo is caused when melanocytes die or when they stop producing melanin. The exact cause of this disease is unknown however, vitiligo may be caused due to several reasons such as immune system disorders, family having a history of being suffered from a disease, or due to stress, sunburn, skin trauma or coming in contact with some chemical. However, vitiligo is caused due to genetic as well as environmental risks which results in initiation of an autoimmune attack on melanocytes present in the skin. The pathogenesis of vitiligo is complex and involves multiple factors whereas, the immunopathogenesis is not completely understood [1,2].

Fig 1: Vitiligo

Types of vitiligo:

Generalized vitiligo: this type of vitiligo is the most common type in which there are wide and randomly distributed areas of depigmentation.

Universal vitiligo: universal vitiligo is the rarest type of vitiligo. In this more than 80% of the skin becomes depigmented.

Focal vitiligo: in focal vitiligo the depigmented patches occur in small area and they are not likely to spread in certain pattern for one to two years.

Segmental vitiligo: this type of vitiligo occurs in one side of the body or in one area, for example face or hands.

Acrofacial vitiligo: this type of vitiligo occurs in fingers and periorificial areas.

Mucosal vitiligo: mucosal vitiligo affects on mucous membranes of the mouth or the genitals.

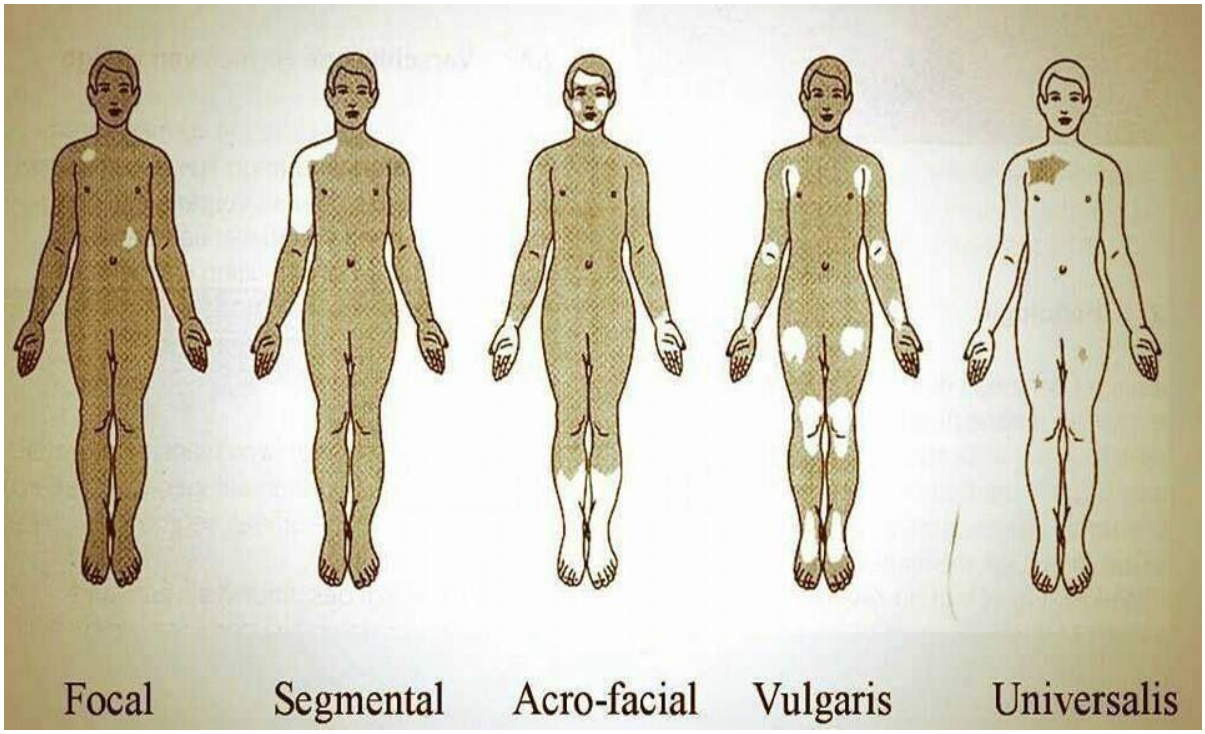

Fig 2: Types of Vitiligo

Affected areas:

