3 Monogenic Obesity

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CONTENTS

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INTRODUCTION

Obesity is increasing dramatically worldwide and is projected to affect 1.12 billion people by 2030 *(1)*. The epidemic of obesity is attributed to recent changes in the environment (easy access to high-energy palatable food, combined with a decreased physical activity), whereas individual differences in obesity risk are attributed to genetic differences between individuals: the heritability of body mass index (BMI) has been estimated to approximate 70% in adults and is even higher (77%) for younger people raised in an increasingly obesogenic environment *(2)*. The prevalence of obesity (defined by a BMI $\geq$ 30 kg/m^2) in the United States increased by 24% between 2000 and 2005; the prevalence of morbid obesity (BMI $\geq$ 40 kg/m^2) and super obesity (BMI $\geq$ 50 kg/m^2) increased by 50 and 75%, respectively, during the same period *(3)*. These data clearly indicate that the current environment acts as a "catalyst" to reveal subjects with higher genetic susceptibility to obesity.

Whereas the key role of heredity in obesity was established from 1986 with seminal twin studies by Stunkard and colleagues *(4)*, the first discovery of a gene involved in human obesity came more recently, with the discovery of mutations in the leptin gene in 1997 *(5)*. Since then, seven additional genes have been linked to human monogenic obesity, illuminating the alteration of central control of food intake as a major causative mechanism leading to obesity (Fig. 1). The recent harvest of polygenic genes influencing obesity and corpulence has confirmed the importance of neuronal influence on food intake regulation in body weight regulation *(6)*. Here we review the main advances in the elucidation of monogenic forms of human obesity and offer a specific focus on the future directions of research in the genetic dissection of obesity single gene disorders.

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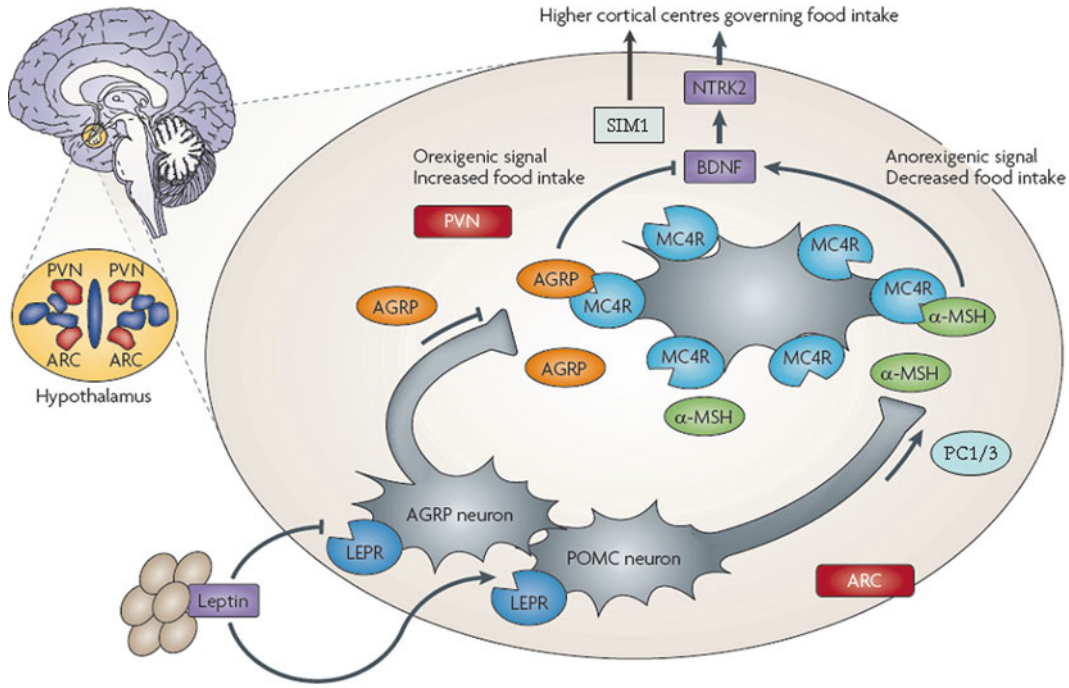

