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Genetic & epigenetic approach to human obesity

Obesity is considered as a metabolic syndrome resulting from a chronic imbalance of energy intake versus energy expenditure, leading to storage of excessive amounts of triglycerides in adipose tissue[1]. It is a major risk factor for multiple disorders, such as type 2 diabetes, cancer, fatty liver disease, hormonal disturbances, hypertension, cardiovascular diseases (CVD), increased and morbidity and mortality rates, *etc*[2-7]. According to the current estimates, by 2015 more than 700 million individuals worldwide will be obese[8]. Obesity rates are also increasing in children and adolescents all over the world, predisposing them to poor health from an early age[8,9].

In clinical practice, it is measured in terms of body mass index (BMI), which gives a surrogate measure of overall obesity and, accordingly the World Health Organization (WHO) classifies a person with a BMI $\geq$25 kg/m^2 as obese and a BMI $\geq$40 kg/m^2 as extremely obese[8]. It is important to note that sex and age are associated with differences in obesity and body composition. For example, women tend to store more fat subcutaneously rather than in visceral adipose tissue. So at the same BMI, women will tend to carry more body fat than men[9]. Two general patterns of fat distribution have been observed *viz*., android type or central obesity (adipose deposition in the abdominal area) common in males and gynoid type (adipose deposition around the hips) common in females[10]. Android/central obesity is an established independent risk factor for CVD and type 2 diabetes, whereas the gynoid pattern is thought to be protective or inversely correlated[11]. To account for these differences in fat distribution, waist-to-hip ratio (WHR) is commonly used and BMI and WHR are correlated (r^2~0.6)[12].

The current lifestyle has driven obesity prevalence to epidemic proportions with a substantial genetic contribution of 40-70 per cent approximately[13,14]. Since human obesity occurs due to sedentary lifestyle, epigenetics (mitotically and meiotically heritable changes in gene expression without any change in the DNA sequence[15]) also play an important role in its establishment. There are two important mechanisms involved in epigenetics regulations *viz*. DNA cytosine methylation and histone modifications[16]. It has also been observed that microRNAs (miRNAs) extended their role to epigenetic regulation[17]. Thus, dietary methyl-groups (choline, methionine, genistein and folate) intake during critical periods of developmental stages can alter promoter DNA and histone methylation resulting in the lifelong changes in gene expression and thereby altering the epigenome towards obesity in adulthood[18,19].

Genetics

Being a very complex disease, obesity does not appear to be limited to a single gene disorder (Table I) but rather found to occur as symptoms of other disorders (Table II) or as a result of multiple gene disorders (Table III). Hence, depending on the suspected aetiology obesity could be classified into three subgroups: Monogenic, Syndromic and Polygenic or Common obesity. The first single gene defect causing monogenic obesity was first described in 1997[20], and to date, about 20 such disorders have been reported for an autosomal form of obesity[1] (Table I). Interestingly, all these mutations lie in the leptin/melanocortin pathway of the central nervous system (CNS) which are critical in the regulation of whole-body energy homeostasis[68]. Obesity in these cases appears to be extremely severe due to increased appetite and diminished satiety. The second type, syndromic obesity arises from discrete genetic defects or chromosomal abnormalities at several genes, and can be autosomal or X-linked[69] (Table II). The third, polygenic/common obesity is due to the simultaneous presence of DNA variations in multiple genes. Potentially, many such polygenic variants (mostly >100) play a role in body weight regulation[70] (Table III). If an individual harbours many such polygenic variants for increased body weight, obesity can ensue and each variant will have a higher frequency than in normal/lean individuals[70]. A polygenic basis of obesity also implies that a specific set of polygenic variants relevant for obesity can vary from individual to individual.

Strategic approaches for detection of obese genes

Human obesity, usually arises from the combined effects of the interactions among multiple genes, environmental factors and behaviour, and this complex aetiology makes the management and prevention of human obesity especially challenging. A genetic basis for obesity exists and has been proven to be a dreadful task. Genetic epidemiologic methods for the gene discovery of complex traits, such as obesity, can be divided into two broad classes: hypothesis-driven (candidate gene or biologic pathway) and hypothesis-free (genome-wide linkage and genome-wide association) approaches.

Candidate gene single nucleotide polymorphism (SNP) analyses: The hypothesis-driven approach (candidate gene or biologic pathway analysis) needs a prior

Table I. List of genes responsible for monogenic obesity: Autosomal reccessive form of obesity

S.No.	Locus mutated	Encoded proteins	Usual physiological functions	References
1.	*LEP*	Leptin (LEP)	Protein hormone produced by adipocytes and regulates eating behaviour	20, 21
2.	*LEPR*	Leptin receptor (LEPR) in hypothalamus	Binds leptin and activates the synthesis of pro-opiomelanocortin (POMC)	22
3.	*POMC*	Pro-opiomelanocortin (POMC)	Precursor protein α-melanocyte stimulating hormone (α-MSH) along with other protein hormones	23, 24
4.	*PC 1*	Prohormone convertase-1 (PC 1)	Catalyzes post-translational cleavage of POMC into α-MSH	25, 26
5.	*MC4R*	Melanocortin-4 receptor (MC4R)	Binds of MC4R to α-MSH receptor, expressed in hypothalamus to activate anorexigenic signals	27-37

