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Research Article

Integrated Whole Exome and Transcriptome Sequencing in Cholesterol Metabolism in Melanoma: Systematic Review

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Abstract

Background: Melanoma is a highly malignant form of skin cancer that exhibits remarkable metabolic adaptability. Melanoma cells exhibit the capacity to adapt to specific conditions of the tumor microenvironment through the utilization of diverse energy sources, thereby facilitating the growth and advancement of the tumor. One of the notable characteristics of metabolic reprogramming is the heightened rate of lipid synthesis. This review was conducted to illustrate how the integration of whole exom and transcriptome sequencing will enhance the detection of the effect of cholesterol metabolism in melanoma.
Methods: The Cochrane database, Embase, PubMed, SCOPUS, Google Scholar, Ovid, and other databases were thoroughly searched for works addressing integrated whole exome and transcriptome sequencing in cholesterol metabolism in melanoma. Skin malignancy, melanoma progression, transcriptome sequencing, whole exome sequencing, transcriptome sequencing by RNA sequencing, and integrated transcriptome and whole exome sequencing were the key phrases employed. This article underwent a phased search for pertinent literature using a staged literature search methodology. Each section's relevant papers were identified and summarized independently. The results have been condensed and narratively given in the pertinent sections of this thorough assessment.
Results: DNA-based analysis has proven to be ineffective in identifying numerous mutations that have an impact on splicing or gene expression. RNA-Sequencing, when combined with suitable bioinformatics, offers a reliable method for detecting supplementary mutations that aid in the genetic diagnosis of geno-dermatoses. Therefore, clinical RNA-Sequencing expands the scope of molecular diagnostics for rare genodermatoses, and it has the potential to serve as a dependable initial diagnostic method for expanding mutation databases in individuals with inheritable skin conditions.
Conclusion: The integration of patient-specific tumor RNA-sequencing and tumor DNA whole-exome sequencing (WES) would potentially enhance mutation detection capabilities compared to relying solely on DNA-WES.

Keywords: skin malignancy, melanoma progression, whole exome sequencing

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1. Introduction

1.1. Melanoma

Eighty percent of skin cancer-related fatalities are caused by melanoma [1], a skin cancer that develops from melanocytes. Environmental elements and genetic changes interact intricately throughout the development of melanoma. On skin that has been exposed to the sun, melanoma is most frequently detected in Caucasians. Melanoma typically exhibits somatic mutations in BRAF, NRAS, or neurofibromatosis 1 (NF1), and it has been demonstrated that the frequency of these mutations is influenced by the total exposure to UV light and the anatomical site [2].

The occurrence of melanoma is attributed to the alteration and proliferation of melanocytes [3]. The incidence of melanoma exhibits a notable rise with advancing age, while the likelihood of survival tends to decrease, even when considering variables such as tumor grade and stage [4]. According to available data, the number of fatalities associated with melanoma in the United States in 2020 was estimated to be 6850 [5]. In contrast to the decreasing incidence rates observed in breast and lung cancers, there has been a consistent upward trend in the occurrence of melanoma over the past four decades. During the period 2006–2015, there was a statistically significant rise of 3% in the occurrence of melanoma among individuals aged 50 and older, including both males and females. The typical age at which melanoma is diagnosed was determined to be 62 years [6]. In addition, it has been noted that older individuals display a greater occurrence of metastases, encounter lower rates of overall survival, and manifest a reduced response to targeted therapy in comparison to younger individuals [7, 8]. Melanoma tumors exhibit significant heterogeneity and often comprise cell populations with distinct transcriptional states, referred to as melanoma phenotypes. Significantly, the range of individual phenotypes, spanning from an undifferentiated to a fully differentiated state, can have a notable influence on therapy [9, 10] as well as on the invasion and metastasis of melanoma [11].

1.2. Sterols

Sterols are a category of organic compounds belonging to the isoprenoid family. Cholesterol is the primary sterol present in mammalian tissues. Cholesterol is important in maintaining the integrity and fluidity of cellular membranes. Furthermore, it serves as a vital constituent of lipid rafts, thereby exerting regulatory control over processes such as endocytosis, membrane trafficking, cellular signaling, and cell motility. The enzyme HMGCR(3hydroxy-3-methylglutaryl-COA reductase) catalyzes the reduction of HMG-CoA

(3-hydroxy-3-methylglutaryl CO enzyme A) to mevalonate, serving as the initial rate-limiting step in the biosynthesis of cholesterol. Additionally, this enzymatic pathway also enables the production of squalene [12, 13].

Cholesterol is synthesized endogenously through the action of HMGCR in melanocytes. However, it is important to acknowledge that the uptake of exogenous cholesterol can also occur via the LDL receptor (LDLR)/Apo-B100 pathway [14]. The regulation of the process of cholesterol biosynthesis and uptake in non-cancerous cells is tightly controlled through a negative feedback mechanism that detects intracellular cholesterol levels [15]. The upregulation of ATP-binding cassette (ABC) transporters is observed to enhance the process of cholesterol efflux, and

a decrease in cholesterol uptake [13]. Acyl-CoA-cholesterol acyl transferases (ACATs) possess the capacity to enzymatically transform cholesterol molecules into cholesteryl esters, which are less toxic and typically stored in lipid droplets (LD). Alternatively, ACATs possess the capability to enzymatically transform cholesterol into oxidized derivatives, which are subsequently metabolized into bile acids that are excreted via the digestive system [16].

1.3. Potential roles of cholesterol in melanoma progression

Due to their high proliferative nature, tumor cells need cholesterol to support their rapid growth and fulfill their significantly increased nutrient needs for membrane generation, which promotes carcinogenesis and progression. Indeed, the proliferation, motility, stemness, and treatment resistance of tumor cells can be controlled by cholesterol, its derivatives, and its production intermediates [17].

1.3.1. The critical importance of disturbance of cholesterol homeostasis in cancer

The regulation of cholesterol homeostasis is disrupted in cancer cells, leading to significant effects on cancer progression, such as alterations in cell proliferation, migration, and invasion [18]. These alterations encompass heightened biosynthesis of cholesterol, augmented uptake of exogenous cholesterol by LDLR, enhanced esterification of cholesterol by ACAT1, and increased production of oxysterols [19]. The activation of the SREBP (sterol regulatory element-binding protein), pathway which is a transcription factor that binds to the sterol regulatory element DNA sequence, and the establishment of a positive feedback loop between SREBP-dependent lipogenesis and PI3K-AKT-mTORC1 signaling (PI3K: Phosphatidyl inositol-3-Kinase, ATK: Protein Kinase B, mTOR: mammalian target of rapamycin are intracellular signaling pathways important in regulating the cell cycle) contribute to the proliferation of melanoma cells, both in vitro and in vivo [20]. Of note, the activation of the SREBP pathway was observed regardless of the presence of the oncogenic BRAF mutation. The examination of data obtained from the Cancer Genome Atlas (TCGA) indicates that approximately 60% of melanoma samples demonstrate heightened expression or chromosomal copy number variations in one or more genes linked to the process of cholesterol synthesis. Upregulation of number of these genes exhibited a significant association with reduced survival rates among individuals diagnosed with melanoma [21]. Furthermore, it has been reported that the oxysterol 27-hydroxycholesterol (a derivative of cholesterol obtained by oxidation involving enzymes and/or pro-oxidants) plays a role in enhancing the proliferation of melanoma cells by sustaining the AKT/MAPK (AKT: Protein Kinase B, MAPK: Mitogen Activated Protein Kinase) signaling pathway [22, 23]. Cumulatively, these studies provide evidence of significant associations between heightened cholesterol metabolism and the advancement of melanoma.