Fig. 1. The leptin–melanocortin pathway (Modified from Walley et al., Nat Rev Genet 2009, with the permission of Nat Rev Genet). The melanocortin 4 receptor (MC4R) is highly expressed in the paraventricular nucleus (PVN) of the hypothalamus, where it has a key role in the control of appetite. Leptin released from adipose tissue binds to leptin receptors (LEPR) on agouti-related protein (AGRP)-producing neurons and proopiomelanocortin (POMC)-producing neurons in the arcuate nucleus (ARC) of the hypothalamus. Leptin binding inhibits AGRP production and stimulates the production of POMC, which undergoes post-translational modification by prohormone convertase 1/3 (PC1/3) to generate a range of peptides, including alpha-, beta-, gamma-melanocyte-stimulating hormone (MSH). AGRP and alpha-MSH compete for MC4R–AGRP binding suppresses MC4R activity and alpha-MSH binding stimulates MC4R activity. Decreased receptor activity generates an orexigenic signal, whereas increased receptor activity generates an anorexigenic signal. Signals from MC4R govern food intake through secondary effector neurons that lead to higher cortical centres, a process that involves single-minded homologue 1 (SIM1), brain-derived neurotrophic factor (BDNF), and neurotrophic tyrosine kinase receptor type 2 (NTRK2; also known as tropomyosin-related kinase B, TRKB).

MONOGENIC OBESITY CAUSED BY MUTATIONS IN THE LEPTIN/MELANOCORTIN PATHWAY

1-Leptin and Leptin Receptor Deficiency

Leptin is a cytokine secreted by adipocytes in proportion to body's fat content. It binds to receptors in two different specific populations of neurons (neuropeptide Y/agouti-related peptide-expressing neurons and proopiomelanocortin/cocaine and amphetamine-related transcript-expressing neurons) of the arcuate nucleus of the hypothalamus. The key role of leptin and its receptor in energy metabolism was first demonstrated by the successful positional cloning of two mouse models of obesity (the *ob/ob* and *db/db* mice). These seminal studies were rapidly followed by the analysis of candidate genes in obese patients, which led to the identification of pathogenic mutations in both leptin (*LEP*) and its receptor (*LEPR*) in extreme forms of early-onset obesity *(5,7)*.

Thus far, 13 patients of Turkish, Pakistani, and Egyptian origin have been found to have *congenital leptin deficiency*. They are all homozygous carriers of a frameshift (deltaG133) or two missense (R105Y and N103K) mutations in the *LEP* gene, resulting in very low circulating leptin levels.

The first patients with *congenital leptin receptor deficiency* were three sisters from a consanguineous Algerian pedigree who had a homozygous G→A mutation in the splice donor site of exon 16 that results in a truncated leptin receptor lacking both the transmembrane and the intracellular domains *(7)*. The prevalence of patients with pathogenic *LEPR* mutations (homozygous/compound heterozygous) was 2.7% in a highly consanguineous cohort of 300 severely obese children *(8)*. Frameshift or nonsense mutations result in the loss of all isoforms of the leptin receptor, whereas missense mutations affect only the extracellular domain of the receptor *(8)*.

Subjects with *congenital leptin deficiency* exhibit normal weight at birth but gain weight rapidly in the early postnatal period. Leptin deficiency is associated with marked hyperphagia, impaired satiety, and excessive fat deposition in the trunk and limbs *(5)*. Complete leptin deficiency is also associated with hypothalamic hypothyroidism (low free T4 and mildly elevated serum TSH) and hypogonadotropic hypogonadism, with delayed or absent pubertal progression. Linear growth and serum IGF-1 are normal but final height is reduced because of the absence of a pubertal growth spurt. Children with leptin deficiency have altered T-cell number and function and suffer high rates of childhood morbidity and mortality from infectious disease *(9)*.