Table II. List of syndromic obesity in humans: Autosomal or X-linked

S. No.	Syndrome	Symtoms	Locus	Gene	References
Autosomal dominant					
1.	Prader-Willi syndrome (PWS)	Short physique with psychological defects hypotonia and hypogonadism.	15q11.2-q12	Unknown	38-43
2.	Albright's hereditary osteodystrophy (AHO)	Short physique with skeletal defects and defective olfaction	20q13.2	*GNAS1*	43, 44
3.	Fragile X syndrome	Psychological and speech defects with macro-orchidism	Xq27.3	*FMR1*	45
4.	Ulnar-mammary syndrome	Postponed puberty with imperfect ulnar and hypoplastic nipples	12q24.1	*TBX3*	46
Autosomal reccessive					
5.	Bardet-Biedl syndrome	Psychological and renal defects with retinal dystrophy and hypogonadism	11q13 (BBS1)	*BBS1*	47
			16q21 (BBS2)	*BBS2*	48
			15q22 (BBS4)	*BBS4*	49
			20p12 (BBS6)	BBS6 (MKKS)	50-52
6.	Alström syndrome	Retinal dystrophy with neurosensory deafness and diabetes	2p13	*ALMS1*	53, 54
7.	Cohen syndrome	Prominent central incisors with opthalmopathy, and microcephaly	8q22	-	55
X-linked					
8.	Börjeson-Forssman-Lehmann syndrome	Psychological defects with large pinna and hypogonadism	Xq26	*PHF6*	56
9.	MEHMO syndrome	Psychological defects with epilepsy, hypogonadism, microcephaly	Xp22.13	-	57, 58
10.	Simpson-Golabi-Behmel, Type 2	Skeletal and visceral abnormalities	Xp22	-	59
11.	Wilson-Turner syndrome	Psychological defects with tapering fingers, and gynaecomastia	Xp21.2	-	60

knowledge of the cause(s) for the genetic polymorphisms in a candidate gene or a biologic pathway being studied and their effect(s) on a particular phenotype of interest. This approach has been considered to be an efficient strategy for identifying genetic variants with small or modest effects that underlie susceptibility to common disease, including obesity. The selection of candidate gene(s) should, therefore, consider both the significance of the candidate gene to the pathogenesis of the disease of interest and its functional effects due to a particular polymorphism. So the design of the candidate gene approach is simple; the fundamental requirements are the identification of a gene that is involved in the disease phenotype, a polymorphic marker within that gene and a suitable set of subjects to genotype for that marker. But the identification of the potential candidate gene(s) is the main stumbling block.

There are two main types of candidate genes that are generally considered in such studies: functional and positional. Functional candidates are the genes with products that are involved in the pathogenesis of the disease. Obviously, this is highly dependent on the current state of knowledge about a disease, and in the case of obesity, the discovery of signaling molecules such as leptin and pro-opiomelanocortin (POMC)

Table III. List of genetic modifications (SNPs) showing polygenic effects on body weight in terms of BMI in humans

