Whole Exome Sequencing and Whole Genome Sequencing for Investigation of the Genetic Basis of Obesity: A Rapid Review

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ABSTRACT

Objective: In recent decades, the prevalence of obesity has reached a global epidemic level. This study was done to assess the whole exome sequencing and whole genome sequencing methods used to investigate the genetic basis of obesity.

Materials and Methods: Different studies on Pubmed, Scopuis, Google Scholar and other data bases were extracted and their findings were analyzed.

Results: Childhood obesity has risen to alarming levels as World Health Organization (WHO) estimates that there were 38.2 million children under the age of 5 years with overweight or obesity in 2019. It has been shown that genetic factors also play a key role in the risk of obesity, and strong evidence suggests that BMI is highly heritable. However, in both adults and children, a major part of the genetic aetiology of obesity is still unknown. Recent advances and increasing affordability of whole exome sequencing (WES) and/or whole genome sequencing (WGS) have provided a rapid and comprehensive method for identifying the novel genes in obesity, particularly in children with severe early-onset obesity. Conclusion: This rapid review aimed to review a variety of literature reporting novel candidate genes for non-syndromic obesity identified through applying WES and WGS techniques in humans.

Keywords: Genetic Research, Obesity, Whole Exome Sequencing, Whole Genome Sequencing

INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to an extent that it may adversely affect health and/or reduce life expectancy[1,2]. It has destructive medical and social impacts and has turned to a severe health issue in both developed and developing nations[3]. The levels of global obesity have increased in recent years to a level of an epidemic health problem[4]. From 2008 to 2016, the global estimate of obesity in adults, clinically defined as a body-mass index (BMI) > 30 kg/m^2, has increased from 500 million to 671 million and a further one billion adults are currently overweight (25 kg/m^2 ≤ BMI < 30 kg/ m^2)[5,6]. Regarding this rising trend in obesity, studies warn that over a billion people worldwide would be classified as clinically obese in 5 to 10 years, resulting in tremendous burdens on medical and public health burdens[7]. Trends in childhood obesity levels are similar to adults and the prevalence of global obesity in children and adolescents aged 5-19 has risen tremendously from under 1% in 1975 to more than 124 million (6% of girls and 8% of boys) in 2016. According to WHO, in 2020, the number of overweight or obese children under 5 was 39 million, almost half of them living in Asia[4].

Obesity leads to serious health complications, (such as type 2 diabetes mellitus, dyslipidaemia, hypertension, obstructive sleep apnea, and fatty liver), and in some countries, such as the United States, it is considered the greatest preventable contributor to morbidity and death[8]. Obesity is also associated with psychological impacts such as low self-esteem, difficulties in social relationships, and weight bias[9,10]. Recently, the escalating obesity epidemic has been blamed for the higher rates of hospitalization and mortality in patients with COVID-19[11,12].

Poor eating habits and reduced physical activity are widely accepted as the two main causes of obesity. However, in recent decades, it has been shown that genetic factors also play a key role in the risk of obesity, and there is a high heritability for BMI variation[13]. From a genetic point of view, human obesity can occur in both polygenic and monogenic forms[14]. Polygenic obesity, which accounts for common obesity in adults, is resulted from interactions of obesity causing variants with each other and/or with environmental factors, while monogenic obesity is a type of obesity caused by pathogenic mutations in a single gene and occurs in two distinct forms of syndromic and non-syndromic[14,15].

In this review, we have discussed the genetics of obesity and summarized the novel genes identified for polygenic and non-syndromic monogenic forms of obesity through whole exome sequencing (WES) and/or whole genome sequencing (WGS) in recent years.