Leptin-deficient patients can be treated with daily injections of recombinant human leptin, which reverses the obesity and associated phenotypic abnormalities *(10,11)*. Leptin administration dramatically reduces food intake through the modulation of neural activation in key striatal regions, suggesting that the hormone acts centrally to diminish the perception of food reward and to enhance the response to satiety signals during food consumption *(12)*. Leptin administration is also associated with decreased food preference for carbohydrates *(13)*. Long-term (4 years) leptin administration also confers sustained beneficial effects on fat mass, hyperinsulinemia, and hyperlipidemia. Appropriately timed pubertal development and restoration of T-cell responsiveness are also observed, allowing the withdrawal of T4 treatment *(14)*. Leptin replacement in patients with congenital leptin deficiency is proposed to increase grey matter concentration in the anterior cingulate gyrus, the inferior parietal lobule, and the cerebellum *(15)* and to induce changes in rates of development in many neurocognitive domains *(16)*.

Heterozygous carriers of deleterious mutations in LEP have a partial leptin deficiency associated with an overweight or obese phenotype (85%) *(17)*. Peripheral leptin supplementation was shown to induce significant weight loss in the subgroup of subjects with low levels of leptin *(18)*, suggesting that leptin administration may be warranted in subjects with partial leptin deficiency to reduce their propensity to develop obesity.

Patients with *congenital leptin receptor deficiency* consume three times the amount of energy eaten by controls in a test meal, and they all become severely obese during childhood *(8)*. They present alterations in immune function and frequent childhood infections of the respiratory tract associated with high rates of premature death. They also manifest delayed puberty due to hypogonadotropic hypogonadism, and some are hypothyroid *(7)*. However, their clinical features are usually less severe (mean BMIz +5.1) than those of subjects with congenital homozygous leptin deficiency (mean BMIz +6.8), and hypothyroidism is less common *(8)*. Importantly, serum leptin levels in patients with leptin receptor mutations (36–365 ng/ml at age 4–18 years) were not significantly different than those in comparably obese subjects with no apparent mutations of the leptin receptor *(8)*. Interestingly, the body fat content is higher in *heterozygous carriers of LEPR mutations* than in their wild-type relatives *(8)*. Leptin treatment is ineffective.

2-Proopiomelanocortin (POMC) Deficiency

POMC/CART neurons are activated by leptin (Fig. 1). In contrast to CART, there is clear evidence that POMC peptides play critical roles in feeding behaviour. POMC is processed by prohormone convertases 1/3 and 2 into five biologically active proteins: adrenocorticotropic hormone (ACTH), alpha-, beta-, and gamma-melanocyte-stimulating hormone (MSH), and beta-endorphin. Mouse models with disruption of both alleles of the *POMC* gene are characterized by obesity, defective adrenal development, and altered pigmentation *(19,20)*. Krude and colleagues provided the first description of human obesity associated with congenital deficiency of all *POMC* gene products *(21)*. To date, six patients carrying either homozygous or compound heterozygous *POMC* mutations have been reported. Patients with complete POMC deficiency present in early life with hypoadrenalism secondary to ACTH deficiency, leading to hypoglycaemia, jaundice, and in one case neonatal death associated with severe liver cholestasis. Treatment with glucocorticoids reverses hypocortisolemia in these patients, but they develop severe early-onset obesity associated with hyperphagia.

As a result of the lack of ligand for melanocortin 1 receptors, POMC-deficient patients of European ancestry have pale skin and red hair *(21)*. However, two subjects of Turkish or Algerian origin had normal hair and skin pigmentation despite congenital POMC deficiency *(22,23)*. Chemical analysis of hair pigment revealed an increased production of both pheomelanin and eumelanin, but these subtle pigmentary features were not distinguishable during a clinical examination *(23)*. This observation is concordant with a polygenic control of hair and skin pigmentation *(24)* and suggests that the molecular screening of POMC can be considered in patients with early-onset adrenal insufficiency and obesity, even in the presence of normal pigmentation.

A Turkish pedigree including 1 homozygous and 12 heterozygous carriers of the C6906del mutation of POMC was recently reported *(22)*. The mutation was predicted to lead to the loss of all POMC-derived products. Interestingly, 11 of 12 heterozygous carriers were obese or overweight, strongly suggesting that loss of one copy of the POMC gene predisposes to obesity *(22)*.