S. No.	Single nucleotide polymorphism (SNP)	Chromosome no.	Locus	Adjacent gene	Sample size	Allelic frequency	BMI outcome	References
1.	rs2815752	1p31	72,524,461	*NEGR1*	32,387	62% (A)	+0.10 kg/m^2 per A alleleb	61
2.	rs2568958		72,537,704		25,344	58% (A)	+0.43 kg/m^2 for AA genotype	62
3.	rs10913469	1q25	176,180,142	*SEC16B, RASAL2*	25,344	20% ©	+0.50 kg/m^2 for CC genotype	62
4.	rs6548238	2p25	624,905	*TMEM18*	32,387	84% (C)	+0.26 kg/m^2 per C allele	61
5.	rs7561317		634,953		25,344	84% (G)	+0.70 kg/m^2 for GG genotype	62
6.	rs7647305	3q27	187,316,984	*SFRS10, ETV5, DGKG*	25,344	77% ©	+0.54 kg/m^2 for CC genotype	62
7.	rs10938397	4p13	45,023,455	*GNPDA2*	32,387	48% (G)	+0.19 kg/m^2 per G allele	61
8.	rs4712652	6p22.2-p21.3	22,186,593	*PRL*	2,796	41% (A)	+0.031 kg/m^{2}per A allele in children	63
9.	rs10508503	10p12	16,339,956	*PTER*	2,796	8.5% ©	+0.144 kg/m^2 per C allele in childrend	63
10.	rs6265 (V66M)	11p14	27,636,492	*BDNF*	25,344	85% (G)	+0.67 kg/m^2 for GG genotype	62
11.	rs10838738	11p11.2	47,619,625	*MTCH2*	32,387	34% (G)	+0.07 kg/m^2 per G allele	61
12.	rs7138803	12q13	48,533,735	*BCDIN3D, FAIM2*	25,344	37% (A)	+0.54 kg/m^{2}for AA genotype	62
13.	rs7498665	16p11.2	28,790,742	*SH2B1 ATP2A1*	32,387	41% (G)	+0.15 kg/m^2 per G allele	61
14.	rs7498665		28,790,742		25,344	44% (G)	+0.45 kg/m^2 for GG genotype	62
15.	rs8050136	16q22.2	52,373,776	*FTO*	25,344	41% (A)	+1.07 kg/m^2 for AA genotype	62
16.	rs9939609		52,378,028		38,759	40% (A)	+0.40 kg/m^2 per A allele	63
17.	rs9939609		52,378,028		32,387	41% (A)	+0.33 kg/m^2 per A allele	61
18.	rs1421085		52,358,455		2,796	40% (C)	+0.112 kg/m^2 per C allele	63
19.	rs1424233	16q22-q23	78,240,251	*MAF*	2,796	43% (A)	+0.091 kg/m^2 per A allele in Children	63
20.	rs1805081	18q11.2	19,394,429	*NPC1*	2,796	44% (A)	-0.087 kg/m^2 per A allele in children	63
21.	rs17782313		56,002,077	*MC4R*	16,876	24% (C)	+0.22 kg/m^2 per C allele	64
22.	rs17782313		56,002,077		32,387	22% (C)	+0.22 kg/m^2 per C allele	61
23.	rs17782313		56,002,077		2,796	17,5% (C)	+0.097 kg/m^2 per C alleled	63
24.	rs12970134	18q22	56,035,730		25,344	30% (A)	+0.36 kg m^{2}for AA genotype	65
25.	rs52820871 (I251L)		56,189,806		16,797	0.75% (251L)	-0.35 SD of their BMI Z-score per 251L allele	67
26.	rs2229616 (V103I)		56,190,256		7,713	2% (103I)	-0.48 kg/ m^2 per 103I allele	67
27.	rs29941	19q13.11	39,001,372	*CHST8, KCTD15*	25,344	70% (C)	+0.46 kg/ m^{2}for CC genotype	62
28.	rs11084753		39,013,977		32,387	67% (G)	+0.06 kg/ m^2 per G allele	61

NEGR1, neuronal growth factor regulator 1; SEC16B, cerevisiae, homologue of, B; RASAL2, RAS protein activator like 2; TMEM18, transmembrane protein 18; INSIG2, insulin induced gene 2; SFRS10, splicing factor, arginine/serine-rich, 10; ETV5, ets variant 5; DGKG, diacylglycerol kinase, gamma, 90kD; GNPDA2, glucosamine-6-phosphate deaminase 2; PRL, prolactin; PTER, phosphotriesterase related; BDNF, brain derived neurotrophic factor; MTCH2, mitochondrial carrier homologue 2 (*C. elegans*); BCDIN3D, BCDIN3 domain containing; FAIM2, Fas apoptotic inhibitory molecule 2; SH2B1, SH2B adaptor protein 1; ATP2A1, ATPase, Ca^{2+} transporting, cardiac muscle, fast twitch 1; FTO, fat mass and obesity associated; MAF, v-maf musculoaponeurotic fibrosarcoma oncogene homologue (avian); NPC1, Niemann-Pick disease, type C1; MC4R, melanocortin 4 receptor; CHST8, carbohydrate (N-acetylgalactosamine 4-0) sulphotransferase 8; KCTD15, potassium channel tetramerisation domain containing 15

has provided a great stimulus to the field. Positional candidates are the genes that lie within genomic regions that have been shown to be genetically important in linkage or association studies, or by the detection of chromosomal translocations that disrupt the gene[71].

Candidate gene analysis is an indirect test of association to examine the relation between a dense map of SNPs and disease, while candidate SNP analysis is a direct test of association between putative functional variants and disease risk. The advantage of indirect association is that it does not require prior determination of which SNP might be functionally important; however, the disadvantage is that larger numbers of SNPs need to be genotyped[72]. A combination of functionally important SNPs with a collection of tag SNPs covering the entire candidate gene has been used in many candidate gene association studies[73]. Genetic variants in multiple candidate genes within the same biologic pathway can be examined, and their interaction can be tested in pathway analysis. But those genetic variants in multiple candidate genes of different biologic pathways and their interactions are very difficult to study through only candidate gene/SNP analysis as in the case of human obesity. Several genes have been analyzed in humans because these were found to be involved in central or peripheral pathways controlling energy intake and expenditure in animal models. Enormous association studies for obesity involving cases and controls or, less regularly, families comprising one or more affected children and both parents have been performed. But only for a small number of genes meta-analyses have been carried out and a list of latest positive results is available at *http://obesitygene.pbrc.edu/* which showed positive associations of obesity phenotypes with a total of 113 candidate genes, of which only for 18 genes a minimum of five positive studies had been reported as often the study groups were small[74]. The first truly validated polygenic effect on body weight detected via a candidate gene analysis was Val103Ile polymorphism in melanocortin-4 receptor (*MC4R*) gene[75].