Genetic Contribution to Obesity: Inappropriate eating habits and reduced physical activity are widely accepted as the two main causes of the global obesity epidemic. However, in recent decades, it has been shown that genetic factors also play a key role in the risk of obesity, and strong evidence suggests that BMI is highly heritable[16]. Stunkard et al., in 1986, provided the first strong evidence of the genetic effects on human obesity through a twin study showing that monozygotic (MZ)

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twin pairs have a greater correlation with obesity levels than dizygotic (DZ) pairs[17]. Adoption studies, alongside with twin studies, endorsed the genetic contribution to obesity by revealing correspondence of BMI with biological parents rather than the adoptive parents[18,19]. Furthermore, epidemiology studies of obesity have shown that concordance for obesity levels decreases in parallel with the degree of relatedness[20]. In addition, obesity prevalence varies substantially between ethnic groups living in similar "obese" environments[21]. Finally, the elucidation of mutations in a single gene that leads to monogenic or Mendelian forms of obesity has provided direct evidence for the involvement of genetic factors in the development of obesity[22]. Based on these studies and similar findings, it is estimated that about 35 to 75% of the differences in the BMI of people living in the same environment are due to genetic differences[13].

Molecular Genetics of Human Obesity: Obesity is clinically divided into two types: syndromic and non-syndromic. In the non-syndromic form, obesity is the only main feature in the patient, while in the syndromic form it occurs along with other disorders such as mental retardation, dysmorphic features, and organ-specific abnormalities. From a genetic point of view, syndromic obesity can have different inheritance modes of sporadic, autosomal dominant (AD), and autosomal recessive (AR). Non-syndromic obesity can be divided into two distinct groups based on genetic aetiology: polygenic (or common) obesity and monogenic non-syndromic obesity[14].

Polygenic (or Common) Obesity: Polygenic obesity is resulted from interactions of obesity causing variants with each other and/or with environmental factors. Two hypotheses have been proposed to explain the etiology of polygenic diseases, including common obesity[15]. The common disease-common variant (CD-CV) hypothesis suggests that complex (common) diseases are usually caused by a number of common genetic variants (variants with allele frequency above 5%)[23]. According to this hypothesis, genome-wide association studies (GWAS) have been extensively used to reveal the major causative genetic contributions to common diseases and traits. Nevertheless, the CD-CV hypothesis has been challenged by increasing evidence of allelic complexity at the loci predisposing to common diseases and by the notion that natural selection should, in fact, retain the frequency of deleterious alleles in the human genome at low levels[24]. Moreover, most loci identified through GWAS studies have failed to explain the heritability of common diseases. For example, so far, GWAS studies have been able to explain only less than 5% of the heritability of common obesity[20]. Therefore, an alternate common disease-rare variant (CD-RV) hypothesis has been offered, suggesting that a large part of the genetic variance underlying complex diseases could be due to risk alleles that are deleterious and thus, present at lower frequencies in the population[25]. It has been argued that, in recent two centuries, the explosive accelerated growth of population size has increased a load of rare variants, as there is little time for natural selection to operate and eliminate them unless they are extremely deleterious[26]. The proponents of the CD-RV hypothesis also argue that rare variants are rare because they are likely to be selected against due to their deleterious nature, while common variants are common probably because they are not functional enough to have been subjected to negative selection. Therefore, the notion that multiple, very recent rare variants contribute to the diseases arising in the past two centuries is more compatible with human population pathobiology than the CD-CV hypothesis[27]. Ultimately, it is important to realize that these hypotheses are not mutually exclusive, and a combination of both common and rarer variants may contribute to complex diseases[27].

Monogenic (Mendelian) Non-Syndromic Obesity: Monogenic or so-called Mendelian non-syndromic obesity is a type of obesity caused by pathogenic mutations in the genes that play a key role in the hypothalamic control of energy balance and/or in the regulation of food intake[28]. It often presents as early-onset severe obesity associated with intense hyperphagia and with a massive impact on patients' morbidity and mortality[28]. Furthermore, several endocrinopathies, such as hypothyroidism, growth hormone deficiency, hypogonadism, and corticotropic insufficiency are often present[29]. Monogenic non-syndromic obesity has been identified in 5 to 10% of obese subjects of European descent, but it could be higher depending on the population and the extent of the diagnosis effort[28,30]. Additionally, there is growing evidence that some of the common variants identified by GWAS as associated with a slight increase in BMI are located within or adjacent to the genes underlying monogenic non-syndromic obesity[31,32].