The phenomenon of exogenous cholesterol has been noted to augment the melanogenesis process in melanocytes and intermediate pigmented melanoma cells; this effect is believed to be mediated through the production of cyclic adenosine monophosphate (cAMP), which subsequently activates the CREB/MITF/tyrosinase pathway (CREB: cAMP dependent protein Kinase, MITF: melanocyte stimulating hormone). Additionally, Schallreuter and colleagues postulate that exogenous cholesterol may potentially play a role

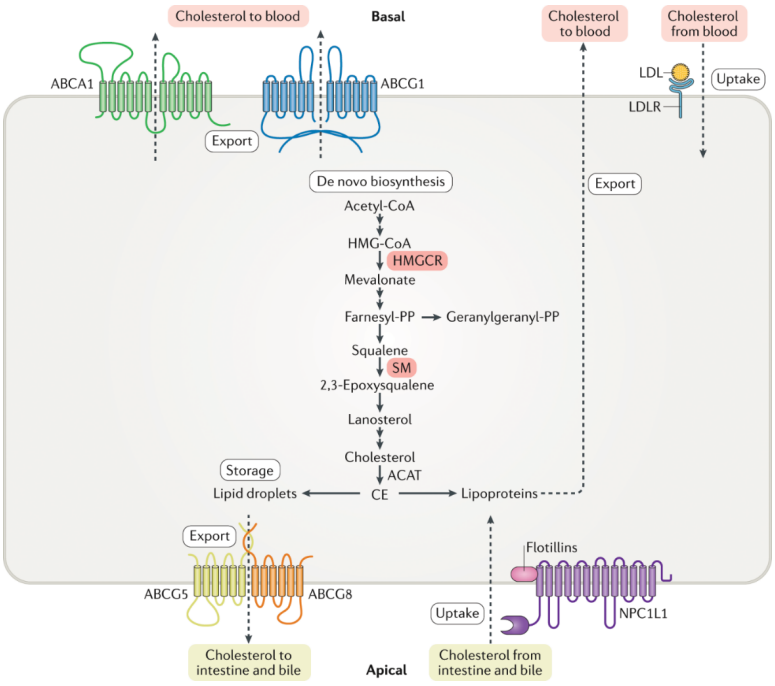

Figure 1: Major pathways of cholesterol metabolism [15].

in this biological phenomenon by enhancing the stability of cellular membranes and protecting melanogenic enzymes from degradation by the proteasome [14]. This finding implies a potential increased requirement for cholesterol in the early stages of melanosomes. The potential association between cholesterol levels and the process of melanoma cell dedifferentiation remains uninvestigated in scientific literature. It is noteworthy that the temporary increase in CD271 expression, which is a marker of melanoma cells that have undergone dedifferentiation and possess stem-like characteristics and invasive capabilities, resulted in the upregulation of genes associated with the synthesis of cholesterol [24].

1.3.2. Lipid metabolic reprogramming and role in melanoma progression

It is apparent that specific proteins involved in the metabolic pathway of cholesterol have been observed to contribute to the enhancement of melanoma's aggressiveness, irrespective of their established metabolic functions. The scavenger receptor class B type I (SR-BI) has been demonstrated to facilitate the specific internalization of high-density lipoprotein (HDL) cholesteryl ester into various cell types, such as hepatocytes and steroidogenic cells [25]. Moreover, it has been observed to induce a process resembling epithelial–mesenchymal transition (EMT) [26]. The investigation of SR-BI has elucidated its impact on the regulation of MET, a proto-oncogene that serves as a target gene for MITF and plays a critical role in extracellular vesicle (EV) formation. The examination of SR-BI's potential role in facilitating the production of extracellular vesicles (EVs) warrants careful consideration [26].

Metastatic colonization pertains to the mechanism through which cancerous cells disseminate from the primary neoplasm to remote locations within the body. However, the existing evidence does not currently establish the potential role of SR-BI as a regulatory factor for MITF. Numerous studies have provided evidence for the existence

of a regulatory/feedback mechanism involving SR-BI and MITF [27]. This finding aligns with the significant correlation observed in the expression of these two entities in accordance with the strong correlation observed in samples from patients with melanoma [26].

The application of methyl-β-cyclodextrin (MβCD), a derivative of cyclodextrin, to mice with melanoma resulted in the inhibition of tumor growth and an increase in the lifespan of the animals. In terms of its mechanism of action, it has been demonstrated that MβCD effectively inhibits the activity of protein kinase B/AKT. Apoptosis was induced in human melanoma cells by MβCD, as evidenced by previous studies [28]. Additionally, the cellular morphology and migration of these cells were also influenced by MβCD [29]. To be more precise, the utilization of MβCD led to the deactivation of Src by causing its separation from lipid rafts and ultimately, the process of focal adhesion disassembly is suppressed [29]. Furthermore, the reduction of cholesterol levels significantly affects the proton pumping functions of the V-ATPase, resulting in a decline in the migratory and invasive capabilities of B16F10 melanoma cells [30]. The proton pump plays a crucial role in the regulation of acidity within the tumor microenvironment (TME), thereby facilitating the optimal functioning of proteolytic enzymes like metalloproteinases and cathepsins. As a result, this phenomenon establishes a favorable microenvironment that facilitates the process of cellular migration and invasion [31].

The studies presented collectively provide evidence for the crucial involvement of lipid rafts containing cholesterol in preserving cellular morphology and enabling the requisite functions for the metastatic advancement of melanoma. However, the specific mechanisms underlying this phenomenon remain unidentified.

Cholesterol plays a crucial role in both the development and functioning of invadopodia, specialized membrane microdomains that are rich in cholesterol and necessary for localized degradation of the extracellular matrix (ECM). The formation, function, and structural integrity of invadopodia in human melanoma cells have been demonstrated to rely on the levels of cholesterol in the plasma membrane. Additionally, caveolin 1 serves as a crucial mediator of these processes [32]. Moreover, it is widely acknowledged that cholesterol plays a crucial role in the regulation of membrane fluidity [33]. Recent research has revealed that mobile cancer cells have a tendency to display reduced levels of membrane cholesterol. This reduction in cholesterol is believed to enhance the fluidity of the plasma membrane, thereby facilitating the cells' capacity to infiltrate different types of tissues [34, 35]. The expression of the ABCA1 transporter, which is recognized for its involvement in cholesterol transport reversal, has been discovered to occur during epithelial–mesenchymal transition (EMT) and to facilitate the development of metastatic traits in both experimental settings and living organisms [35].