Generally, these association studies have not given consistent results. Therefore, it is very difficult to get any convincing meta-analysis of variants in candidate genes that are explicitly linked to the genetic risk for obesity[76]. There is a wide difference in the obesogenic environments from where the subjects are recruited for the study. In many cases, data could not be replicated because of the various limitations in studies with the cohort size in which the association of variant(s) with the disease(s) was first detected. Because the contribution of any given gene to the phenotype of a complex trait is often minimal, a large cohort size is required if statistical significance is to be achieved. Another disadvantage of candidate gene analysis is that it depends on a prior hypothesis about disease mechanisms, therefore, the discovery of new genetic variants or novel genes would be excluded. Thus, the candidate gene approach is more appropriate for single gene disorder and not for the obesity like complex diseases. This type of approach may not give full resolution to the problem, but may help in establishing relationship between disease susceptibility and genetic variation. Hence, the only way forward seems to be investigation of the functional roles of the current candidate genes in model organisms and *in vitro* cell systems using Genome-Wide Approaches (GWA) which lead to the development of functional assays to test putative activator/inhibitor molecules as potential therapeutics.

Genome-wide approaches

Through genome wide association studies (GWAS) up to 2,000,000 genetic variants can now be analyzed for association with a given phenotype and have been proven extremely successful for various phenotypes[77]. This approach can be pursued through two ways, *viz*., Genome-wide linkage scans (GWLS) and Genome-wide association studies (GWAS). The GWLS identify chromosomal regions having gene(s) pertinent for a particular phenotype via linkage data. The regions underlying linkage peaks are narrowed down by fine mapping, so that the candidate gene analyses can be pursued. The first candidate gene for early onset of obesity detected via GWLS was ectonucleotide pyrophosphatase/phophodiesterase 1 (*ENPP*1) and attempts are ongoing to validate the association[78]. More than 40 microsatellite-based GWLS have been performed and none of the single candidate genes detected have been validated explicitly which further shows that either the effect sizes of genes influencing adiposity are small or the substantial heterogeneity exists or both. Therefore, to avoid such types of uncertainty GWLS analysis requires large samples size.

The GWAS provide a better devise to identify common variants with low to rational penetrance and are significant as risk factors for the trait of interest. Within a short duration, GWAS have proven to be very successful for the detection of polygenic variants. The advent of high density SNP-chips has made GWAS practicable and has given a new dimension to the study of complex diseases through the increased identification of confirmed genes and thereby revolutionized the molecular genetic analyses of complex disorders. GWAS performed for obesity or BMI (Table III) have established it as an extremely powerful tool to detect genetic variants for the complex phenotype(s). For example, presently known polygenic variants have a mean effect on BMI of approximately 0.03-0.5 kg/m^{2}which appears reasonable as these effect sizes represent the upper limit of such common variants[64,79,80]. So far, independent gene variants with small but replicable effects on body weight have been identified unambiguously in 17 gene regions[79]. *FTO* gene is the first example of a non-syndromic obesity gene studied thoroughly via GWAS of T2D[81]. Despite the initial success of the GWAS strategy, the established loci together explain less than 2 per cent of the inter-individual BMI variation and less than 1 per cent of the inter-individual WHR variation[12,82]. With heritability estimates of 40-70 per cent for BMI and 30-60 per cent for WHR, there must be many more susceptible loci to be discovered[32]. These types of susceptible loci can only be unraveled if GWAS are expanded to different ethnic groups through large-scale international collaboration and meta-analysis of existing data (Table IV), selection of defined scientific procedures like CT scan, dual-energy X-ray absorptiometry (DEXA) or magnetic resonance imaging (MRI) and consideration of rare and low frequency variants and non-coding RNAs. Though GWAS approach seems to be a strong way of analysis for complex disorders, it has to be extended for different environmental conditions as it is very evident that gene-environment (GXE) interactions also regulate the mechanisms of gene expression[90].

