

## Symposium on ‘Genes, behaviour and environment’

### Genetics of human obesity

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The rapid development of new concepts and tools has led to a change in the way in which researchers carry out nutrition-related research. Obesity is determined by the interaction between predisposing genetic and environmental aspects, but at present the gene–gene and gene–environment interactions contributing to the development of this complex disease cannot be analysed in detail. The purpose of the present paper is to provide some examples of the knowledge that is available in the field of obesity genetics, and also the new strategies being developed that are aimed at studying the relative contribution of numerous genes to obesity and their responses to environmental changes. In the rare cases of monogenic obesities in which a major gene is the cause the molecular approach has proved extremely powerful in the identification of the genes responsible and in defining new syndromes. However, in the common forms of obesity (polygenic obesity) most studies have analysed genotype–phenotype associations without sometimes taking into account the influence of environmental factors (diet, sedentary lifestyle). Among the aspects limiting this integrated approach to obesity are the difficulty of having large enough samples and the expansion of biocomputing tools developed for accessing the question of multiple interactions with no *a priori* hypotheses. This picture is rapidly changing. Large databases of clinical data and DNA and biological sample banks with more precise environmental information and patient phenotypes are being compiled. The capacity for studying multiple genes simultaneously at the DNA or RNA levels is also possible. Finally, the tremendous progress in biocomputing will allow the integration of these different types of data (relating to environment, phenotype, genotype, gene expression) and will improve the ability to deal with this complex disease.

#### Obesity genetics: Monogenic obesity: Polygenic obesity: Candidate genes: Environmental factors

In recent years obesity has become a major public health problem because of its prevalence (>25 % in certain countries) and its alarming increase in children (Troiano & Flegal, 1998; Flodmark *et al.* 2004). The molecular mechanisms at the origin of fat mass storage and maintenance are still not understood. Obesity results from the interaction between environmental factors (overeating and/or reduction in physical activity) and hereditary factors, as has been shown by numerous epidemiological studies carried out in several populations (twins brought up together or separately, adopted children, nuclear families etc.; Stunkard *et al.* 1990). Clinically and physiologically, obesity is a very heterogeneous disease. Its development is

classically associated with energy imbalance as a result of the interaction between individual susceptibility (partly genetic; Clément & Ferre, 2003) and lifestyles that encourage energy intake and lack of physical activity. Numerous factors come into play, including the role of environmental, behavioural and socio-economic factors in individuals with different physiological susceptibilities. Studies have indicated that 30–80 % of the weight variation is determined by genetic factors (Bouchard, 1991; Carmelli *et al.* 1994).

In recent years the molecular approach to obesity has advanced the understanding of some of the causes and mechanisms of this disease, with the aim ultimately of

**Abbreviations:** BBS, Bardet-Biedl syndrome; MC4R, melanocortin-4 receptor; MKKS, McKusick-Kaufman syndrome; PC1, proconvertase 1; POMC, pro-opiomelanocortin; SNP, single nucleotide polymorphism.

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developing more appropriate forms of treatment. Currently, the contribution of genetic factors to obesity can be summarized as:

- (a) single rare mutations in certain genes explain, wholly, the development of obesity (monogenic obesity). These forms of obesity are rare, very severe and generally start in childhood (Farooqi & O'Rahilly, 2004);
- (b) several genetic variants interact with an 'at risk' environment (polygenic obesity). In this case each susceptibility gene, taken individually, would only have a slight effect on weight, and the cumulative contribution of these genes would only become important when there is an interaction with environmental factors predisposing to their phenotypic expression (overeating, reduction in physical activity). This hypothesis relates to common obesities. The common disease–common variant hypothesis has been proposed (Lander, 1996), in which the genetic risk for obesity is considered to be a result of disease-producing common alleles. As a consequence, the percentage of obesity attributed to these alleles (attributable risk) would be high. However, even the firm supporters of this hypothesis agree that in certain circumstances rare variant alleles may be involved in common obesity. Here, the risk for common obesity could be a result of a large number of loci, each with multiple disease-predisposing alleles of low frequency (Pritchard, 2001).