The current body of evidence pertaining to the interaction between cholesterol functions and the progression of cancer suggests a complex relationship between cholesterol and disease that is not fully understood. We set out to produce this review article in an effort to close the knowledge gap surrounding integrated whole exome and transcriptome sequencing in cholesterol metabolism in melanoma because there are currently not enough reviews or research works on the subject.

Our study's goal is to explain how, in comparison to relying simply on DNA-WES, the integration of patient-specific tumor RNA-sequencing and tumor DNA whole-exome sequencing (WES)

might improve the ability to detect mutations in melanoma patients with high cholesterol levels.

2. Methods

2.1. Search strategy

A comprehensive literature search was done on PubMed, SCOPUS, Embase, Cochrane database, Google Scholar, and Ovid to identify articles discussing integrated whole exome and transcriptome sequencing in cholesterol metabolism in melanoma. Keywords used were skin malignancy, melanoma progression, whole exome sequencing, RNA sequencing, transcriptome sequencing, and integrated whole exome and transcriptome sequencing. The search terms were used as keywords and in combination as MeSH terms to maximize the output from literature findings. Using a staged literature search, this article was searched for relevant literature in a phased manner, in which each section was searched separately, and all relevant studies were identified and summarized separately for each section. Studies published in English language were selected, and no restrictions were imposed on the duration of publication. The authors of this study have diligently identified and evaluated all pertinent articles, and the findings have been succinctly presented in a narrative format within the relevant sections of this comprehensive review.

2.2. Inclusion and exclusion criteria

Studies were included if they have reviewed a correlation between a melanoma and the cholesterol. Exclusion criteria were editorials, consensus documents, commentaries, and studies with no particular definition of the role of cholesterol in melanoma.

2.3. Data extraction

All articles were screened. The study was conducted as follows: We began by writing a brief paragraph discussing melanoma, potential role of cholesterol metabolism in melanoma progression, the principles and methods of whole exome sequencing, and the principles and methods of transcriptome sequencing. Then, we discussed the applications of whole exome sequencing to identify rare and low frequency coding variant associated with LDL cholesterol, whole-exome sequencing reveals novel genetic variants associated with diverse phenotypes of melanoma cells, genomic classification of melanoma, transcriptomic classification of melanoma, novel gene fusion in melanoma by transcriptome analysis, and integrated whole exome and transcriptome sequencing.

3. Results and Discussion

3.1. The principles and methods of Whole exome sequencing

3.1.1. Exome sequencing in genetic disease: Recent advances and considerations

The historical emphasis in the field of disease etiology has been on identifying a specific causal variant that is responsible for a particular phenotype. This methodology has demonstrated efficacy in the context of diseases that are seemingly governed by a single gene, such as cystic fibrosis [36] or Huntington's disease [37]. Linkage analysis was initially employed to investigate the segregation of genetic variants associated with a specific phenotype within a pedigree [38]. While early linkage studies were crucial in identifying connections between basic

genetic factors and diseases, they often required additional experiments to precisely locate disease loci and identify specific protein-altering variants within genes. The majority of disease-causing variants, comprising more than 60% of all known causative genomic variation, can be attributed to protein-coding variants that are either inherited or acquired[39, 40]. Exome sequencing (ES) refers to the focused sequencing of the majority of protein-coding regions within the genome [41, 42]. In the context of variant discovery, it is common practice to employ either hybridization capture or multiplex primer-based amplification techniques to produce libraries containing exonic sequences. These libraries can then be aligned to the reference genome in order to identify variants. Due to the extensive availability of information pertaining to protein-coding genes in comparison to other segments of the genome, ES effectively utilizes thoroughly sequenced and mapped regions of the genome in conjunction with computational predictions of protein functionality. The discipline of genetics has transitioned from a method involving the identification of multiple loci through a series of steps, followed by subsequent resequencing, to a more contemporary approach of simultaneously testing a comprehensive set of protein-coding genes [43].

As the field of genetic research advances, the scope of diseases and syndromes that can be attributed to a single variant or a solitary altered gene diminishes progressively. In contemporary research, there is a growing interest in investigating the impact of genetic modifiers on the severity and age at onset of diseases that were traditionally classified as monogenic[44, 45]. Monogenic or familial variants of a heterogeneous disease typically represent a minority within the overall population affected by the disease [38]. The genetic basis of amyotrophic lateral sclerosis (ALS) serves as an illustrative example for the proposed multi-step model, which aims to integrate the influence of genetic variants and environmental exposure on disease risk [46]. In the proposed model, it is postulated that a single genetic variant may be responsible for multiple, or all of the essential processes required to initiate the onset of a disease. Nevertheless, it is possible for the same disease to be acquired through various lower penetrance variants or through a complex interplay between genetic and environmental factors.

3.1.2. Whole exome sequencing

The human genome is estimated to comprise approximately 3 X 10^9 bases and encompasses approximately 180,000 coding regions, commonly referred to as the exome. These coding regions make up approximately 1.7% of the entire human genome. According to estimates, approximately 85% of mutations that lead to disease are found within the exome. Due to this rationale, the process of sequencing the entire exome holds the capacity to reveal a greater number of pertinent variants at a significantly reduced expense compared to the sequencing of the entire genome. The utilization of whole exome sequencing is widely regarded as a highly effective and robust method for the identification of genetic variations that impact heritable phenotypes. This includes significant mutations responsible for disease manifestation as well as naturally occurring variations that can be leveraged for the enhancement of agricultural produce and livestock [47, 48].

The methodology of Whole Exome Sequencing entails the application of exome capture technology to selectively enhance the exonic regions of the genome, followed by the sequencing of these targeted regions in a highly efficient and automated manner. In order to provide precise

details, the initial step involves fragmenting DNA samples, followed by the utilization of biotinylated oligonucleotide probes (referred to as baits) to selectively hybridize with the exome region within the genome. Subsequently, Magnetic streptavidin beads are utilized to facilitate the binding process with biotinylated probes. The elimination of the non-targeted fraction of the genome is achieved through a washing procedure, which is subsequently followed by the application of polymerase chain reaction (PCR) to amplify the concentration of DNA derived from the specific region of interest. Following this, the sample undergoes sequencing using the Illumina HiSeq platform. The implementation of this particular approach has the potential to yield a substantial enhancement in gene coverage for the entirety of the human genome, reaching up to a 100-fold increase. The validated sequencing data is subsequently employed for variant analysis and the generation of clinical statements. [47, 49]

3.1.3. The utilization of whole exome sequencing offers several advantages in the field of genomics

The utilization of cost-effective methods , the widespread accessibility of resources, enhanced sequencing coverage, surpassing a minimum of 120X, the identification of coding single-nucleotide polymorphism (SNP) variants with a level of sensitivity comparable to that of whole genome sequencing, a reduced dataset that facilitates quicker and more convenient analysis in contrast to whole genome sequencing and the potential applications of this technology in the fields of medicine and agriculture [50].