The zones of skin most generally influenced by vitiligo include:

1. Mouth and eyes
2. Fingers and wrists
3. Armpits
4. Groin
5. Genitals
6. Inside of the mouth
7. Hair (hair roots or scalp)

Vitiligo consistently starts as a pale fix of skin that gradually turns absolutely white. The point of convergence of a fix may be white, with paler skin around it. In case there are veins under the skin, the fix may be hardly pink, instead of white[1]. The condition changes from individual to person. A couple of individuals simply get a few little, white patches, yet others get more prominent white repairs that sign across tremendous zones of their skin.

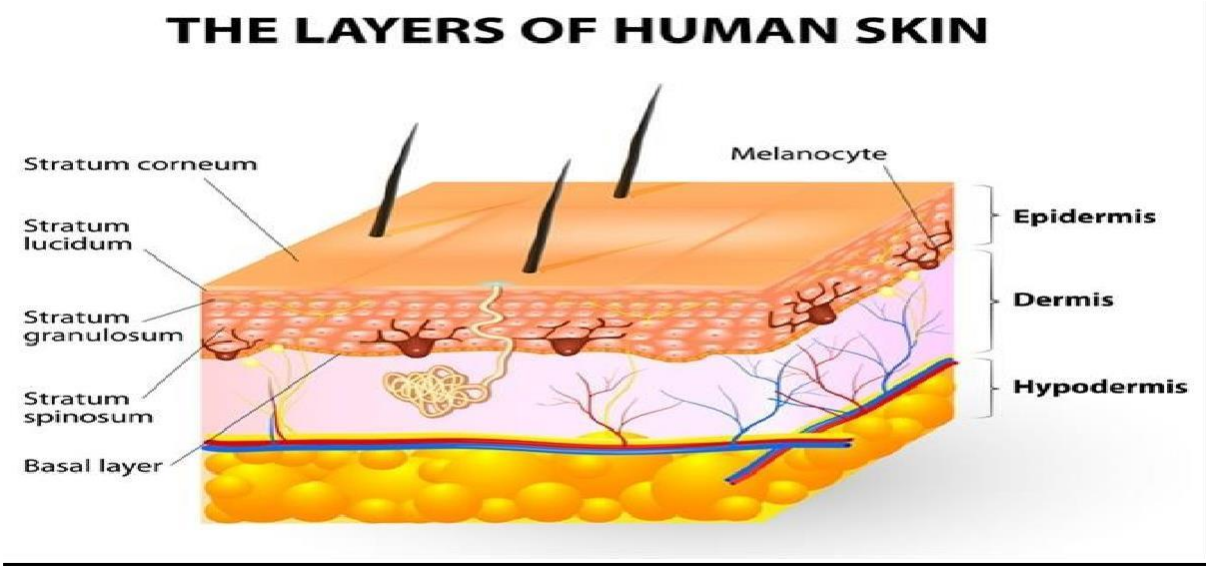

Fig 3: Melanocytes responsible for patchy skin colour loss, source: wikipedia

However, there are several problems associated with this disease that people may experience such as:

The colorless patches of the skin are more sensible to the sunlight than to the normal skin.

Also, people suffering from vitiligo has some kind of abnormalities in their retinas and there is some color variation in the irises however, vision is not affected by this. Another problem associated with person having vitiligo is that they are more likely to be affected by other autoimmune disease. For eg. Hypothyroidism, Diabetes, etc. People suffering from vitiligo may also experience anxiety or embarrassment due to the color of their skin. This may lead them to depression or may lower their self-esteem and affects quality of life.

OBJECTIVE

- **To study the role of autophagy in vitiligo.**
- **To study the role of autophagy related gene 7 in vitiligo.**
- **To find the ATG7 variants and their role in vitiligo susceptibility**

REVIEW OF LITERATURE

THEORIES FOR PATHOGENESIS OF VITILIGO:

The pathogenesis of vitiligo is complex and it involves various factors, however the exact pathogenesis for vitiligo is unknown.