### Monogenic obesities

#### *Genetic obesity syndromes*

Obesity is associated with many genetic syndromes. In order to identify new pathways involved in the control of weight, several research teams are attempting to identify the genes and mutations responsible for these syndromes. The Online Mendelian Inheritance in Man database (<http://www.ncbi.nlm.nih.gov/entrez/Query.fcgi?db=OMIM>) lists these syndromes and provides access to the clinical description of these diseases. Among those listed the most well known are the Prader-Willi, Cohen, Alström and Bardet-Biedl (BBS) syndromes. The rarity of these diseases has made it difficult to identify the genes implicated, but genome screening in affected families has made it possible to detect certain genes and mutations responsible for these complex diseases. Some examples will be provided.

Genes responsible for the BBS and Alström syndromes have been characterized. The BBS is characterized by early-onset obesity associated with rod–cone dystrophy, morphological finger abnormalities (polydactyly, syndactyly), learning difficulties and renal disease, amongst other clinical traits. Although a homogeneous clinical entity was described initially, BBS has been associated with eight different chromosomal locations, with several mutations identified within some of them (BBS1 on 11q13; BBS2 on 16q21; BBS3 on 3p13; BBS4 on 15q22·3; BBS5 on 2q31; BBS6 on 20p12; BBS7 on 4q27 and BBS8 on 14q32·11; Katsanis *et al.* 2001b). While BBS has for a long time been

considered as a syndrome with autosomal recessive transmission, it has been shown that the clinical symptoms of certain forms of BBS are related to recessive mutations on one of the loci implicated in BBS in association with a mutation on a second locus. The hypothesis of a triallelic transmission has been suggested (Katsanis *et al.* 2001a). At least four genes have been characterized in BBS, without their function necessarily being known. For BBS6, a positional cloning approach has implicated the McKusick-Kaufman syndrome (MKKS) gene, situated on chromosome 20, which codes for a chaperone protein. Chaperone proteins facilitate the three-dimensional folding of other proteins. The mutations identified in MKKS result in a shortened chaperone protein and represent 5–7% of BBS cases, indicating the molecular heterogeneity. The links between MKKS, its protein targets and the MKKS clinical traits remain to be determined. Unlike BBS6, the genes implicated in BBS1, BBS2 and BBS4 are very different from MKKS genes, but it is possible that they code for MKKS protein substrates (Slavotinek *et al.* 2002). Very recently, studies performed in single-cell organisms have shown that certain BBS genes are specific to ciliated cells (Fan *et al.* 2004). These cells play a fundamental role in development in mammals, contributing to the right–left symmetry that enables the organs (heart, liver, lungs) to be correctly positioned. Such dysfunction in the processes affecting the ciliated cells may contribute to alterations in pigmentary epithelia and to the structural anomalies in certain organs encountered in BBS. Why these dysfunctions should lead to obesity is an open question.

Similar observations may be derived from the study of Alström disease. Alström syndrome is an autosomal recessive disease of childhood, which, in addition to obesity, is associated with retinal cone dystrophy, dilated cardiomyopathy, type 2 diabetes and other clinical traits of variable severity such as hypothyroidism, small size, hypogonadism, anomalies of hepatic function and sometimes mental retardation. Family studies have demonstrated that mutations of the Alström syndrome 1 gene are implicated. This gene encodes a protein with ubiquitous expression, the function of which is also unknown (Mykytyn *et al.* 2002).

These examples emphasize the necessity for multicentre studies that group together the families affected by these syndromes (often already grouped within an association) in order to characterize the genes responsible for these rare diseases. Although certain of these genes have been identified, the physiopathological links between their protein products and the development of diseases characterized by multiple clinical traits, but also by overlapping clinical traits (retinal disease, mental retardation, insulin resistance), remain to be determined (Stefan & Nicholls, 2004). In addition, the possibility of these genes contributing, in a minor way, to the development of common obesity should not be excluded.

#### *Obesity by alteration of the leptin and melanocortin pathways*

Another strategy, which has resulted in substantial success, has been the study, in severe early-onset obesity, of



**Table 1.** Mutations in human obesity affecting the leptin and the melanocortin pathways

Gene	Transmission	Obesity	Associated phenotype
Leptin	Recessive	Severe, from the first days of life	Gonadotropic insufficiency
Leptin receptor	Recessive	Severe, from the first days of life	Gonadotropic, thyrotropic and somatotropic insufficiency
Pro-opiomelanocortin	Recessive	Severe, from the first month of life	ACTH insufficiency Ginger hair
Proconvertase 1	Recessive	Considerable, from the first month of life	Gonadotropic and corticotropic insufficiency Hyperproinsulinaemia
Melanocortin-4 receptor	Dominant	Early onset, variable severity, Large size	No

ACTH, adrenocorticotropin.