Epigenetic modifications

Modifications that affect gene expression but do not alter the DNA sequence are termed epigenetic modifications. These include DNA methylation and histone modifications, which are expected to have regulatory roles in the inheritance and vulnerability to obesity, by affecting the expression(s) of associated gene(s). Also, intrauterine atmosphere during specific developmental phases can vary the epigenetics of an individual and may work as a foundation for the obesity and other phenotypes during later stage of life[91,92]. For example, variations in birth weight are affected by several factors such as maternal genes, maternal *in utero* and placental factors, maternal BMI, maternal smoking, maternal alcohol consumption, maternal drug use, exercise during pregnancy, paternal genes, birth weight, *etc*. In the similar manner as during the early post-natal period poor nutrition also affects the mother metabolism to acclimatize to favour the storage of nutrients[93]. Another study on Pima Indians showed paternally imprinted gene located on chromosome 11 position 80 cm was influencing birth weight[94]. Similarly, in a study on Mexican Americans quantitative trait loci (QTL) on chromosome 6q was found to be associated with birth weight regulation[95]. Hence, gene-environment (GXE) interactions play a significant role in the aetiology of obesity may be via modifications in DNA methylation patterns. Thus, apart from variations at DNA sequence level, epigenetic incidents also seem to contribute to the epidemiocity of obesity which are evident from modern day sedentary living style. It has been observed that during the early years of life monozygotic (MZ) twins are epigenetically indistinguishable from each other. But, with increasing age remarkable differences in their overall content and genomic distributions of 5-methylcytosine DNA and histone acetylations become noticeable and similarly environment could have an influence on individual's BMI[91]. A comparative study of epigenetic metastability of 6,000 unique genomic regions between matched monozygotic (MZ) and dizygotic (DZ) twins demarcated epigenetic differences in both the MZ and DZ twins[96]. Molecular mechanisms of heritability may not be limited to differences in DNA sequence only, rather epigenetic modifications are also acting as one of the very important governing factors in unraveling the secrets behind the blue prints of DNA sequence.

Mitochondrial contribution to obesity

Being the centre of all the metabolic processes and very susceptible to change, mitochondria play a crucial role in the development of any disorders. Since only mother contributes mitochondria to the next generation, there is a chance of maternal inheritance of diseases

Table IV. Summary of Genome-wide association studies (GWAS) or meta-analysis for obesity in humans

S. No.	Different GWAS study	Sample size in detected cohort	Predecessors of detected cohort	Parameter(s)	References
1.	WTCCC	1924	Europeans	BMI for quantitative analysis	64
2.	Sardinia	4741	Europeans	BMI for WC and quantitative analysis	75
3.	LOLIPOP	2684	Indian Asians	Analysis of IR and related quantitative phenotypes	79
4.	-	16 876	Northern European	BMI for quantitative analysis	64
5.	The CHARGE consortium	31 373	Europeans	WC for quantitative analysis	83
6.	The GIANT consortium	38 580	Europeans	WC and WHR for quantitative analysis	12
7.	-	775 cases and 3197 unascertained controls	Europeans	Extreme obesity or BMI	84
8.	-	1380 and 1416 age-matched normal-weight control	Europeans	Early onset and morbid adult obesity	63
9.	DeCODE	37 347	Europeans & African Americans	BMI for quantitative analysis	62
10.	The GIANT consortium	32 387	Europeans	BMI for quantitative analysis	61
11.	-	487 extremely obese young cases and 442 healthy lean controls	Europeans	Extreme obesity or BMI	85
12.	-	453 extremely obese young cases and 435 healthy lean controls	Europeans	Extreme obesity or BMI	86
13.	KARE	8842	Asian	BMI, WHR for quantitative analysis	87
14.	MAGIC	77 167	Europeans	WHR for quantitative analysis	88
15.	-	123 865	Europeans	BMI for quantitative analysis	89

BMI, Basal metabolic index; WC, waist circumference; WHR, waist to hip ratio; IR, insulin resistance

affecting physiology of mitochondria in mother. For example, maternal obesity (*in utero* environment) plays a detrimental role in the development of early embryo(s) during embryogenesis[97]. It was further elaborated in a study including maternal-diet induced obesity in a murine model through the association of altered mitochondrial activities and redox status of oocytes and zygotes[98]. These altered mitochondrial properties involved an increase in mitochondrial membrane potential, mitochondrial DNA (mtDNA) content and biogenesis, generation of reactive oxygen species (ROS) and depletion in glutathione level leading to more oxidized - redox state. These effects resulted in oxidative stress which led to the significant developmental impairment at early embryogenesis and might be the explanation for the reduced reproductive status observed in obese women[99]. Thus, mitochondria were found to be the liable candidates for compromised metabolism in the embryo, and are maternal in origin. Mitochondria also execute various regulatory roles during oocyte maturation, fertilization, initiation and progression of pre-implantation of embryos[100,101]. As power house of the cell, the central and most vital role of mitochondria is the synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation, a mechanism coupling the oxidation of nutrients and