The first direct evidence for monogenic obesity in humans came in the late 1990s when a recessive homozygous mutation in the leptin

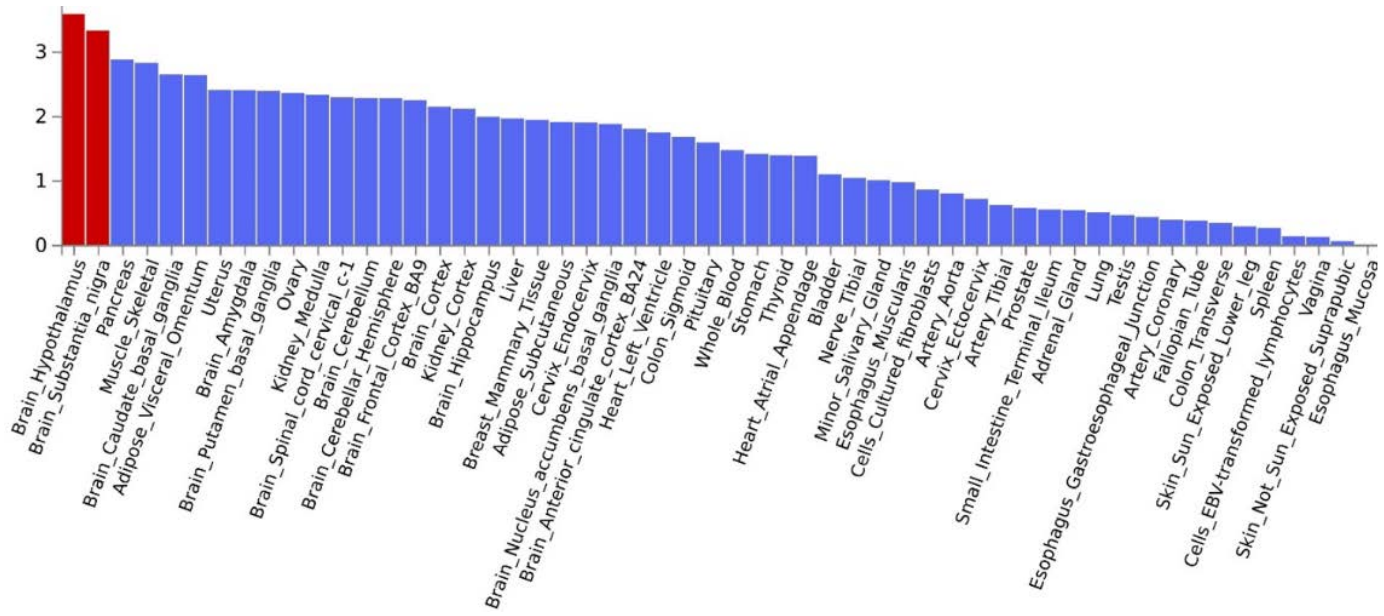

Figure 1: Tissue enrichment analysis for 32 genes listed in table 1

Table 1: Novel candidte genes identified for non-syndromic obesity in humans using WES or WGS techniques