#### *Other monogenic obesities*

In addition to the mutations of the leptin and melanocortin pathways, other rare types of obesity have been described in man that have increased the understanding of the physiopathological mechanisms. One example is a patient with severe obesity from the first month of life who has a *de novo* translocation between chromosomes 1p22.1 and 6q16.2. This translocation causes a deletion of the single-minded type 1 gene on chromosome 6. This gene codes for a transcription factor involved in the development of the paraventricular nuclei of the hypothalamus in the mouse (Holder *et al.* 2000). These obesity situations, even if they are exceptional, merit being recognized by clinicians, so that new genes that have a role in energy homeostasis and are potentially candidates in common obesity can be identified.

A spontaneous heterozygous mutation in the neurotrophic tyrosine kinase, receptor, type 2 gene has also been identified in a patient with early-onset obesity and hyperphagia combined with mental retardation and anomalies of higher neurological functions (Yeo *et al.* 2004). This gene codes for the receptor for the brain-derived neurotrophic factor, itself involved in the regulation of dietary intake (Xu *et al.* 2003), that is expressed throughout the central nervous system. Studies have shown that the mutation leads to marked functional alteration of the receptor for the brain-derived neurotrophic factor, suggesting the functional role of this variant. Several other mutations of the receptor for the brain-derived neurotrophic factor have been found in patients with early-onset obesity and retarded development, but their functional effect and their involvement in obesity remains to be demonstrated. Whether these genes may interact with the capacities of the leptin and melanocortin axes in controlling food intake and energy balance is probably a question being investigated by the specialists in the field.

#### *Obesity associated with mutation of the melanocortin-4 receptor; an example of oligogenic obesity?*

One of the important participants in the melanocortin pathway is the melanocortin-4 receptor (MC4R). MC4R, a G-protein-coupled receptor with seven transmembrane domains (Cone, 2000), is expressed in the brain, mainly in the hypothalamus. The importance of MC4R in

controlling weight homeostasis has been demonstrated in animals. Mice with a genetic deletion of MC4R (knock-out) develop morbid obesity. Mice that are heterozygous for this deletion present with intermediate obesity of various extents of severity. The use of pharmacological agonists of MC4R in rodents reduces food intake, while antagonists of this receptor increase food intake (Huszar *et al.* 1997). Based on the importance of the melanocortin pathway in the control of food intake, the MC4R gene has become a candidate of choice for the genetic study of human obesity. Several different mutations that result in a change of amino acid in the protein have been described in different populations of German, French, English, Danish and American children and adults (Vaisse *et al.* 1998, 2000; Hinney *et al.* 1999; Farooqi *et al.* 2000; Dubern *et al.* 2001; Jacobson *et al.* 2002; Miraglia Del Giudice *et al.* 2002; Larsen *et al.* 2005). The frequency of these mutations has been assessed as being between 0.5 % and 2 % for moderate forms of obesity and could reach 4 % for severe forms. In these families the obesity usually has an autosomal dominant mode of transmission. Penetrance of the disease is incomplete and the clinical expression is variable. In rare cases subjects with homozygous MC4R mutation have been characterized; these subjects develop extremely severe forms of obesity (Farooqi *et al.* 2000; Lubrano-Berthelier *et al.* 2004).

Subjects with heterozygous MC4R mutations are not always obese; if they are obese, the severity of the condition has mostly been reported to be variable. A recent study has shown that MC4R mutations are strong predictors for obesity and also suggests that the role of the environment is not negligible, nor is that of other potentially-modulating genetic factors (Dempfle *et al.* 2004). The phenotype of subjects with an MC4R mutation has still not been specified, except when early-onset obesity occurs. Only one study, in English children, has suggested that bone mineral density and size increase if there is an MC4R gene mutation (Farooqi *et al.* 2003a), but this finding has not been confirmed in the other cohorts studied. Recently, an association between feeding behaviour disorders of the 'binge eating' type and MC4R mutations has been described (Branson *et al.* 2003). Again, this finding has not been confirmed in other populations and currently remains very controversial (Farooqi *et al.* 2003b; Gotoda, 2003; Herpertz *et al.* 2003; Hebebrand *et al.* 2004). Generally, clinical analysis does not allow detection



of those forms of obesity that resemble common forms of early-onset obesity. They are known as non-syndromic and are placed between the exceptional forms of monogenic obesity with complete penetrance and the polygenic forms of common obesity.