The neural theory:

The neural theory of vitiligo explains that the melanin production is decreased due to the accumulation of neurochemical substances. The abundant norepinephrine acts as an extrinsic factor which is secreted by nerve endings or keratinocytes results in the direct melanocytes impairment which are different from neural crest cells. It was found in one of the studies that levels of norepinephrine (NE), epinephrine (E), normetanephrine (NMN), metanephrine (MN) was found to be comparably higher in patients suffering from vitiligo than control. Also, the impairment of sympathetic nervous system's role (SNS) activity affect production of melanin and results in depigmentation.

The autoimmune hypothesis:

This theory explains that melanin producing cells are killed by autoimmune effector mechanisms, either by memory cytotoxic T cells or as a result of tolerance breakage autoantibodies which are directed surface antigens of melanocytes might kill melanocytes. Also, it is known that vitiligo is related to various autoimmune diseases and it has been found that Hashimoto thyroiditis which is autoimmune thyroid disease is the most common type of disorders associated to vitiligo. In 2003, Gauthier et al. proposed melanocythorragic hypothesis in which it was mentioned that non-segmental vitiligo (NSV) occurs as a result of melanocytorrhagy or chronic melanocyte detachment and trauma loss or various other stress related elements including autoimmune elements. However, the etio-pathogenesis of generalized or non-segmental vitiligo can be explained in a better way with the help of autoimmune mechanisms as vitiligo often has autoimmune comorbidities and it responds to immunosuppressive treatments.

The biochemical theory-reactive oxygen species model:

This theory suggests the imbalanced redox (reduction-oxidation) state of the depigmented skin. As a result of imbalanced redox state there is formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2). Therefore, these reactive oxygen species oxidizes the cell components which results in melanocytes destruction and creates the depigmented patches in the skin.

However, among all the theories this theory plays an important role in the onset and progression of the disease.

Viral theory:

This theory explains about the strong association between vitiligo and chronic hepatitis C virus (HCV) infection and autoimmune hepatitis. In 2006, Akcan et al. reported a low hepatitis B virus (HBV) sero-positivity in patients suffering from vitiligo. Also, the previous or concurrent cytomegalovirus (CMV) infections may induce the etio-pathogenesis or deterioration of Vitiligo.

However, other viruses such as hepatitis E virus, herpes virus, HIV may also have association with vitiligo. [4,5]

AUTOPHAGY:

Autophagy may be a vital process during which the body's cells "clear out" any unnecessary or damaged components.

Autophagy is nothing but a process in which a cell work towards their own cells and remove unnecessary and damaged component of the body. It is a process which can be used in maintain the homeostasis of the body and for cellular recycling and cell degradation. The process of autophagy can mainly be seen during starvation of cells , in which the cells undergo ketosis (through the process of autophagy) to keep the process of homeostasis in control[6]. Any defects in this process are directly correlated to different kind of human sickness, neuro degeneration and malignant tumours.

Autophagy can mainly be classified into three groups: macroautophagy , microautophagy and chaperone mediated autophagy .In infection , autophagy has been viewed as a versatile reaction to the stress ,advancing endurance of cell, but in different cases it seems to showcase necrobiosis and dismaleness. However during extreme cases, it can lead to breakdown of the cell for the cellular survival[7].