reducing equivalents [NAD(P)H, $FADH_2$] with the phosphorylation of adenosine diphosphate (ADP). Consumption of the energy rich diets results in the excess production of these reducing equivalents [NAD(P)H, $FADH_2$] which ultimately leads to the increased pumping of protons (H^+) out of the mitochondrial matrix through mitochondrial electron transport chain (ETC) and results in hyperpolarization of the inner mitochondrial membrane thereby generating excess of proton motive force (PMF) and excess of ATP. Besides being used for energy production, these reducing equivalents [NAD(P) H and $FADH_2$] are known to regulate the intracellular redox status. NADH oxidation in the mitochondria will produce ROS whereas NADPH oxidation (in cytosol and mitochondria) serves to restore the antioxidant protection by reducing per-oxiredoxins, thioredoxin and oxidised glutathione. Thus, mitochondria have dual regulation on the intracellular redox state via regeneration of antioxidants and via ROS production[102]. Apart from centre of ATP production, mitochondria are also a source of cellular guanosine-5'-triphosphate (GTP) production as well as the site of amino acid synthesis and the reservoir of cellular calcium (Ca^{2+}). Therefore, any change(s) in mitochondrial activity can alter cellular functions. Importance of mitochondria in oocyte quality and embryo development has been highlighted in many studies showing the various impacts of mitochondrial biogenesis defect(s) on mitochondrial mass, failure of oocyte maturation, abnormal embryo development[103,104], *etc*. Both the quality and the quantity of mitochondria are an important prerequisite for successful fertilization and embryo development[105]. *In vitro* studies including have emphasized the vulnerability of mitochondria towards various environmental stress either within the oocyte or in the developing embryo and it has been shown that even low-level of acquired mitochondrial injuries may persist into embryonic life[106,107]. Latent influences of maternal nutritional status during obesity had been indicated in studies showing that periconceptual exposure to high energy substrates such as fatty acids and proteins resulted in perturbed mitochondrial metabolism in oocyte as well as in embryo[108,109]. Hence, mitochondrial abnormalities in oocytes and in early embryos have been found as a direct environmental consequence of maternal obesity. It is, therefore, noteworthy that alterations in mitochondrial activities (*i.e*., dysfunctional mitochondria) are not only restricted to the maternal obesity but rather obesity itself results in the development of dysfunctional mitochondria or vice versa. This ultimately leads to various types of obesity associated complications such as development and progression of T2D complications, early mitochondrial adaptations in skeletal muscles to diet-induced obesity, mitochondrial remodelling in adipose tissue associated with obesity, inflammation and mitochondrial fatty acid β-oxidation linking obesity to early tumour promotion in pancreas, *etc*[110-114]. It has been observed that mitochondrial genome polymorphisms are also involved in the development of obesity syndrome such as a mtSNP, 8684C>T (T53I) in the mitochondrial ATP synthase subunit 6 gene (ATP6) was detected in five patients of type-2 diabetes and was not detected in any of the young obese adults. Similarly, two mtSNPs, 3497C>T (A64V) in NADH dehydrogenase subunit 1 gene (ND1) and 1119T>C (472U>C) in the 12S rRNA gene, were detected in five young obese adults and were not found in any of the diabetic patients[115]. Further, several studies have also shown the associations of different mtSNPs with the incidence of obesity in various human ethnic groups during the course of evolution. These include three human mtSNPs *viz*., ND2, COX2 and ATP8 within genes encoding proteins of electron transport and oxidative phosphorylation in sub-haplogroups of the Pima Indians. These were adaptations toward an energy-efficient metabolism when this population migrated to the desert and adopted a restricted caloric intake. Today these may contribute to obesity[116]. Polymorphisms of the *UCP*2 gene (rs660339 and rs659366) were found to be associated with body fat distribution and risk of abdominal obesity in Spanish population[117]. The *UCP*2 A55V variant was found to be associated with obesity and related phenotypes in an aboriginal community in Taiwan[118]. *UCP*1 variants, g.IVS4-208T>G SNP was associated with obesity in Southern Italy severe obese population[119]. Two mtSNPs (mt4823 and mt8873) and mtDNA haplogroup X were observed to be significant markers associated with reduced body fat mass[120]. Linkage and association analyses of the *UCP*3 gene showed an association with obesity phenotypes in Caucasian families[121]. A common polymorphism in the promoter of *UCP*2 gene was found to be associated with obesity and A allele associated with obesity and hyperinsulinaemia in north Indians[122]. In another study, obesity and hepatosteatosis were observed in human 8-oxoguanine-DNA glycosylase 1 (hOGG1)