The functional study of MC4R mutations has, however, confirmed the role of these mutations in causing obesity in patients carrying them. Studies of links between ligands and MCR4 or the production of intracellular cAMP in response to  $\alpha$ -melanocyte-stimulating hormone have demonstrated a broad heterogeneity in the activation of different MC4R mutants in response to  $\alpha$ -melanocyte-stimulating hormone, ranging from normal or partial activation to a complete absence of activation (Vaisse *et al.* 2000). An intracellular transport defect of the mutated receptor (by intracytoplasmic retention) has been described for the majority of MC4R mutations found in childhood obesity (Lubrano-Berthelier *et al.* 2003). In addition, MC4R has a constitutive activity, a basal activity not necessitating ligand presence, for which agouti-related peptide acts as an inverse agonist (Nijenhuis *et al.* 2001). Thus, in the absence of ligand MC4R has an inhibitory action on food intake. The systematic study of basal activity in the mutation has shown that an alteration in this activity may be the only functional anomaly found, particularly for mutations situated in the N-terminal extracytoplasmic part of the receptor (Srinivasan *et al.* 2004). This finding suggests that a tonic satiety signal, provided by the constitutive activity of MC4R, could be required in the long-term regulation of energy balance.

### Polygenic obesities known as common obesities

#### *General and theoretical approach*

As mentioned earlier, there are two hypotheses relating to the contribution of heredity to the development of common forms of obesity, the common variant–common disease hypothesis and the rare variant hypothesis. At the present time, both hypotheses are valid, because the genetic approach has not established one hypothesis to be more acceptable than the other.

The genetic study of common obesity is based on the analysis of variations in genomic DNA (genetic polymorphisms or single nucleotide polymorphisms (SNP)) located within or near candidate genes. The aim of genetic studies of different types, depending on whether they are carried out in related (family) or unrelated obese subjects, and also on the statistical methods employed (studies of family links, association studies in unrelated patients), is to determine whether an association exists between an allele of a gene and obesity traits (Hebebrand *et al.* 2003; Snyder *et al.* 2004). For this purpose, DNA and clinical data banks have been compiled in several countries in Europe and the USA. The results of these genetic studies relate to an enormous number of genes and chromosomal regions, and are reported every year in the international journal *Obesity Research* (Snyder *et al.* 2004). Although details of these studies will not be provided in the present paper, some important outcomes and examples will be mentioned.

#### *The genome regions linked to obesity*

With the improvements in molecular screening tools and the greater understanding of genetic polymorphism, it has been possible to explore the genome of the families of patients with common obesity. The objective is a systematic examination of all the chromosomes in families of obese subjects without preconceptions about the functions of these genes, and thus identification of known or unknown genes predisposing to obesity, using powerful statistical tools that allow their position to be established. These approaches have been developed for different types of cohorts; families with members who have developed extreme obesity (French and American studies), families coming from the general population (Quebec family study) and also particular ethnic groups (Pima Indians, Mexican Americans, African Americans and Amish).

This strategy has identified several chromosomal locations linked to obesity. At least seven genes situated on chromosomes 2, 5, 10, 11, 19 and 20 may be implicated in common obesity (Hager *et al.* 1998; Perusse & Bouchard, 2000, 2003; Hebebrand *et al.* 2003; Arya *et al.* 2004; Bell *et al.* 2004; Snyder *et al.* 2004). Some other loci may be more specific for morbid obesity or for obesity with early onset (Saar *et al.* 2003). Certain of these regions have been confirmed in different populations; the 2p21 region would seem to play a role in the variability of circulating leptin levels in French Caucasians, Mexican Americans and African Americans. The region situated on chromosome 10 is linked to obesity in, for example, the French, German Caucasians and in the Amish. At least sixty other chromosomal regions (virtually no chromosome except Y is spared!) have also been linked to different phenotypes such as fat mass, the distribution of adipose tissue, resting energy expenditure or even the levels of circulating leptin and insulin. Finally, interactions between several chromosomal regions have been observed, such as those on chromosomes 10 and 20, and more recently those on chromosomes 13 and 2. These regions may interact to influence extreme human obesity (Dong *et al.* 2005).