3.1.4. Principle and workflow of whole exome sequencing

The exome is typically described as the portion of the genome that encompasses all exons of protein-coding genes, as well as noncoding elements like microRNA or lncRNA. The analysis of the exome enables the detection of distinct genetic loci that are associated with different pathological conditions. When researchers intend to investigate the exonic information within the human genome, the expenses associated with whole genome sequencing are likely to be remarkably high, given the substantial size of the human genome, which consists of over 3 billion base pairs [51]. In the investigation of uncommon mendelian disorders, exome sequencing has proven to be a superior method for the detection and characterization of genetic variants. The progress of whole exome sequencing has been greatly influenced by the development of target-enrichment strategies and DNA sequencing techniques [52].

3.1.5. Principle of exome sequencing

Exome sequencing comprises two fundamental procedures, namely target-enrichment and sequencing. Target-enrichment is a technique utilized to selectively isolate and capture the exome regions of DNA samples. There are two main methodologies employed to attain exome enrichment [47].

3.1.4.1. Array-based exome enrichment

involves the utilization of high-density microarrays that are equipped with probes designed to capture the exome. A microarray is a two-dimensional array that is commonly constructed on a glass slide, or a substrate made of silicon thin-film. This array is

Figure 2: The sequential steps involved in the process of whole exome sequencing.

composed of oligonucleotides that are specifically designed to be complementary to specific regions of the target genome. During the process of microarray analysis, fragmented DNA samples are subjected to the complementary pairing effect, which leads to the binding of the exome at the microarray. Consequently, the remaining portions of the genome become dissociated, resulting in the separation of the exome from other genomic regions [53].

3.1.4.1. The process of in-solute capturing

relies on the utilization of magnetic beads. The magnetic bead is classified as a magnetic nanoparticle that integrates functional chemical constituents in order to facilitate the binding process with specific target substances. In the present context, magnetic beads are utilized to facilitate the binding of exome. The narrative maintains coherence with the array-based approach, in which the exome is specifically attracted and bound to magnetic beads, while other segments of the genome remain un-associated. An important advantage of utilizing the in-solute capturing method is the improved reaction efficiency achieved by employing magnetic beads, which can facilitate agitation or heating of the system [53].

'Both techniques have demonstrated efficacy in the extraction of exome from the genome. It can be asserted that the sensitivities of both entities are sufficiently elevated. Nevertheless, the issue at hand pertains to the concept of specificity. Certain regions of the genome exhibit a resemblance in the sequence of specific exons. Certain regions of the genome have the potential to form associations with microarray or magnetic beads, which can lead to the occurrence of false positive results [54].

The process of sequencing involves determining the specific arrangement of deoxyribonucleotides within the exome, which can provide insights into potential pathophysiological changes associated with certain diseases. The prominence of whole exome sequencing has been heightened due to the decreased cost associated with this technology. It is anticipated that the cost of sequencing the human genome is two to three times that of sequencing the entire exome. Therefore, it would be advantageous to conduct additional iterations of whole exome sequencing in order to acquire a larger number of samples, thereby enhancing the statistical significance of the obtained results [55].

3.1.6. General workflow of exome sequencing

The following is a depiction of a commonly employed workflow for exome sequencing. The subsequent section will provide a detailed analysis of the primary procedures involved in the workflow.

3.1.7. DNA fragmentation

Most of the experiments involving DNA commence with the process of DNA fragmentation. It is imperative to fragment DNA into appropriate segments due to the typically excessive length of

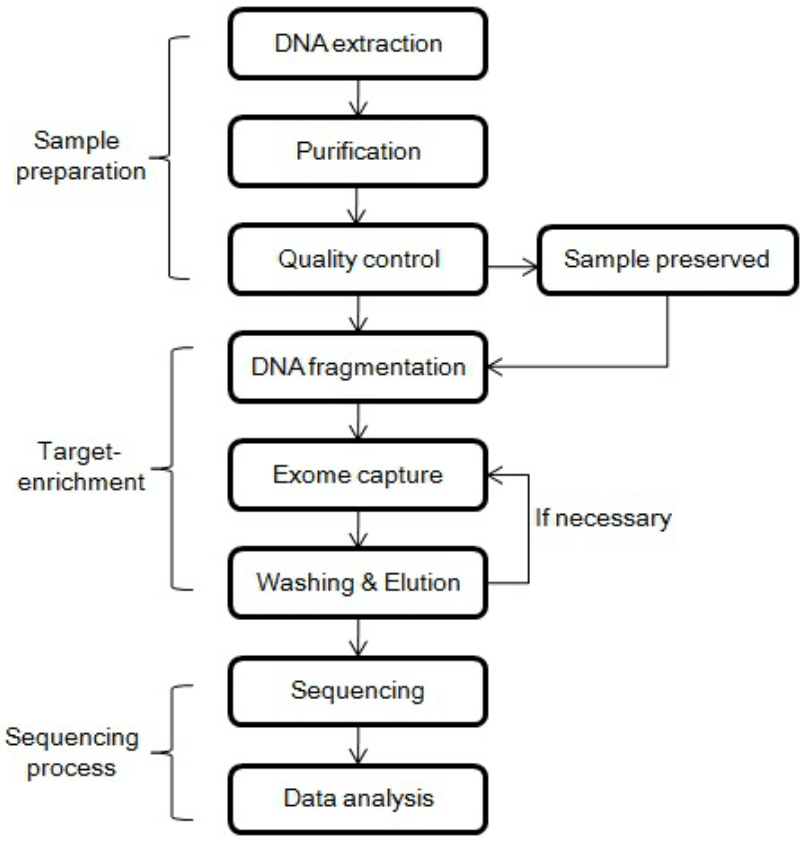

Figure 3: General workflow of exome sequencing [56].

DNA samples obtained from tissues or cells. The process of shearing DNA is commonly referred to as DNA fragmentation. The determination of the optimal target length is contingent upon the selection of the sequencing instrument. In order to enhance the efficiency of whole exome sequencing, there are multiple primary techniques available for the fragmentation of DNA samples [57].

3.1.5.1. Physical fragmentation

Involves a range of techniques, including acoustic shearing, sonication, and hydrodynamic shear. Two commonly used methods for DNA fragmentation are acoustic shearing and sonication. The process of ultrasound exposure leads to the fragmentation of DNA samples through the mechanisms of acoustic cavitation and hydrodynamic shearing.

3.1.5.2. Enzymatic Methods:

Enzymes commonly employed for the fragmentation of DNA molecules into smaller fragments encompass nuclease and transposase. The nuclease enzyme facilitates the hydrolysis of phosphodiester bonds that connect nucleic acid molecules, leading to the degradation of DNA. In particular, restriction endonucleases are enzymes that catalyze the cleavage of DNA molecules at specific recognition sequences known as restriction sites. The transposase enzyme is employed to facilitate transposition events, which involve the relocation of a specific DNA segment within the chromosome. Additionally, the role of transposase in DNA fragmentation is evident when suitable DNA samples are prepared. Instead of undergoing reinsertion, the fragmented DNA is connected with adapters, leading to fragmentation [58].

Following the process of fragmentation, the DNA samples are prepared for the subsequent target-enrichment procedure.