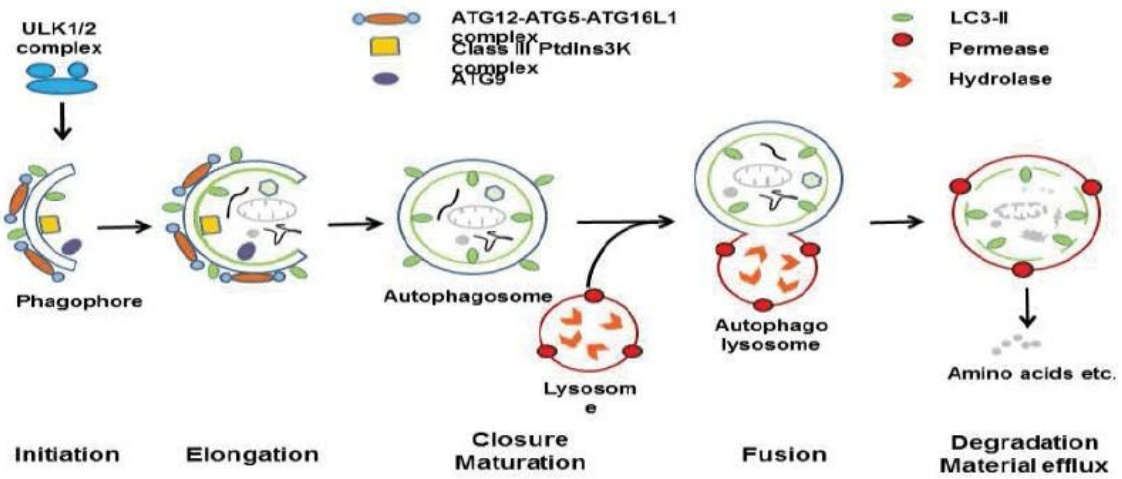

Figure 1.

A dynamic process in autophagic flux.

Fig 4: Process of autophagy (self-cleaning mechanism) ; source:wikipedia

Autophagy is the regular, directed system of the cell that takes out trivial or futile fragments. It allows the orderly debasement and reusing of cell fragments. Autophagy moreover expects a huge capacity in the homeostasis of non-starved cells.

Autophagy can be of various sorts:

1. **MACROAUTOPHAGY**: it is a crucial pathway, used to annihilate hurt cell organelles and proteins which are of no used. this type of pathway is used to degrade large complexes but they do not directly fuse with lysosome. they first, fuse with the autophagosome and then when the cell matures, they combine with lysosome to start the process of autophagy.

2. **MICROAUTOPHAGY**: this type of pathway also degrades large complexes but the difference between the micro and macro autophagy is that in micro autophagy the material to be destroyed directly fuses with the lysosome but this is not the case in macroautophagy**.**

3. **Chaperone mediated autophagy:** the chaperones are the molecules which are generally associated with protein folding and destruction of such molecules can lead to destabilization in the membrane and the structures**.** This type of pathway includes the acknowledgement by the hsc -70 containing complex, which can further be associated with the chaperone substrate and chaperone complex.

4. **Mitophagy:** autophagy of the components of the mitochondria and their degradation to maintain the homeostasis of the nonstarved cells.

5. **Lipophagy**: it is the debasement of lipids via autophagy.

ABOUT *ATG7*:

Autophagy related 7 is a protein in individuals is encoded by ATG7 gene itself . It is related to GSA7; APG7L; APG7-LIKE. ATG 7, present in both plant and animal genomes, goes probably as a crucial protein for cell corruption and its reusing. The gathering accomplices of this protein with the ubiquitin-proteasome structure (UPS), is required for the novel improvement of an autophagosomal layer [3]. This gene is present on the chromosome number 3 in humans and chromosome number 6 in mice.

Autophagy is a significant cell measure that helps in looking after homeostasis It can be used for degrading the cellular components and for their recycling. During the beginning of autophagy, ATG7 acts like an E-1 substance for ubiquitin-like proteins (UBL) , which includes ATG12 and ATG8. ATG7 helps the UBL proteins in zeroing in on their molecule by official to them and impelling their trade to an E-2 substance [2,7]. .ATG7's part in both of these

autophagy-express UBL structures helps in putting together all the elements of autophagososme .

ATG 7 is consistently associated with ATG12/ATG5 sequenced ubiquitination course. Additionally in presence of p53 cell cycle pathways during centered and supplement vulnerable conditions..

ROLE OF *ATG7* IN AUTOPHAGY:

This gene encodes an E1-like initiating catalyst that is fundamental for autophagy and cytoplasmic to vacuole transport. The encoded protein is additionally thought to regulate p53-subordinate cell cycle pathways during delayed metabolic pressure[7] .It has been related with various capacities, including axon film dealing, axonal homeostasis, mitophagy, fat separation, and hematopoietic immature microorganism upkeep.