Title of paper	Summary of approach	Identified candidate gene(s)
Functional analysis of variance for association studies[43]	Using WES data for a case-control study of the association between BMI and members of the ANGPTL family which contribute to the control of plasma triglyceride levels	*ANGPTL 4* and *ANGPTL 3*
Whole exome sequencing identifies variation in CYB5A and RNF10 associated with adiposity and type 2 diabetes[44]	WES in 177 Pima Indians followed by genotyping of selected variants in up to 5,880 subjects with longitudinal measures of BMI	*CYB5A* and *RNF10*
Rare Variant Analysis of Obesity-Associated Genes in Young Adults With Severe Obesity From a Consanguineous Population of Pakistan[45]	Conventional or augmented whole-exome analysis for point mutations and copy number variants (CNVs) in 126 randomly selected young adult obese subjects from a consanguineous population in Pakistan	*ASNSD1* and *IFI16*
Genetic interaction of DGAT2 and FAAH in the development of human obesity[46]	Analysis of WES data obtained from 227 young obese subjects and 219 lean controls	*FAAH* and *DGAT2*
A low-frequency variant in MAPK14 provides mechanistic evidence of a link with myeloperoxidase: a prognostic cardiovascular risk marker[47]	Characterization of gene variants in MAPK11/14 genes by exome sequencing and follow-up genotyping or imputation in participants well-phenotyped for cardiovascular and metabolic traits	*MAPK14*
The genetic and epigenetic association of LDL Receptor Related Protein 1B (LRP1B) gene with childhood obesity[48]	Exome sequencing of the *LRP1B* gene from a childhood severe obesity cohort	*LRP1B*
Coding Variants are Relevant to the Expression of Obesity-Related Genes for Pediatric Adiposity[49]	Investigation of WES data from 76 children with obesity and 74 children with normal weight, and their associations with obesity-related traits in an additional 6,334-child cohort	*SULT1A2* and *MAP3K21*
Functional variants in cytochrome b5 type A (CYB5A) are enriched in Southwest American Indian individuals and associate with obesity[50]	Identification of variants that are enriched in Southwest American Indian (SWAI) individuals using WGS data followed by testing for association with BMI	*CYB5A*
Identification of low-frequency and rare sequence variants associated with elevated or reduced risk of type 2 diabetes[51]	WGS of 2,630 Icelanders and imputation into 11,114 Icelandic cases and 267,140 controls followed by testing for association with BMI in Danish and Iranian samples	*CCND2*
Whole-exome sequencing study reveals common copy number variants in protocadherin genes associated with childhood obesity in Koreans[52]	Association analysis of copy number variation (CNV) using WES data from a total of 102 cases and 86 controls	*PCDHB7* and *PCDHB8*
Exome Sequencing Identifies Genes and Gene Sets Contributing to Severe Childhood Obesity, Linking PHIP Variants to Repressed POMC Transcription[53]	Exome and targeted sequencing in 2,737 severely obese cases and 6,704 controls	*PHIP, DGKI,* and *ZMYM4*
Predicting novel candidate human obesity genes and their site of action by systematic functional screening in Drosophila[54]	Exome sequencing of severely obese children for identification of rare homozygous gene variants followed by assessing the function of these genes in vivo in Drosophila	*TAOK2*
Exome sequencing followed by genotyping suggests SYPL2 as a susceptibility gene for morbid obesity[55]	WES of 100 morbidly obese adult subjects and 100 controls followed by genotyping of identified obesity-enriched variants in 494 adult subjects with morbid obesity and 496 controls	*SYPL2*
Exome sequencing in Thai patients with familial obesity[56]	WES of two obese and one normal subject belonging to the same Thai family followed by genotyping of the selected variants in 12 obese and 5 normal individuals of this family	*HCRTR1, COL9A2,* and *TRPM8*
Exome sequencing reveals novel genetic loci influencing obesity-related traits in Hispanic children .	Exome sequencing of 293 Hispanic families each with a proband with obesity between the ages 4-19 years	*PEX1*
Sequencing of 640,000 exomes identifies GPR75 variants associated with protection from obesity[57]	WES of 645,626 individuals from the United Kingdom, the United States, and Mexico and estimating the associations of rare coding variants with BMI	*GPR75*
A form of the metabolic syndrome associated with mutations in DYRK1B[58]	Linkage analysis and WES in three large families with coinheritance of early-onset coronary artery disease, central obesity, hypertension, and diabetes	*DYRK1B*
Two novel candidate genes identified in adults from the Newfoundland population with addictive tendencies towards food[59]	A combination of exome sequencing method and a candidate gene association approach in 24 subjects including 8 obese with high and 8 obese with low/zero food addiction (FA) clinical symptom score, and 8 healthy controls with normal BMI and low/zero FA symptom score.	*DRD2* and *TIRAP*
Exome Sequencing Identifies A Nonsense Variant in DAO Associated With Reduced Energy Expenditure in American Indians[60]	WES in 373 healthy Pima Indians informative for 24-hour energy expenditure during energy balance	*DAO*
Whole-Exome Sequencing Suggests LAMB3 as a Susceptibility Gene for Morbid Obesity[61]	WES of 200 morbidly obese subjects and 100 control subjects followed by genotyping of obesity-enriched low-frequency coding variants in 1,911 morbidly obese and 1,274 control subjects	*LAMB3*
Loss-of-function mutations in ADCY3 cause monogenic severe obesity[62]	WES analysis in 138 probands who presented with severe, early-onset obesity and their available family members ($n = 117$) from consanguineous families	*ADCY3*

gene was identified in two severely obese cousins from a highly consanguineous Pakistani family[33]. Treatment of these individuals with daily subcutaneous administration of recombinant leptin dramatically reduced their body fat to normal levels[34]. Since these findings, investigations into the contributions of other genes in human obesity have led to the discovery of about 20 genes, which are mostly involved in the leptin/melanocortin pathway[28,35].