3.1.8. Isolation of exome: Target-enrichment methods

In order to proceed with sequencing, it is imperative to isolate the exome from the entirety of the human genome, as the exome represents a mere 1% of the complete genome. The process of selectively capturing specific genomic regions is commonly known as target-enrichment. Target-enrichment is a fundamental concept that involves the isolation of specific entities of interest from a mixture by exploiting their distinct physicochemical properties in comparison to other substances. Several commonly used target-enrichment methods exist. Regardless of the specific kit selected, the variability in capture has a significant impact on exome sequencing. The consideration of DNA sample quality, quantity, and fragment sizes is of utmost importance [59].

3.1.9. Washing and elution

After the isolation of the exome from non-target regions of the genome, it is imperative to conduct several iterations of rinsing. The act of washing can be understood as the literal removal of undesirable elements in order to preserve the desired object or substance. In this particular context, the inclusion of substances such as additional genomic components, proteins, and electrolytes is considered un-favorable. In general, the eluent of choice for eluting the target substance is commonly distilled water. Nevertheless, the utilization of particular reagent kits may require the application of a specific eluent [60].

3.1.10. Sequencing technology

The Sanger Sequencing technology has not made a substantial contribution to biological and clinical studies as a result of its time-consuming nature and lengthy requirements. However, the advent of next generation sequencing (NGS) technologies changed this scenario. The utilization of dyed dideoxynucleotides (ddNTPs) is a crucial aspect of next-generation sequencing (NGS) technologies, specifically within the Sanger sequencing method. One notable advancement is that Next-Generation Sequencing (NGS) enables the simultaneous combination, amplification, and detection of DNA strands, resulting in a rapid enhancement of sequencing efficiency and the ability to accommodate longer sequence requirements[61]. In essence, the underlying concept of Next-Generation Sequencing (NGS) involves the utilization of specific platforms, such as the Illumina Hiseq flowcell and Roche-454 magnetic beads, to effectively bind exome samples. These samples are subsequently replicated through PCR-in-situ, thereby amplifying the signal during each round of elongation. Subsequently, The detection of deoxyribonucleotide triphosphates (ddNTPs) takes place after every subsequent round of elongation. In the final analysis, the comprehensive sequence is incorporated through the utilization of a biological information algorithm. Next-generation sequencing (NGS) greatly improves operational effectiveness and facilitates the identification of a larger quantity of data. which is why it is commonly referred to as high-throughput sequencing and is extensively utilized [62].

In addition to next-generation sequencing (NGS), the third generation of sequencing technology is currently undergoing rapid development, exhibiting significantly enhanced efficiency compared to NGS. Single-molecule sequencing is considered

the principal characteristic of third generation sequencing. The aforementioned technology significantly decreases the duration needed for the process of whole genome sequencing to a matter of minutes. The effectiveness of methodologies employed by many companies has been demonstrated, suggesting that the third generation of sequencing technology holds promise for a substantial revolution in the domain of exome sequencing [63].

3.1.11. Data analysis

The data obtained from sequencing experiments can be challenging to comprehend and interpret without the aid of bioinformatics analysis. This is primarily due to the fact that many sequencing techniques generate relatively small fragments of sequence information. Consequently, the process of sequence assembly becomes necessary in order to derive meaningful and conclusive outcomes from the data. Researchers with an interest in performing Whole Exome Sequencing (WES) analysis for the purpose of variant calling and investigating genetic diseases can effectively employ the following pipeline [64].

3.2. Bioinformatics workflow of whole exome sequencing

A standard procedure for WES analysis encompasses several key stages: initial assessment of raw data quality, preprocessing of the data, alignment of sequences, subsequent processing of aligned sequences, identification of genetic variants, annotation of these variants, and finally, filtration and prioritization of the identified variants. The following topics will be discussed in the subsequent sections.

3.2.1. Raw data quality control

There are two commonly used standard formats for sequence data, namely FASTQ and FASTA. FASTQ files are capable of storing Phred-scaled base quality scores, which serve the purpose of enhancing the accuracy of sequence quality assessment. Hence, the consensus regards it as the prevailing convention for NGS raw data formatting. Numerous methodologies have been devised to assess the caliber of raw data obtained from next-generation sequencing (NGS) techniques. The aforementioned tools encompass FastQC, FastQ Screen, FASTX-Toolkit, and NGS QC Toolkit.

Examine quality control parameters:

The distribution of base quality scores, distribution of quality scores for sequencing data, the length distribution was examined, the distribution of GC content, the level of sequence duplication, the problem encountered during PCR amplification, the phenomenon of introducing bias to k-mers and the sequences that are present in a disproportionately high number compared to their expected frequency [65].

3.2.2. Data pre-processing

By conducting a thorough examination of the quality control (QC) report, which typically encompasses the aforementioned parameters, researchers are able to ascertain the need for data pre-processing. The pre-processing steps typically encompass the removal of 3' end adapters, filtering of reads with low quality or redundancy, and trimming of undesired sequences. Various tools are available for the purpose of data pre-processing, including Cutadapt and Trimmomatic. Both PRINSEQ and QC3 have the capability to

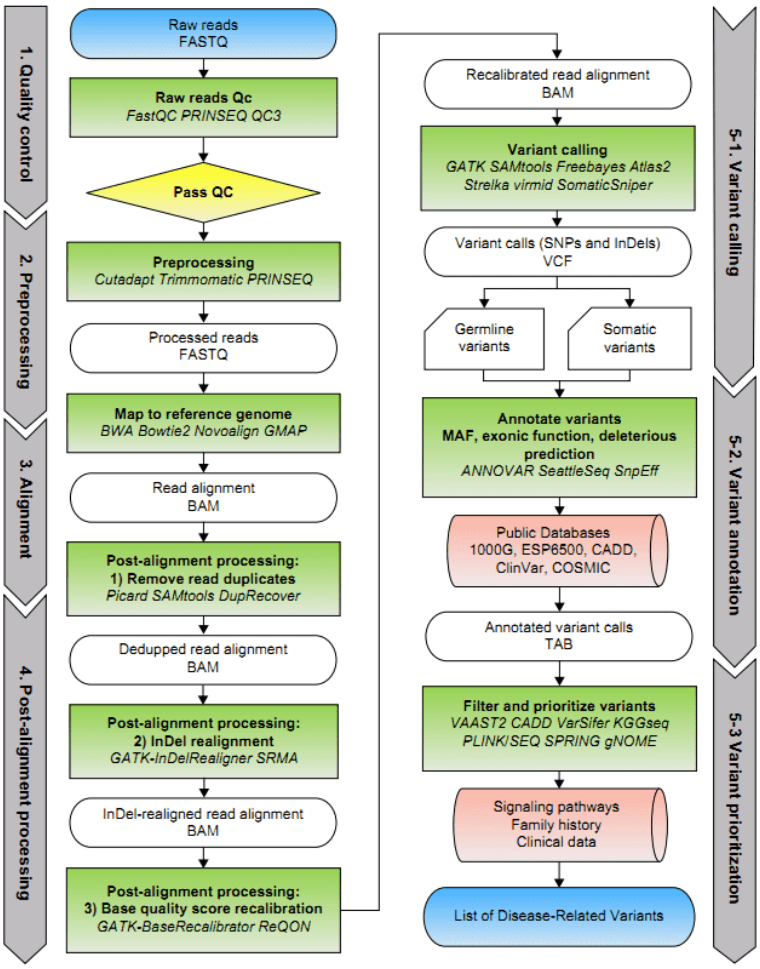

Figure 4: Bioinformatics workflow [66].

perform quality control and pre-processing tasks [67].