Autophagy with its marker autophagy-related quality 7 (ATG7) assumes a significant part in the pathogenesis of vitiligo through its impact on melanogenesis and MC untimely senescence[6].

SINGLE NUCLEOTIDE POLYMORPHISM:

SNPs are commonly known as single nucleotide polymorphism, is nothing but a change in nucleotide in the genome which can lead to genetic variability. This change can be because of many biological and chemical reasons but in general it can occur every once in 1,000 nucleotides which means there are roughly 4 to 5 million SNPs in a person's genome. For example, if A nucleotide appears in most of the individuals which is occupied by G in some minority groups then this means that SNP at this specific position have two possible nucleotide variations i.e. G and A.

IMPORTANCE OF SNPs:

1. They can help predict response of individuals to a certain drug.

2. They are generally associated with some major diseases like diabetes, cancer, rheumatoid arthritis, sickle cell anemia etc. so detecting the change in nucleotide position can help detect the root-cause of these diseases in individuals.

3. They play a major role in forensics.

4. SNPs can be used to as one of the crucial tools responsible for population differences worldwide. This can be considered with an example of albino's differing from Africans.

Types of SNPs:

There are two types of SNPs which includes:

1. **Coding SNPs**
2. **Non coding SNPs**

   While the SNPs in the coding region can further be divide into **synonymous** and **non-synonymous** SNPs.

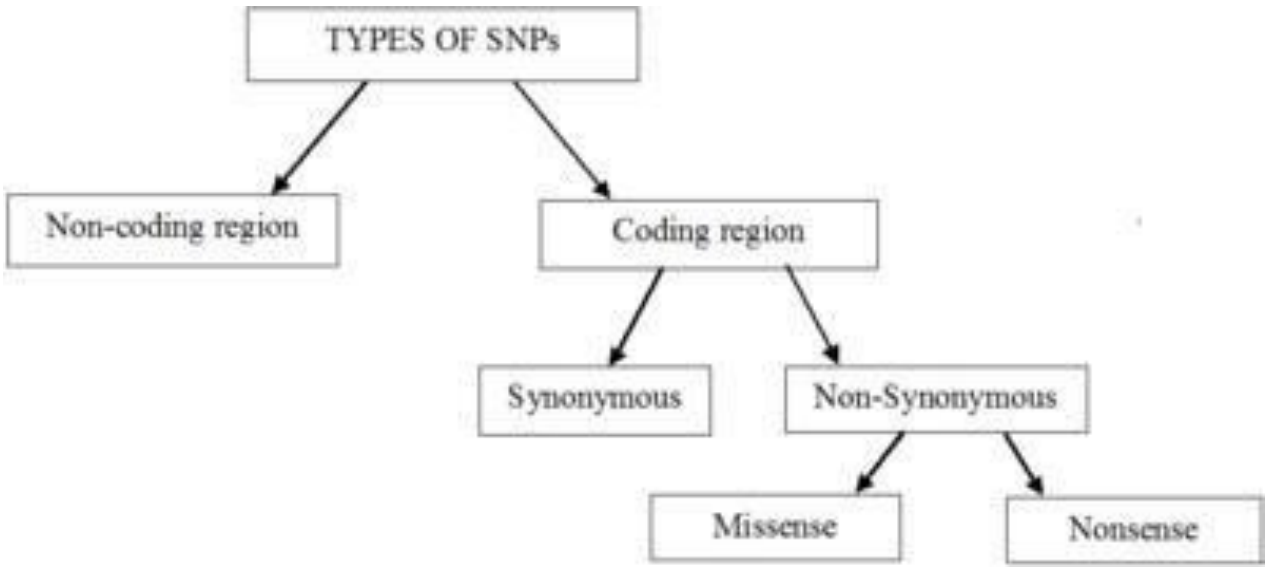

Fig 5: shows different types of SNPs; source: Wikipedia

The non- coding SNPs are more dangerous than the coding SNPs because they can lead to increased probability of cancers and can affect mRNA structure and disease susceptibility. While the SNPs in the coding region do not necessarily change the amino acid sequence due to degeneracy of codon.