Work being carried out in several European countries and in the USA is attempting to identify the genes implicated by these statistical links. When this work has been completed the mutations must be investigated and their involvement in the development of the disease confirmed. Several interesting candidates have been identified in these regions (see p. 137).

#### *Candidate genes*

In general, the classic 'candidate gene' approach used for several years has not revealed the predominant and unambiguous role of candidates in the genesis and development of obesity. The choice of a candidate is based on several factors, including the physiological role of its protein product in the mechanism of obesity, its chromosomal location in a region linked to obesity in man or animals (regions known as quantitative trait loci), the consequences of its genetic deletion (knock-out) or its overexpression (transgenesis) in the rodent and even the *in vitro* functional characteristics of mutations or variations

**Table 2.** Examples of genes frequently studied in human obesity\*

Genes	Locus	Animal model†	Obesity locus	Functional genetic variant‡
<b>Food intake</b>				
Leptin	7q31	<i>ob/ob</i> mice	Yes	No
Leptin-R	1p31	<i>db/db</i> mice	Yes	No
DRD2	11q22	KO non-obese	No	↓ nb receptor
<b>Energy metabolism</b>				
UCP2	11q13	KO non-obese	Yes	No
UCP3	11q13	KO non-obese	Yes	No
B3-AR	9p12	KO ↑ adiposity	Yes	↓ Activity
<b>AT metabolism</b>				
PPAR $\gamma$	3p25	KO ↓BAT	No	↓ Activity
GRL	5q31		Yes	No
TNF- $\alpha$	6p21	KO ↑ adiposity AT under high-fat diet	Yes	No
B2-AR	5q31	KO ↑ adiposity under high-fat diet	Yes	↓ Activity
<b>Lipid metabolism</b>				
LDL-R	19p13	No	Yes	No

Leptin-R, leptin receptor; DRD2, dopamine receptor D2; UCP2 and 3, uncoupling proteins 2 and 3; B3-AR,  $\beta$ 3 adrenergic receptor; GRL, glucocorticoid receptor; B2-AR,  $\beta$ 2 adrenergic receptor; LDL-R, LDL receptor; KO, knock-out; BAT, brown adipose tissue; AT, adipose tissue; ↑, increased; ↓, decreased; nb, number.

\*The genes selected are among the most studied ones in the genetics of human obesity (more than five studies showing a positive association).

†Shows a spontaneous animal model or one created by genetic manipulations of deletion (KO) or overexpression. Positive as well as negative associations have been found between single nucleotide polymorphisms located in these genes and obesity phenotypes.

‡Shows the existence of genetic variants with functional consequences described *in vitro*.

of the DNA studied. The expression characteristics of transcripts of these genes in key tissues for weight control, or even the modification of expression in response to the environment, are more rarely taken into account.

To date, a large number of genes and polymorphisms have been tested (more than fifty in 250 studies in clinically-heterogeneous populations). These genes have been implicated in controlling food intake, energy expenditure and lipid and carbohydrate metabolism (Perusse & Bouchard, 2000, 2003; Hebebrand *et al.* 2003; Snyder *et al.* 2004). Table 2 shows some examples of the most studied genes in populations of different origins. However, for a given polymorphism the effects reported are sometimes uncertain, even conflicting, and illustrate the complexity of obesity as well as statistical difficulties often associated with the lack of power of the analyses. While no predominant role has been established for these genes in the development of obesity, certain genetic variants are associated with different phenotypes of obesity such as early onset, aggravation with time, associated metabolic and cardiovascular complications, feeding behaviour characteristics and the interaction between excess body weight and body fat and the extent of physical activity.