3.2.3. Sequence alignment

Various algorithms have been developed for the purpose of mapping shot reads, Examples of algorithms that can be mentioned include the Burrows-Wheeler Transformation (BWT) and the Smith-Waterman (SW) algorithm. Bowtie2 and BWA are commonly employed alignment tools for short reads, both of which utilize the Burrows-Wheeler Transformation (BWT) algorithm. MOSAIK, SHRiMP2, and Novoalign are widely acknowledged as prominent tools utilized for the alignment of short reads. as they serve as implementations of the Smith-Waterman algorithm with enhanced precision. Moreover, the utilization of multithreading and MPI implementations results in a substantial decrease in the overall execution time. One tool that distinguishes itself from the others mentioned is Bowtie2, primarily due to its rapid execution time, notable sensitivity, and exceptional accuracy [66].

3.2.4. Post-alignment processing

After the read mapping process, the aligned reads are subjected to post-processing in order to remove undesirable reads or alignments. These undesirable reads include those that exceed a predetermined size and polymerase chain reaction

(PCR) duplicates. Specialized software tools, such as Picard MarkDuplicates and SAMtools, can be utilized to accurately distinguish PCR duplicates from authentic DNA materials. Subsequently, the subsequent step entails improving the accuracy of gapped alignment by means of indel realignment. Several aligners, including Novoalign, and variant callers, such as GATK HaplotypeCaller, employ strategies to improve the alignment of insertions and deletions (indels). In order to improve the accuracy of base quality scores prior to variant calling, it is recommended to employ BQSR (BaseRecalibrator from the GATK suite) subsequent to the indel realignment process [68].

3.2.5. Variant calling

Variant analysis is of utmost importance in the process of identifying and characterizing diverse genomic variants, encompassing single nucleotide polymorphisms (SNPs), single nucleotide variants (SNVs), insertions and deletions (indels), as well as copy number variations (CNVs)., and structural variations (SVs). This analytical approach holds particular significance in the domain of cancer research. Differentiating between somatic and germline variants is crucial. Somatic variants are exclusively found in somatic cells and exhibit tissue-specific characteristics, whereas germline variants are hereditary mutations that occur in germ cells and are associated with the familial medical background of the patient. The process of variant calling is employed for the purpose of detecting single nucleotide polymorphisms (SNPs) and short insertions or deletions (indels) in exome samples. Several studies have assessed the performance of these variant callers. Liu and colleagues suggested the use of GATK for the given task, while Bao and colleagues proposed a combination of Novoalign and FreeBayes as an alternative approach [69].

3.2.6. Variant annotation

Once variants have been identified, it is necessary to annotate them in order to enhance comprehension of disease pathogenesis. The process of variant annotation typically encompasses the inclusion of data pertaining to genomic coordinates, gene positioning, and the specific type of mutation. Numerous investigations primarily center on the non-synonymous single nucleotide variants (SNVs) and insertions/deletions (indels) occurring within the exome. These genetic alterations constitute approximately 85% of identified pathogenic mutations associated with Mendelian disorders, as well as a substantial proportion of mutations implicated in complex diseases[70].

In addition to the fundamental process of annotation, numerous databases exist that offer supplementary information pertaining to genetic variants. ANNOVAR is a software tool that effectively integrates an extensive compilation of over 4,000 publicly accessible databases. Its primary objective is to provide annotations for genetic variants. The databases encompass prominent resources such as dbSNP, 1000 Genomes, and NCI-60 human tumor cell line panel exome sequencing data. This tool possesses the capacity to be employed for the estimation of minor allele frequency (MAF), deleteriousness, conservation status of the mutated site, experimental evidence corroborating disease variants, as well as prediction scores derived from GERP, PolyPhen, and other pertinent software. Other commonly utilized databases include OncoMD, OMIM, SNPedia, 1000 Genomes, bdSNP, and personal genome variants [71].

3.2.7. Variant filtration and prioritization

The WES system has the capability to produce a large number of potential candidates with numerous variations. The numerical value can be decreased through the process of variant prioritization, resulting in a concise yet prioritized list of candidate mutations. This list can then be subjected to additional experimental validation. The process of variant prioritization encompasses three essential stages: firstly, the elimination of variant calls that are deemed less reliable; secondly, the reduction of common variants, given the presumption that rare variants have a higher propensity to be pathogenic, this study aims to elucidate the process of ranking variants in relation to disease. This will be achieved through the integration of discovery-based and hypothesis-based methodologies. There exists a variety of tools that can be utilized for the specific objective of variant filtration and prioritization. The aforementioned tools encompass VAAST2, VarSifer, KGGseq, PLINK/SEQ, SPRING, GUI tool, Gnome, and Ingenuity Variant Analysis [71].

3.2.8. WES data analysis

The Bcl files were promptly transformed into FastQ data subsequent to the completion of the run. Table S1 contains information regarding the average size of libraries and the coverage of target regions. The public can access the raw data through the accession numbers E-MTAB-6978 at ArrayExpress and ERP109743 at the European Nucleotide Archive (ENA). The alignment of the data was performed using the BWA package to the reference genome (version GRCh37/hg19). The Genome Analysis Toolkit (GATK) was employed to perform the indel realignment and base recalibration procedures. The generation of VCF files was carried out for the purpose of identifying single nucleotide variants as well as short insertions or deletions (indels). If available, the researchers supplied rs identifiers acquired from the Single Nucleotide Polymorphism Database (dbSNP, version 142). The Variant Effect Predictor (VEP) was utilized to ascertain the distribution of coding consequences resulting from genetic alterations. The Polyphen-2 software, which can be accessed online at genetics.bwh.harvard.edu/pph2/index.shtml, was utilized to computationally predict the functional consequences of single nucleotide polymorphisms. The predictions made using Polyphen-2 were categorized into three groups: benign (scores ranging from 0.000 to 0.449), possibly damaging (scores ranging from 0.450 to 0.959), and probably damaging (scores ranging from 0.960 to 1.000) [72].

In the foreseeable future, it is plausible that whole exome sequencing could become a customary clinical practice for the purpose of disease management. Numerous healthcare facilities have already implemented genetic testing through the utilization of next-generation sequencing (NGS) technologies, such as whole exome sequencing (WES). The forthcoming obstacle will pertain to the effective management of vast quantities of genomic variants, Furthermore, it is important to consider the effective integration of these variants with clinical records and patient information.