In general, genetic mutations underlying monogenic obesity are rare and insufficient to explain the current tsunami of obesity in the world population[36,37]. However, these mutations have very strong biological effects and trigger obesity from a very early age in childhood, meaning that standard care such as lifestyle intervention (reducing food intake and increasing physical activity) and even bariatric surgery are inadequate in inducing significant weight loss. Therefore, investigations on subjects with monogenic obesity are critical in better understanding of the pathophysiology of the disease and the discovery of new pharmaceutical drugs[38,39].

Identifying Genetic Loci for Common Obesity: Until 2007, genome-wide linkage studies and candidate gene studies were the most commonly used strategies for interrogating the genetic basis of common obesity. However, these approaches were most successful in identifying loci associated with rare monogenic diseases rather than common diseases. In more recent times, GWASs have been increasingly used as the method of choice for identifying common variants associated with obesity and other common diseases[40]. Generally, GWASs have been successful in identifying an extraordinary number of obesity loci. However, these loci only explain a small fraction (about 2.7%) of the overall inter-individual BMI variation, suggesting that a large portion of the genetic content of common obesity is yet to be discovered. Another challenge in a GWAS study is that the association signals detected from a GWAS are often not the causal variant, but instead have been identified due to LD with the causal mutation that could lie in any of the regional genes or even reflect long-range interactions. Therefore, the determination of the causal variant or the causal gene may be very complicated[41]. Because of the recent findings favoring the rare variant-common disease hypothesis, an increasing number of studies on common diseases have applied high-throughput sequencing methods such as WES and WGS, which are powerful tools for identifying rare variants.

Identifying Genetic Loci for Monogenic obesity: Monogenic disorders, including monogenic forms of obesity, are often the result of single-point mutations or indels in specific genes resulting in the altered function or dosage of the proteins. Such disorders may also result from microdeletions or microduplications, structural chromosomal aberrations, or imprinting defects. Irrespective of the mutation type, the underlying disease gene in a monogenic disorder can be identified through several approaches. The main traditional approaches that still yield high success rates are the candidate gene approach, functional cloning, and positional cloning. However, with the advent of the next-generation sequencing platforms, new gene discovery studies increasingly utilize high-throughput sequencing techniques such as WES and WGS. These technologies enable the simultaneous analysis of multiple genes in a single test and have dramatically increased the throughput and decreased the cost of gene sequencing. Thus, during the last decade, more and more studies have applied NGS technologies for detection of the novel variants and genes in morbid and/or early onset obesity[42].

In this review, English-language articles were searched on the PubMed database using the following strategy: ((exome sequencing [Title/Abstract]) OR (exome analysis [Title/Abstract]) OR (genome sequencing [Title/Abstract]) OR (genome analysis [Title/Abstract])) AND ((obesity [Title/Abstract]) OR (body mass index [Title/Abstract]) OR (adiposity [Title/Abstract]) OR (weight gain [Title/Abstract])). This search yielded 391 papers which were first screened by title and abstract to exclude papers that have reported the application of WES and WGS for the identification of novel mutations in previously reported genes for obesity. Furthermore, irrelevant papers, studies on non-human species or syndromic forms of obesity, and literature reviews were excluded from the search results. Moreover, studies on obese and/or overweight subjects with underlying diseases such as asthma or cystic fibrosis were removed from further investigation. Finally, 21 papers that reported the novel genes for non-syndromic obesity in humans using WES or WGS techniques were selected (Table 1).