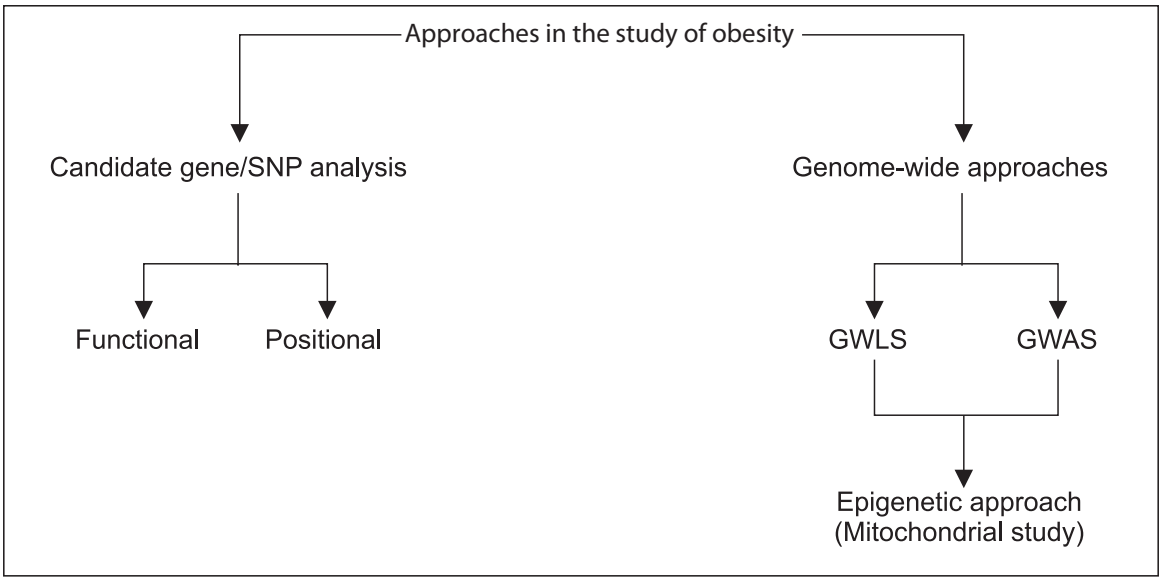

Fig.1. Strategic approach towards analysis of obesity in humans. SNP, single nucleotide polymorphisms; GWLS, genome-wide linkage scans; GWAS, genome-wide association studies.

transgenic (TG) mice with enhanced oxidative damage of mitochondrial DNA due to overexpression of the transgene hOGG1[123]. Thus, mitochondria might be either a culprit or a victim in obesity and the studies focussing on this organelle may help in better understanding of the cause of diet-induced obesity.

The obesity cauldron – the current focus

Obesity, especially central obesity being heritable arises from the interactions of multiple genes and environmental factors (GXE). Since the sequencing of the human genome, researchers and geneticists are busy in documenting the various interactions among genetic and environmental risks of precursors to chronic illness, including the influence of gene-diet interactions in disorders such as obesity and diabetes. This includes systematic genomic variation(s) in identifying various risks for common diseases along with the knowledge of body compositions in different populations with a consideration of individual's ethnic and racial variations. This might lead to the development of more individualized, more predictive, and more preventive therapeutics against obesity. Thus, if a strategy of GWAS along with epigenetic study of a particular disease like obesity is followed, it will help in finding a solution (Figure). For example, a novel genome wide scan employing a high density SNP array led to the identification of a SNP in the vicinity of the insulin induced gene 2 (INSIG2) and was found to be associated with obesity in both children and adults[124,125]. In another report, MC4R coding sequence variants were found to be associated with fat mass, body weight and obesity[80]. The *MC4R* mutations were also found to be linked with dominantly inherited genetic cause of childhood obesity in Americans of European and African ancestry[126].

To detect small gene effects and to narrow down the genomic target region more precisely, the GWAS are expected to be more powerful so that these can analyse a fixed set of genome and try to fix biomarkers. An example is peroxisome proliferater-activated receptor gamma (PPARγ) which is essential for adipocyte differentiation[127]. Here a zinc finger-containing transcription factor abundantly expressed in adipose tissue Krox 20 is expressed early in adipogenesis and is found to trans-activate the CCAAT/enhancer binding proteins β (C/EBP) β promoter[128]. Others like Kruphel-like factor (KLF) 6 and KLF 15 have been shown to promote adipogenesis and KLF15 is noticed to upregulate GLUT 4 expression[129,130]. Recent reports demonstrated that KLF4 functions as an immediate early regulator of adipocyte differentiation. Another one is adiponectin which is an oxidative regulator from adipocytes that modulates insulin sensitivity and thus regulates glucose

and lipid metabolism and leads to a reduction in energy homeostasis in obesity[131]. Hydroxysteroid (11-β) dehydroxgeinase-1 overexpression leads to visceral obesity by regulating glucocorticoid action[132]. Others in the list are mutation of *MC4R*, leptin/leptin receptor, prohormone convertase1 and pro-opimelanocartin (POMC)[20-26,30,31].