3.3. The principles and methods of transcriptome sequencing

3.3.1. Transcriptome sequencing

The transcriptome refers to the complete collection of RNA molecules, encompassing various types such as mRNA, rRNA, tRNA, and noncoding RNA, the entities under consideration are derived

from either an individual cell or a collection of cells. Transcriptomics is a scientific discipline that involves the methodical examination of cellular gene expression in reaction to particular circumstances by means of a thorough analysis of RNA transcripts. This analysis encompasses both messenger RNA (mRNA) and non-coding RNAs. Transcriptomics enables a thorough investigation of transcription throughout the entire genome, with particular attention to individual nucleotides. This methodology enables the evaluation of transcript abundance, impartial identification of alternative splicing events and post-transcriptional RNA editing occurrences, as well as the identification of single nucleotide polymorphisms (SNPs). The method demonstrates advantages when compared to established methodologies such as microarrays and expression sequence tags. The application of this tool exhibits considerable potential in the domains of early embryo development, cellular differentiation, phylogenetic inference, and biomarker discovery [73].

Total RNA is firstly isolated from a sample, then library preparation may involve such steps as poly-A-based mRNAs enrichment or ribosomal-depletion-based RNAs enrichment, RNA fragmentation, reverse transcription to cDNA, adapter ligation, and PCR amplification [74].

The efficacy of accurately characterizing exon structure and discovering new transcription events across various species has been demonstrated through the utilization of large-scale transcriptome sequencing, specifically RNA-seq, employing short paired-end reads [75]. The application of paired-end short-read sequencing of RNA offers a beneficial and efficient approach for identifying gene fusions. The primary reason for this phenomenon can be attributed to the increased number of distinct reads and the improved physical coverage achieved by utilizing longer fragment lengths [76].

RNA-sequencing provides a comprehensive dataset at the sequence level for each individual transcript, in addition to the identification and characterization of chimeric transcripts. Several research groups have developed methodologies for performing focused high-throughput sequencing of genomic DNA. The techniques employed in these methodologies entail the targeted capture of particular genomic regions, typically exons, by means of molecular inversion probes [77] microarray-based capture, or solution-phase hybrid selection. On the other hand, RNA-sequencing offers a methodical strategy for detecting sequence mutations in protein-coding transcripts that are actively expressed, thereby obviating the need for additional selection protocols [78]. The efficacy of identifying mutations from RNA-sequencing data is significantly influenced by the expression levels of the associated genes.

The initial step involves the isolation of total RNA from a given sample. Subsequently, library preparation encompasses various procedures, The process involves various steps, such as the enrichment of messenger RNAs (mRNAs) using poly-A-based or ribosomal-depletion-based techniques, fragmentation of RNA molecules, reverse transcription to produce complementary DNA (cDNA), ligation of adapters, and amplification through the polymerase chain reaction (PCR). Ribosomal depletion is considered to be a more advantageous method compared to poly-A selection, primarily because it results in the enrichment of RNA molecules, regardless of whether they possess poly-A tails or not. Hence, the transcriptome that has undergone rRNA depletion is commonly regarded as encompassing the entirety of RNA molecules, comprising both messenger RNA (mRNA) and non-coding RNA. The deep sequencing procedure described involves the utilization of either the Illumina HiSeq platform,

which can be configured for single-end or paired-end sequencing with short-read capabilities, or the PacBio SMRT platform, The technology facilitates the sequencing of exceptionally long reads. The final stage of transcriptomics research involves the utilization of bioinformatics analysis. The process generally involves multiple essential stages, such as eliminating inferior sequences from the initial data, aligning or mapping the sequences to a known genome or executing de novo assembly, carrying out subsequent manipulations and quality evaluations after mapping, and utilizing sophisticated data mining methods, such as quantifying gene expression [73].

RNA-Sequencing provides an impartial and exceptional high-resolution perspective of the comprehensive transcriptional panorama, this technology facilitates a cost-efficient and accurate approach to measure gene expression levels and perform comparative analysis of gene expression variations among various sample groups. Furthermore, it facilitates the identification of previously unknown gene structures, alternatively spliced isoforms, Gene fusions, single nucleotide polymorphisms (SNPs), insertions/deletions (InDels), and allele-specific expression (ASE) are among the genetic variations that have been extensively studied in the field of genomics [79].

3.3.2. Several advantages associated with the utilization of RNA-Sequencing (RNA-Seq) in scientific research

The technique enables accurate and quantitative measurement of RNA molecules at a resolution of individual base pairs, it facilitates the identification of previously unknown transcripts, splice variants, and gene fusions , the method can be applied to any species, regardless of the availability of a reference genome and the cost of this approach is comparable to or lower than that of many alternative methods.

3.3.3. RNA-sequencing workflow

CD Genomics utilizes a combination of Illumina HiSeq and PacBio platforms to offer a rapid and precise RNA-Sequencing and bioinformatics analysis for various species. The team of experts we have assembled possesses extensive experience and expertise in the field. They diligently adhere to established quality management protocols, meticulously following each procedure to guarantee outcomes that are both reliable and impartial. The subsequent sections provide an overview of the typical workflow employed in RNA-Sequencing [79].

3.4. Whole-exome sequencing identifies rare and low frequency coding variant associated with LDL cholesterol

The level of low-density lipoprotein cholesterol (LDL-C) is a complex trait that can be influenced by various environmental and genetic factors. It is estimated that around 40% to 50% of the observed variation in LDL-C levels can be attributed to hereditary factors[80]. Rare mutations have been identified in families who are impacted by Mendelian forms of lipid-related disorders. Individuals who inherit these rare genetic variations within their familial lineage often display significantly deviant lipid profiles during their formative years. Moreover, those who have elevated levels of low-density lipoprotein cholesterol (LDL-C) are prone to experiencing cardiovascular disease at a premature age. Research in the field of family studies has provided evidence indicating

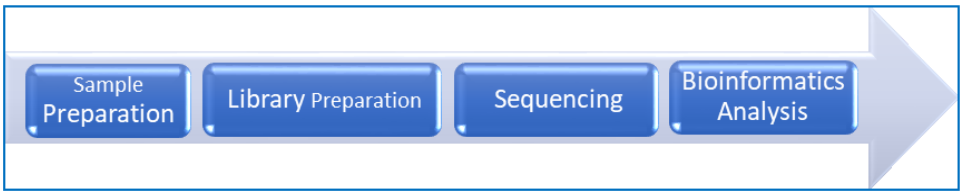

Figure 5: Workflow for RNA sequencing.

that mutations in specific genes, namely LDLR (MIM 606945), PCSK9 (MIM 607786), APOB (MIM 107730), ABCG5 (MIM 605459), ABCG8 (MIM 605460), and LDLRAP1 (MIM 605747), can lead to significantly elevated levels of cholesterol. Conversely, mutations in genes such as PCSK9, MTTP (MIM 590075), APOB [81], and ANGPTL3, (MIM 603874) [82] have been associated with abnormally low levels of cholesterol. Previous research has identified rare mutations in LDLR, PCSK9, and NPC1L1 genes through targeted sequencing studies conducted on individuals with low cholesterol levels. Nevertheless, the degree to which these uncommon and infrequent variations contribute to the overall variability in cholesterol levels among individuals in the population remains inadequately comprehended. The primary focus of genome-wide association studies (GWASs) has been on common variants, resulting in the successful identification of 157 loci that exhibit associations with lipid levels, particularly low-density lipoprotein cholesterol (LDL-C). However, it is important to note that despite the robust evidence of association between these loci and LDL-C, only a small proportion, these common variants can account for approximately 10% to 12% of the total variance in LDL-C. This is in contrast to the estimated heritability of LDL-C, which is approximately 40% to 50% [80]. In this study, we examined the proposition that uncommon or infrequent genetic variants, which are inadequately represented in genome-wide association studies (GWASs) and not readily imputable, are similarly linked to low-density lipoprotein cholesterol (LDL-C) levels. To summarize, This research on exome sequencing presents findings that suggest LDLR, PCSK9, APOB, and PNPLA5 are the genes that show the strongest indications of rare or low-frequency coding variants that have an impact on LDL-C levels, based on the analysis of approximately 17,000 genes [83].