Heterozygosity for a variety of mutations in the region encoding alpha-MSH *(25)* or beta-MSH *(26,27)* is also associated with a high risk of obesity. A missense mutation (R236G) disrupting the dibasic cleavage site between beta-MSH and beta-endorphin resulted in a fusion protein that binds to the melanocortin 4 receptor (MC4R) but has reduced ability to activate it *(28)*. This mutation was fourfold more prevalent in subjects with early-onset obesity than in lean controls *(28)*.

3-Proprotein Convertase 1 Deficiency

Prohormone convertase 1/3 (*PCSK1*) represents the major processing enzyme of prohormones involved in the regulated secretory pathway. This enzyme converts prohormones (like proinsulin, proglucagon, or pro-POMC) into functional hormones that regulate central and/or peripheral energy metabolism. To date, three patients with monogenic forms of human obesity due to *PCSK1* deficiency have been described *(29–31)*. Complete *PCSK1* deficiency due to compound heterozygous or homozygous mutations leads to early-onset obesity *(29–31)*, hyperphagia *(31)*, reactive hypoglycemia *(29,30)*, and an increased ratio of proinsuiln to insulin *(29–31)*. In addition, PC1/3 mutations are associated with an enteropathy with diarrhea, suggesting that enteroendocrine cell expression of PC1/3 is essential for the normal absorptive function of the human small intestine *(30,31)*. Family members who are heterozygous for PC1/3 mutations are clinically unaffected and not obese *(29–31)*. In contrast mice heterozygous for a *PCSK1* mutation are characterized by an intermediate phenotype *(32)*: N222D-heterozygous mice had increased body fat content compared to wild-type mice.

4-Melanocortin 4 Receptor Deficiency

Of the five melanocortin receptors, only the melanocortin 4 receptor has been described as pivotal in the control of energy balance in rodents. The melanocortin 4 receptor is a seven transmembrane-spanning α-helices protein of the class A, G-protein-coupled receptors that include rhodopsin and the adrenergic receptors. Targeted disruption of the melanocortin-4 receptor results in an allelic dosage-dependent obesity phenotype in mice *(33)*.

The first cases of human obesity caused by heterozygous *MC4R* mutations were identified in 1998 *(34,35)*. Since then, more than 100 mutations in the coding sequence of *MC4R* have been reported to cause familial forms of obesity. The prevalence of *MC4R* pathogenic mutations has been reported to be as high as 5.8% in a cohort of children with extreme obesity and consanguinity *(36)*, but more commonly approximates 2% in more general obesity cohorts of European origin *(37,38)*. In contrast, pathogenic mutations are found in only 0.1% of the European general population *(39,40)*. Indeed, the penetrance of obesity in heterozygous mutated individuals is not complete and non-obese mutation carriers have been occasionally described *(37,41,42)*. Modifying factors like gender or generational environment have been proposed to modulate substantially the obesity phenotype associated with partial *MC4R* deficiency *(37,43)*. In contrast, homozygous or compound heterozygous pathogenic mutations lead to an obligatory severe obesity phenotype *(36,37,44)*. Co-dominance is therefore the most likely pattern of inheritance in case of *MC4R* deficiency *(36,37)*.

MC4R deficiency is associated with early-onset hyperphagia (often in the first year of life) and subsequent rapid increase in fat mass during childhood *(36)*. However, hyperphagia and body fat accumulation are also observed in adulthood (mean BMIz homozygotes +4.8–5.0, heterozygotes +2.8) *(8,37)*. Body fat mass averages 42 and 50%, respectively, in heterozygotes and homozygotes. Accelerated linear growth and tall stature are apparent within the first year of life, possibly due to exaggerated secretion of insulin associated with early-onset severe obesity; serum IGF-1 is normal *(36)*. Bone density is increased approximately 1.5 SD. Serum and urinary cortisol and serum lipids are normal and the leptin levels correlate with fat mass. Gonadotropin secretion and pubertal development are also appropriate for age. *MC4R* deficiency is paradoxically associated with lower systolic and diastolic blood pressure (melanocortinergic signalling modulates the control of blood pressure through an insulin-independent mechanism) *(45)*.