Genes associated with β-cell dysfunction have also been identified which include hepatocyte nuclear factor 4 and α1 polymorphism in potassium channel Kir6-2 (KCNJ11) and transcription factor 7 like 2 (TCF 7 L 2). Many candidate genes have also been identified such as calpain 10, adiponectin, PPAR γ co-activator 1 (PGC1) and glucose transporter GLUT 2[133]. Genetic polymorphisms at the perilipin (*PLIN*) locus that is minor allele at PLIN4 (11482G>A) was associated with higher risk of metabolic syndrome (MS) at baseline, whereas the PLIN6 SNP (14995A>T) was found to be associated with weight loss in obese children and adolescents[134]. A positive functional relevance of nicotinamide nucleotide transhydrogenase (NNT) in the development of human obesity and visceral fat distribution has been observed in obese patients and correlated with body weight, BMI, percentage body fat, visceral and subcutaneous fat area, waist and hip circumference, and fasting plasma insulin (FPI)[135]. Association of decreased levels of plasma ghrelin has been observed with obesity in obese Caucasian and Pima Indians[136]. Interleukin-1 (IL-1) gene polymorphisms may be involved in increased central obesity and the genetic influences are more evident among patients who have a higher level of obesity or inflammatory markers[77]. Genetic variation in the McKusick-Kaufman gene (MKKS) gene may play a role in the development of obesity and the metabolic syndrome in Greek population[138]. A study on postmenopausal women suggested that *FTO* gene was a susceptibility locus for both obesity and type-2-diabetes (T2D). Common genetic variants in the intron 1 of *FTO* gene may present a natural predisposition to obesity in an ethnicity-specific manner also[139]. The insulin responsive adiponectin genetic variants of potatin-like phospholipase domain-containing genes 1 (PLPLA1) have moderate effect on obesity and potatin-like phospholipase domain-containing gene 3 (PNPLA3) or adeponectin effects (ADPN) gene shows on insulin sensitivity[140]. Procolipase (CLPS) is secreted from the exocrine pancreas into the gastrointestinal tract and its genetic variability is associated with the secretary function of insulin in non-diabetic humans[141]. SNPs in α-2 subunit of neuronal nicotinic acetylcholine receptor gene *CHRNA2* rs2043063 SNP might be a risk factor for overweight/obesity in Koreans[142]. Folate/vitamin B12 plays vital role in the critical stages of foetus development and involves one carbon pool metabolism which may lead to greater insulin resistance, and further to the development of obesity[143].

According to twelfth update of Human Obesity Gene Map, 52 genomic regions harbour QTLs supported by two or more studies[74]. The number of QTLs reported from animal models has reached to 408. The number of human obesity QTLs derived from genome scans continues to grow, and so far 253 QTLs for obesity-related phenotypes from 61 genome-wide scans have been reported. Association studies between the variation(s) of DNA sequence in specific genes and obesity phenotypes have also been increased considerably (426 findings of positive associations with 127 candidate genes). At our institute, two mutant obese rat strains *viz*., WNIN/Ob (with euglycaemia) and WNIN/GR-Ob (with impaired glucose tolerance) had been developed naturally from a Wistar inbred rat colony[144,145]. These mutants show hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia, and they also have several obese features such as polydipsia, polyuria and proteinuria. From the preliminary studies it has been found that mutant WNIN/Ob does not exhibit any of the conventional defects either in leptin or leptin receptor locus (unpublished data) but showed the defect on chromosome no. 5, in the upstream region of leptin receptor and the studies are still ongoing to identify and sequence the locus.

Conclusions and the way forward

Numerous analyses have been published discussing about the genetic complications and various types of challenges concerned with the biological pathway of common obesity[74,146-149]. However, the major obstruction is the replication of data. The complexity of a trait/disease in an individual's life is a result of accumulation of various interactions of the linked genes to different genetic settings and exposure to diverse environmental factors. Due to scarce knowledge on the extent to which genes finally contribute to complex trait, the significance of subtle environmental factors may not be

valued. Thus, at such an early stage of our knowledge to unravel the genetics of obesity both replicated and un-replicated data should be considered equally important. So far, most of the studies on polygenic obesity are SNP based which are located either within or near a candidate gene. Considering the entire candidate gene studies on animal models and *in vitro* as an initial level studies, their association with the common obese phenotype should be verified through various types of case control and family studies. But, in contrast to monogenic obesity, many genes and chromosomal area contribute to characterize common obese phenotype (polygenic) and have been found linked to an extensive range of biological functions, such as regulation of food intake, energy expenditure, lipid and glucose metabolism, adipose tissue development, *etc*. Even with this ever increasing gene catalogue at our disposal, unraveling the molecular mechanisms of obesity is still challenging. As not only the number of genes associated with obesity is high, but, the modifications in these gene(s) also show the significant polymorphisms in the elucidation of environmental stimuli. Further, *in utero* environment and expression of several genes during embryonic development and specifications play an important role in governing the intensity of obesity. For instance, genetically programmed developmental variations in adipocytes and their precursors in different sections of the body play a significant role in the progression of obesity via a complex network of transcription factors like activators, co-activators and repressors[150].

With the advancement in the knowledge of the human genome, the development of comprehensive technologies, and new analytical approach it will be feasible to address both the genetic and environmental features of complex traits simultaneously. But the success will eventually lie with international consortiums that pool together expertise and resources to describe and interpret the functional role of the various genetic factors underlying the diverse types of obesity. Undoubtedly, family, twin and adoption studies primarily provide sufficient data for moderate to high heritability of BMI and are a focus of molecular research in finding an explanation at DNA level. Epigenetic research will add a new dimension to this by explaining intra-individual variation in body weight. Thus, with the use of advanced technologies epigenetic profile of the associated obesity genes can be discerned and could also be applied in a genome-wide approach.

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