A few examples of these studies will be discussed. While the genes implicated in the monogenic forms of obesity might not play a predominant role in the development of common forms of obesity, they may not be excluded as potential targets for multifactorial obesity (Table 2), as

is the case for the leptin gene (*ob* gene). Although the expression and the secretion of leptin increase in proportion to the fat mass, there are wide inter-individual variations for the same level of fat mass. Interestingly, the chromosomal region of the leptin gene has been linked to obesity phenotypes in several different populations, including a very large Caucasian American population recently screened (Feitosa *et al.* 2002) and other populations (Heijmans *et al.* 2004). These results have been confirmed by a meta-analysis bringing together ten genetic studies (Allison & Heo, 1998). Several SNP located in the leptin gene region, and notably in the promotor region, have been shown to be associated with obesity, raising the possibility of a role for this region in common obesity (Jiang *et al.* 2004). The question arises as to whether SNP situated in the promoter or non-coding regions of the gene can modulate expression of the *OB* gene, and as a consequence circulating leptin levels. In such circumstances the perception by the central nervous system of the organism's energy reserves could be modified and/or there could be inappropriate variation in leptin in response to variations in energy balance.

The gene coding for insulin, considered as another 'adiposity signal' essential for the maintenance of energy homeostasis, has also been implicated in phenotypes linked to obesity. In this gene repetitions in tandem of the DNA bases (variable number of tandem repeats) have been associated with modifications in insulin secretion and a moderate increase in weight in obese adolescents (Le Stunff *et al.* 2000). Other energy-metabolism genes are also among the most-studied genetic factors. Massively-obese French subjects with the Trp64Arg mutation of the  $\beta$ 3-adrenergic receptor and a variant of the regulator region of the gene for uncoupling protein 1 show an increased capacity to put on weight during their lifetime. The  $\beta$ 3-adrenergic receptor and uncoupling protein 1 are factors that contribute to the regulation of energy metabolism in animals but their role in human physiology is being debated (Clement *et al.* 1996).

Identification of the genes implicated in obesity may also be achieved by the strategy of positional cloning. Two recent studies have thus been able to highlight a marked difference in the SNP frequency between populations of obese subjects and controls. These SNP are located in the gene encoding solute carrier family 6 member 14 (Suviolahti *et al.* 2003) and glutamate decarboxylase 2 (Boutin *et al.* 2003). The solute carrier family 6 member 14 gene is potentially interesting as a candidate because it codes for an amino acid transporter that can regulate the availability of tryptophan during the synthesis of serotonin, therefore affecting regulation of appetite and mood. However, it is still necessary to undertake functional studies of the genetic variation in order to demonstrate the physiopathological role of solute carrier family 6 member 14. The glutamate decarboxylase 2 gene codes for the glutamic acid decarboxylase enzyme (glutamate decarboxylase 65) that catalyses the formation of  $\gamma$ -aminobutyric acid, which itself interacts with neuropeptide Y in the hypothalamus to stimulate food intake. In this study (Boutin *et al.* 2003) different SNP analysed have shown an association with morbid obesity in a French population,

**Table 3.** Risk factors of obesity phenotypes evaluated for common genetic variations in French obese subjects and in a meta-analysis (for PPAR $\gamma$ )

Gene and variant	Phenotype	Odds ratio
$\beta$ 3-AR (Trp64Arg)	High weight gain	1.7
UCP1 (-3826A/G)	High weight gain	1.4
GAD2 (risk haplotype)	Morbid obesity	1.3
PTP1B (risk haplotype)	Obesity, dyslipidaemia	1.49
SREBP (risk haplotype)	Obesity, dyslipidaemia	1.53
SLC6A14 (risk haplotype)	Obesity	1.27
PPAR $\gamma$ (Pro12Ala)	Diabetes	1.4 (meta-analysis)

B2-AR,  $\beta$ 2 adrenergic receptor; UCP1, uncoupling protein 1; GAD2, glutamate decarboxylase 2; PTP1B, protein tyrosine phosphatase-1B; SREBP, sterol regulatory element-binding protein; SLC6A14, solute carrier family 6 member 14.

as well as a protective effect of the wild allele. The physiopathological role of one of the SNP studied, situated in the glutamate decarboxylase 2 promoter, is strengthened by a functional study showing that the allele most frequently found in obese subjects exhibits greater transcriptional activity than the wild allele. This activity could result in an increase in hypothalamic  $\gamma$ -aminobutyric acid and stimulation of food intake. However, these potentially-interesting targets must be confirmed in cohorts of independent subjects, and functional findings need to be replicated.