Up to 80% of pathogenic mutations in *MC4R* cause an intracellular retention of the abnormal receptor, but mutations affecting only agonist/antagonist-binding affinity or even causing constitutive receptor activation have also been reported *(46–48)*. Most *MC4R* mutations are more likely to result in obesity through haplo-insufficiency *(47)* with the exception of the D90N mutation, which has a dominant-negative effect due to abnormal receptor dimerization *(49)*.

MC4R-deficient obese children treated with exercise counselling and behavioural and nutritional therapy initially lose weight but fail to maintain weight loss after discontinuation of treatment *(50)*. No specific treatment currently exists to reverse the *MC4R* deficiency-associated obesity phenotype. Nevertheless, small-molecule MC4R agonists might provide a personalized treatment for *MC4R*-deficient patients *(51)*. Interestingly, a recent case report suggests that early diagnosis of MC4R deficiency followed up by lifestyle intervention may prevent the development of obesity *(52)*.

II-MONOGENIC OBESITY WITH NEUROLOGICAL FEATURES

1-Brain-Derived Neurotrophic Factor and Its Receptor TrkB

Brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B (TrkB) are involved in proliferation, survival, and differentiation of neurons during development and postnatal synaptic plasticity in the central nervous system, especially in hypothalamic neurons; BDNF is

expressed at high levels in the ventromedial hypothalamus, where it is regulated by nutritional state and MC4R signalling. Partial deficiency of BDNF or TrkB in mice increases food intake and fat deposition *(53–55)*. In addition, BDNF haplo-insufficiency induces abnormalities in behavioural and locomotor activity *(53,54)*.

Human BDNF haplo-insufficiency was first described in a 8-year-old girl who harboured a de novo chromosomal inversion, 46,XX,inv(11)(p13p15.3), which is the region encompassing *BDNF*. Clinical phenotypes included hyperphagia, severe obesity, impaired cognitive function, and hyperactivity *(56)*. Hyperphagia and obesity are also observed in a subgroup of patients with the WAGR syndrome (the main clinical features are Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation). This syndrome is due to heterozygous, variably sized deletions on chromosome 11p14.1, in the vicinity of the *BDNF* gene. Han and colleagues demonstrated that 58% of the 11p heterozygous deletions they analyzed included the *BDNF* gene. These caused a 50% reduction in serum BDNF concentrations. In patients with the WAGR syndrome and *BDNF* deletions, 100% were obese; in contrast, the rate of obesity was 20% in those without *BDNF* deletions, which corresponds to the obesity prevalence in the United States *(57)*. A child with severe obesity, hyperactivity, and impairments in short-term memory, learning, and nociception was found to be a de novo carrier of a *NTRK2* (the gene coding for TrkB) missense mutation (Y722C) that markedly impaired receptor autophosphorylation and signalling to MAP kinase *(58,59)*.

2-Single-Minded 1 Transcription Factor

SIM1, the mammalian homologue of *Drosophila sim,* is a transcription factor playing a major role in neuronal differentiation of the paraventricular nucleus of the hypothalamus, a critical brain region for food intake regulation. Mice haplo-insufficient for *Sim1* develop hyperphagia and early-onset obesity *(60)*. Holder and colleagues described a de novo balanced translocation disrupting *SIM1* in a patient with hyperphagia and severe obesity *(61)*. Additional evidence of a role of *SIM1* haplo-insufficiency in human obesity was provided by the finding of rare non-synonymous *SIM1* mutations in severely obese patients (6/379) in comparison with lean subjects (0/378) *(62)*. Patients with obesity and a Prader–Willi-like (PWL) syndrome harbour interstitial deletions in the 6q16 region that contains *SIM1*gene *(63)*. The critical region for 6q PWL syndrome encompasses about 10 genes or gene prediction apart from *SIM1 (63)*, but recent data have more specifically linked SIM1 haplo-insufficiency with the PWL syndrome *(64)*.

III-A CONTINUUM BETWEEN MONOGENIC AND POLYGENIC OBESITY?