3.5. Whole-exome sequencing reveals novel genetic variants associated with diverse phenotypes of melanoma cells

Melanoma, a form of malignancy in humans, demonstrates a notable incidence of genetic mutations, with a median count exceeding 10 mutations per megabase. These mutations are frequently linked to the characteristic signature of ultraviolet light exposure [84]. Through a comprehensive analysis of data obtained from The Cancer Genome Atlas (TCGA), it has been determined that melanoma can be classified into four distinct genetic subtypes. These subtypes are distinguished by the presence of recurrent mutations in either BRAF, RAS, NF1, or the absence of mutations in any of these genes [50]. The melanoma molecular classification system classifies melanoma into four distinct subtypes based on the presence of activating mutations in either BRAF (referred to as the BRAF subtype) or RAS (known as the RAS subtype), inactivating alterations in NF1 (referred to as the NF1 subtype), or the absence of mutations in these genes (referred to as the triple wild-type subtype). The

oncoproteins BRAF and RAS exhibit overlapping downstream signaling pathways, wherein ERK1/2 acts as the principal effector molecules. The activation of RAS proteins has the capability to initiate supplementary signaling cascades, such as the phosphoinositide 3-kinase PI3K/AKT pathway. The phenotype of melanoma cells is anticipated to be significantly impacted by modifications in the genes responsible for encoding proteins involved in the MAPK signaling pathway. This signaling cascade plays a crucial role in various cellular processes, such as cell survival and proliferation [85].

3.6. Novel gene fusion in melanoma by transcriptome analysis

The application of high-throughput paired-end sequencing of cDNA, commonly referred to as RNA-sequencing, has become a systematic methodology for identifying chimeric transcripts and other genetic variations that are actively expressed in neoplastic cells. A comprehensive set of 11 unique gene fusions linked to melanoma has been discovered, originating from genomic rearrangements. The identified melanoma gene fusions possess the capacity to function as prominent driver events, exerting a pivotal influence on the advancement of tumors. It is expected that instances of driver events will occur repeatedly in different independent melanomas [86].

3.7. Integrated whole exome and transcriptome sequencing

The utilization of whole-genome sequencing enables the identification of mutations, copy number of variations, and genome signatures throughout the entirety of the genomic landscape [87], Additionally, the measurement of gene expression through RNA sequencing can provide further insights into alterations linked to oncogenic activity, particularly in cancers lacking evident targetable DNA alterations. This can contribute to the enhancement of progression-free survival in rare cancers. Currently, there exists a scarcity of instances that demonstrate the application of whole-genome and RNA sequencing within clinical settings [88].

DNA whole exome sequencing (DNA-WES) is presently a widely utilized technology for the sequencing of cancer genomes, which has resulted in a plethora of significant findings across various cancer classifications. However, the detection of somatic mutations through DNA-WES, while aiming for high sensitivity and specificity, continues to present a significant challenge, This is evident from validation rates of only 73% in repeated sequencing and substantial disagreement among different groups analyzing the same sequencing data [89]. DNA-WES focuses on approximately 200,000 exonic regions and, in practice, can achieve depths of 100X or more over these targeted regions [90]. Nonetheless, DNA-WES has its limitations, such as variable capture-efficiency and incomplete coverage of the exome. In contrast, RNA sequencing (RNA-sequencing) is employed for gene expression analysis, fusion transcript identification, and splicing analysis [91]. In addition to the aforementioned applications, RNA-sequencing offers insights into the DNA sequence of tumors through transcription, enabling the detection of sequence variants. However, RNA-sequencing encounters certain challenges, such as its reliance on gene expression, which restricts the measurement of sequence mutations to specific genes. Furthermore, the accurate identification of variants is contingent upon rigorous quality control measures [92], as failure to adhere to these requirements can lead to a high number of false positive results.

Table 1. Novel melanoma gene fusions

Sample	5′ Gene	Chromosome	3′ Gene	Chromosome	No. of read pairs	No. of fusion-spanning reads	Reading frame
501 Mel	*CCT3*	1	*C1orf61*	1	55	18	In-frame
501 Mel	*GNA12*	7	*SHANK2*	11	18	4	In-frame
501 Mel	*SLC12A7*	5	*C11orf67*	11	32	23	In-frame
501 Mel	*PARP1*	1	*MIXL1*	1	2	4	In-frame
M000216	*KCTD2*	17	*ARHGEF12*	11	3	2	Out-of-frame
M000921	*TMEM8B*	9	*TLN1*	9	2	1	In-frame
M000921	*RECK*	9	*ALX3*	1	23	6	Out-of-frame
M010403	*SCAMP2*	15	*WDR72*	15	2	2	In-frame
M980409	*GCN1L1*	12	*PLA2G1B*	12	3	2	Out-of-frame
M990802	*ANKHD1*	5	*C5orf32*	5	9	20	Out-of-frame
M990802	*RB1*	13	*ITM2B*	13	14	2	In-frame

Novel gene fusions harboring at least two distinct discordant read pairs and at least one fusion-spanning individual 51-mer read are shown. Each gene fusion was validated by RT-PCR followed by Sanger sequencing of the product.

Figure 6: The presented findings exhibit novel gene fusions that contain a minimum of two dissimilar read pairs and at least one individual 51-mer read spanning the fusion site. The validation of each gene fusion was conducted through [86].

[93]. Due to these factors, RNA-sequencing has not attained the status of a conventional method for the identification of somatic mutations. The integration of patient-specific tumor RNA-sequencing with tumor DNA whole-exome sequencing (WES) would yield superior mutation detection capabilities in comparison to relying exclusively on DNA-WES.

4. Conclusion

Melanoma is a highly malignant form of skin cancer that exhibits remarkable metabolic adaptability. The process of metabolic reprogramming plays a substantial role in the progression and diversity of melanoma, ultimately impacting its pathogenesis. One of the notable characteristics of metabolic reprogramming is the heightened rate of lipid synthesis.

Clinical RNA-Sequencing expands the scope of molecular diagnostics for rare genodermatoses, and it has the potential to serve as a dependable initial diagnostic method for expanding mutation databases in individuals with inheritable skin conditions.

The integration of patient-specific tumor RNA-sequencing and tumor DNA whole-exome sequencing (WES) would potentially enhance mutation detection capabilities compared to relying solely on DNA-WES.

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Ethical Considerations

Not applicable.

Competing Interests

The author has no competing interests.

Availability of Data and Material

Data will be available upon request.

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