The SNPs in the coding region can be of two types:

1. Synonymous SNPs : These are those SNPs which after translation do not result in the change of amino acid in the protein chain formed but still can affect the function in other ways .This can be seen in the MDR1 gene in which there is a change at the 412 amino acid position, where glycine changes to isoleucine .

2. Non synonymous SNPs: These are those SNPs which create a change in the amino acid by two ways
    - Missense changes
    - Nonsense changes

Missense changes on the other hand can lead to a single change in the base results in the change of amino acid of the protein which causes diseases. For example, LMNA gene.

While in non- sense mutation a point mutation in the DNA sequence can result in generation of premature stop codon. This can be seen in cystic fibrosis caused by G542X gene.

ANALYSIS OF SNPS:

SNP analysis can be done easily by using techniques such as DNA sequencing, capillary electrophoresis, mass spectrometry, gel electrophoresis, denaturing HPLC, restriction length polymorphism (RFLP), hybridisation etc.

Using these techniques, we can detect the single nucleotide change in the genome and analyse the difference by comparing it with the genome of other individuals, any abnormal change in nucleotide can be detected by these techniques.

Prediction for SNPS:

There are various programs that are used to detect various missense and point change mutations which can lead to various diseases. By using various machine learning derived rules of a programs SNPs can be detected by using:

1. **Sorting intolerant from tolerant (SIFT):** used to detect missense or non-synonymous mutations

2. **Local identity and shared taxa (LIST):** It depends with the understanding that varieties saw in firmly related species are more huge while evaluating protection contrasted with those in remotely related species.

3. **Missense 3-D:** is a tool which provides a stereo chemical report on the effect of missense variants on protein structure.

4. Some other includes **SNAP2, Predict SNP, PolyPhen 2 (Polymorphism phenotyping v2), SuSPect** etc.

ROLE OF SNPs IN SUSCEPTIBILITY TO VITILIGO:

Vitiligo is a disease which can be caused because of various factors, it can be environmental, genetic, metabolic and can also because of immune triggering incidents.

According to the paper, Genetic polymorphisms of GZMB and vitiligo, published in 2018, the GZMB gene that encode for granzyme B plays an important role in the cytotoxic T cell induced apoptosis. There are multiple hypothesises that indicate the cause of vitiligo because of the mis-sense mutations in the genome. The missense SNP, rs8192917 (Arg55Gln), was identified to be significantly associated with status of vitiligo.

Thus, it can be concluded that various mutations in the genome can lead to susceptible cause of vitiligo.

SNPs and ATG7 rs 35807939:

The specific ATG7 strain which is studied is rs35807939 , the rs here stands for ref SNP;s .

SNPs are abbreviated as single nucleotide polymorphisms ,which is single point mutation responsible for change at a single point at genome .The population differences observed is because of these SNPs .

The rs35807939 on the hand, is not studied in depth but it can be concluded that it's mutant strain is associated in causing diseases such as diabetes, liver cancer and Huntington disease[8].

METHODOLOGY & CONCLUSION

After searching the web, to get the full understanding of the project topic, we came across some research papers and review articles of the publishers mostly associated with Autophagy , Atg7 gene in vitiligo susceptibility , vitiligo causes and symptoms. However, there is only one research paper about ATG7 variant rs358079. It is found that there around 474 SNPs and further these SNPs can be analysed using different tools to find the susceptibility of SNPs towards vitiligo.

We used search engines which mostly included GOOGLE SCHOLAR and NCBI, also the open library, science.gov and Wikipedia. Some key words which were used for searching on these search engines included:

1. **MELANOCYTES**
2. **CHAPERONS**
3. **HOMEOSTASIS**
4. **UBIQUITINE-LIKE PROTEIN**
5. **AUTOIMMUNE DISEASES**
6. **AUTOPHAGOSOME**
7. **PHAGOPHORE**

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