Novel Genes Identified Through WES and WGS for Non-Syndromic Obesity: WES provides a unique opportunity for screening a patient's entire exome in genetically heterogeneous diseases and thus has the dual potential to either detect mutations in all known genes or discover novel loci. This technique is able to reliably cover close to 95% of protein-coding regions, which contain approximately 85% of disease-causing mutations in Mendelian disorders[63]. In 2018, Saeed et al. successfully identified *ADCY3* gene loss of function mutations in monogenic obesity using WES in 138 Pakistani children with severe early-onset obesity and their available family member[62]. Most recently, three novel genes (*PHIP, DGKI,* and *ZMYM4*) were linked to severe childhood obesity through WES analysis of 927 individuals with severe early-onset obesity of European ancestry and 4,057 UK healthy blood donors[53]. A combination of exome sequencing in a limited number of samples and following genotyping of candidate variants in a larger set of samples is a cost-saving method for the identification of novel genes underlying polygenic disorders. In 2014 and 2016, exome sequencing followed by the targeted genotyping of candidate variants in obese and control Swedes led to the discovery of SYPL2 and *LAMB3* as susceptibility genes for morbid obesity[55,61]. Moreover, *CYB5A* and *RNF10* were reported in association with BMI through WES in Pima Indians followed by genotyping of selected variants in up to 5,880 subjects with longitudinal measures of BMI[44]. In another type of WES study, the rare variants for potential candidate genes for obesity are extracted from the available WES data and subjected to association tests with BMI. Recently, through this approach, *TMAPK14*, *LPR1B*, *ANGPTL4*, and *ANGPTL3* genes have been identified as candidate genes for the development of obesity[43,47,48]. There are other kinds of WES studies that have analyzed the association of gene variants not with BMI and/or obesity but with traits that are related to the development of obesity, such as food addiction or energy expenditure rate[59,60].

WGS has the potential to identify nearly all forms of genetic variants in both nuclear and mitochondrial genomes. This technology has been shown to be more efficient for the detection of copy number variation (CNV) or structural variation (SV) compared to WES and has the potential to identify trinucleotide repeat expansions, which are typically located in non-coding regions and therefore missed in WES[64]. In recent years, the reduction in the cost of WGS has led to the widespread adoption of this technology in genomic research and public health. However, so far, there are only a few reports of it being used in obesity studies. In 2014, Steinthorsdottir et al. reported the association of *CCND2* with BMI using WGS data of 2,630 Icelanders followed by testing for association with BMI in Danish and Iranian samples[51]. Moreover, *CYB5A* has been associated with BMI through WGS in Southwest American Indian (SWAI) individuals using WGS data analysis followed by testing for association with BMI[50]. It is predicted that, with improvements in sequencing platforms and bioinformatic pipelines and a continuous decrease in the cost, genomic investigations on obesity rely more on WGS technology in the near future.

Pathway and Tissue Enrichment Analysis: We used FUMA platform (https://fuma.ctglab.nl/) to conduct the pathway and tissue enrichment analysis for 32 genes listed in table 1[65]. As expected, most of these genes are specifically expressed in the regions of the brain which have a substantial role in the control of appetite (Figure 1). Only 9 out of the 32 genes (HCRTR1, FAAH, SYPL2, DRD2, SULT1A2, TAOK2, ADCY3, LRP1B, DGKI) have been reported to be associated with BMI in the GWAS catalog, showing that WES and WGS have higher power for identifying candidate genes for obesity than GWAS studies. Pathway analysis failed to identify significantly enriched biological pathways involving the 32 candidate genes identified through WES or WGS. This could be due to the following limitations of this review: including only papers that have conducted WES or WGS for humans, searching exclusively in the Pubmed database, excluding the studies that report intronic genetic varaints, and finally excluding WES or WGS studies conducted on obese cases with a background disease such as asthma. We assume that further WES or WGS studies in the near future may reveal novel genes and subsequently novel pathways for obesity development in humans.

CONCLUSION AND RECOMMENDATION

WES and WGS are powerful techniques in identifying the novel candidate genes underlying human obesity. However, the number of studies applying these technologies is limited due to their high economic costs. We assume that with increasing affordability of the WES and WGS, more candidate genes will be identified for human obesity through applying WES and WGS analysis in obesity studies in developing countries.

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