Up till now, eight genes (*LEP*, *LEPR*, *MC4R*, *POMC*, *PCSK1*, *BDNF*, *NTRK2*, and *SIM1*) have been convincingly linked to human monogenic obesity. In addition, the recent wave of genome-wide association studies (GWAS) has increased our knowledge of the polygenic background of more common forms of obesity *(65)*. A striking observation from GWAS is the existence of a partially overlapping continuum between monogenic and polygenic forms of obesity. At least five genes causing monogenic obesity also increase the risk for polygenic obesity. They are *MC4R (66,67)*, *POMC (68)*, *PCSK1 (69)*, *BDNF (70)*, and *SIM1 (71)*. The case of *MC4R*, a "three-headed" Cerberus obesity gene, is really illustrative from this point of view. Whereas loss-of-function mutations in the *MC4R* gene are the commonest cause of monogenic forms of obesity *(36)*, the two infrequent gain-of-function V103I and I251L coding non-synonymous polymorphisms have been associated with protection against obesity *(66)*. Furthermore, a SNP located 188 kb downstream of the *MC4R*-coding sequence has been consistently associated with a modest increase in the risk for obesity *(67)* and an altered eating behaviour pattern *(72)*.

CONCLUSIONS AND PERSPECTIVES

Candidate gene approaches based on information from obesity mouse models have shown that defects in eight genes involved in the neuronal differentiation of the paraventricular nucleus and in the leptin/melanocortin pathway lead to monogenic forms of early-onset severe obesity with hyperphagia as a key feature (Fig. 1). Elucidation of these genes delineates obesity as an inherited disorder of central regulation of food intake *(73)*. Recent progress in the elucidation of polygenic predisposition to obesity also points to a key role of the central nervous system in body weight regulation *(65)*. This is not totally surprising, since food intake-related parameters are heritable *(74)* and are strongly correlated to body mass index *(75)*.

It remains for us to establish the proportion of "random" obese patients from different ethnic backgrounds who carry rare pathogenic mutations in these eight genes. The presence of specific features in some of these obese subjects (such as a low level of circulating leptin despite severe obesity, a susceptibility to infections, intestinal dysfunction, reactive hypoglycaemia, red hair and pale skin, adrenal insufficiency) can guide our approach to gene sequencing (Fig. 2).

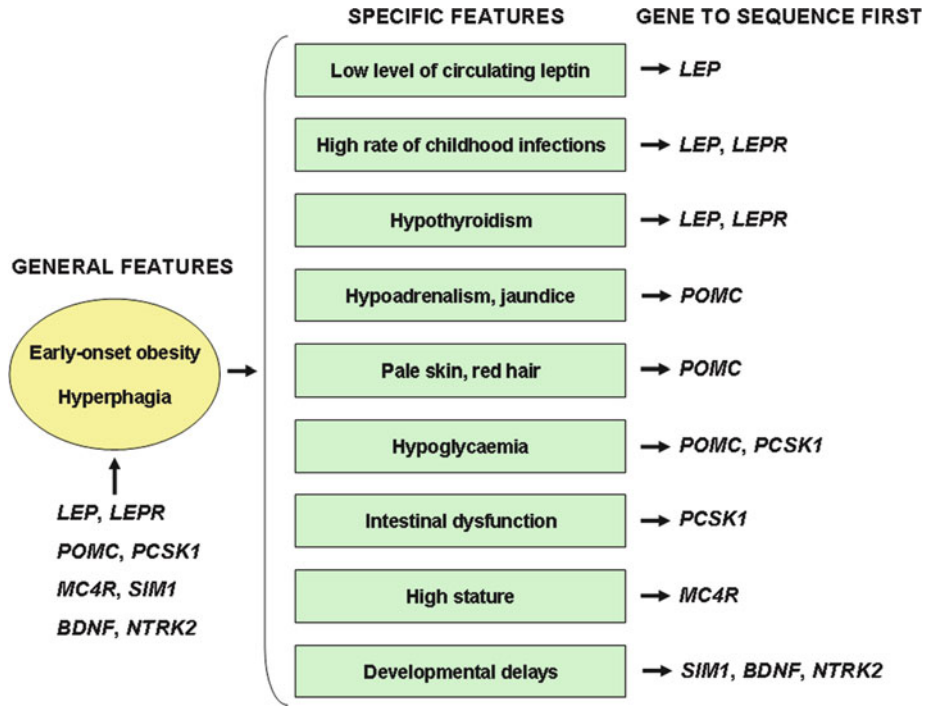

Fig. 2. Monogenic gene screening strategies during clinical examination. Early-onset obesity and hyperphagia are general features of monogenic obesity. Specific features can be useful to prioritize which gene can be sequenced first.

The most effective preventive strategy may be stringent restriction of food access restriction for monogenic mutation carriers. This will require the training and active participation of the parents and the identification of critical environmental components (physical activity, rural/urban environment, physical activity, dietary profile, tobacco consumption, family structure, socioeconomic status, social network, and gender) that modulate the penetrance of obesity associated with pathogenic mutations. Beyond the eight currently known genes, the high occurrence of Mendelian patterns of inheritance observed in multigenerational pedigrees with extreme obesity suggests that many monogenic cases remain to be elucidated *(76)*.

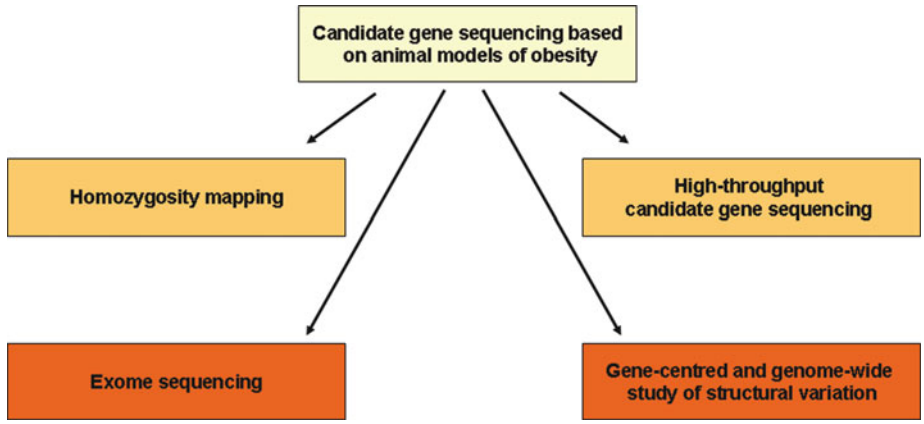

Fig. 3. Past and ongoing strategies for the identification of novel obesity single gene disorders.

Several innovative strategies may shortly lead to a more exhaustive picture of monogenic obesity (Fig. 3). High-resolution homozygosity mapping in large consanguineous pedigrees is a powerful approach to discover novel obesity loci with a recessive mode of inheritance, as recently exemplified in syndromic forms of obesity *(77)*. High-throughput gene resequencing strategies are now available with the new generation of sequencers (Illumina/Solexa Genome Analyzer, Roche SOLID) and can be used in different situations: candidate gene approach *(78)* (e.g. 103 genes are associated with a frank obesity phenotype in mouse models of obesity and represent valuable candidate genes for mutation screening in extremely obese humans); genes identified from genome-wide association studies for BMI and obesity; and resequencing in regions of homozygosity *(79)* or regions with evidence of linkage in multiple independent samples *(80)*. Exome capture and parallel sequencing strategies in carefully selected unrelated cases and controls have proven successful for gene identification *(81)* and this approach should be successfully extended in the future to pedigrees with extreme obesity and a Mendelian pattern of inheritance. Almost 20% of the heritable variation in gene expression has been attributed to structural variation (e.g. copy number variations) *(82)*. Recently, a 45 kb deletion in the *NEGR1* gene region has been associated with polygenic obesity risk *(83)*. Structural variation has been recently linked to Mendelian disorders *(84)*; gene-centred (e.g. the currently identified monogenic obesity genes) or genome-wide studies of structural variation in pedigrees may help to identify additional Mendelian obesity genes.

Although we are aware that the elucidation of monogenic forms of obesity is only a first step in a better prevention and management of this epidemic disease; it should provide novel hypotheses and bio-markers that should help us to translate to the era of genomic personalized medicine.

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