More important, perhaps, than the complex picture of obesity-related susceptibility genes and the inconsistencies in results, will be the challenge to redefine the predisposing alleles in terms of multiple risk factors. Table 3 shows gene variants that were studied 10 years ago in the French population as well as more recently studied variants. The Pro12Ala mutation of the PPAR $\gamma$  gene has also been studied in different populations (Altshuler *et al.* 2000). Apart from issues relating to statistics, which will be discussed, it should be noted that (as probably expected) these studies have produced a list of common variants that may modify the risk of the disorder occurring, albeit with a small extent of certainty. However, these variants have to be considered together with other risk factors associated with obesity development (i.e. environmental factors; Ardlie *et al.* 2002). In the future, this approach will raise the issue of the appropriateness of genetic testing in this complex context. The genetic background as well as epigenetic or environmental factors, which may include diet, exercise, stress and the developmental stage of these epigenetic events, may be involved in the development of obesity. For a given genetic susceptibility, depending on the potential of the individual to react to life events and the stage at which the event occurs during the lifespan of the subject, the involvement of these factors will determine whether obesity develops (Hoh & Ott, 2004).

Thus, although the essential role of gene–gene and gene–environment interactions is emphasized in the development of obesity, it is amazing to find that these interactions have in fact received little attention in genetic

studies, because of the complexity involved in designing these studies and processing the data (Snyder *et al.* 2004). The genetic map published each year suggests that few studies deal with this question, usually analysing the effects of two polymorphisms on one or more phenotypes, or the interaction between a given gene variation and diet or level of physical activity. For example, the simultaneous presence of the two SNP for uncoupling protein 1 and the  $\beta$ 3-adrenergic receptor (gene–gene interaction) in already-obese subjects would aggravate weight gain during their lifetime.

Other work in French obese patients has shown that a variant of the promoter of the uncoupling protein 3 gene may contribute to the excess body weight and body fat of obese subjects and also have effects that depend on the extent of physical activity of patients (gene–environment interaction). The presence of a frequent allele of this variant would indeed reduce the beneficial effects of physical activity on excess body weight and body fat. The physical activity–genotype interaction has also been well illustrated by the glutamine–glutamic acid common variant of the  $\beta$ 2 adrenergic receptor that has been studied in many populations. The glutamine variant allele appears to be associated with obesity, but this effect is seen only in a group of individuals who are physically active. The well-known Pro12Ala mutation of the PPAR $\gamma$  gene also demonstrates this complexity. The Ala allele is associated with low BMI when subjects eat food enriched with unsaturated fatty acids, while it is associated with higher BMI when food has low unsaturated fatty acids:saturated fatty acids. Many other genes are currently being studied, particularly in the wide range of European cohorts in which numerous environmental factors have been characterized (for review, see Verdich *et al.* 2004).

One of the future objectives will be to determine the combinations of genes and their polymorphisms predisposing to the development of obesity and the environmental pressure associated with this outcome. Well-controlled intervention studies are currently underway in a wide range of populations in Europe. In this context of gene–environment interaction recommending procedures for genetic association studies in complex diseases, especially those related to sample size, multiple testing and replication, will be a challenge. Studies dealing with large populations, in which the environment is well controlled, are generally very difficult, but can be performed in the European trials (Verdich *et al.* 2004). Progress in the knowledge of the human genome, the development of biocomputing tools and new analysis strategies taking into account hundreds of items of genetic and environmental information will be necessary to tackle these questions.

Finally, in relation to the number and type of susceptibility genes described, one key issue is whether there are common genetic factors that may specifically intervene in the development of human obesity. Indeed, many susceptibility genes have also been analysed in different common diseases, revealing an association or linkage. Genetic linkage maps for obesity, diabetes or CVD frequently overlap at some loci. As an example of a candidate gene, a recent study suggested that a common polymorphism of IL-6 was associated with weight gain and insulin



sensitivity (Wolford *et al.* 2003; Wernstedt *et al.* 2004). The same variant has also been associated with many other complex diseases such as chronic alcoholic liver disease, asthma, psoriasis or chronic bronchitis (Bennermo *et al.* 2004). As suggested by Becker (2004), the impact of common disease-influencing alleles should be examined when they are in different genetic backgrounds, in other genetic combinations, or influenced by different epigenetic or environmental factors. If these susceptibility genes are not causative and modify obesity risk in a given environment, what are they doing in the meantime? Are they neutral or deleterious for other diseases? It will probably be necessary to carefully analyse the overlapping results across different common diseases to obtain a global view.

### New strategies for the genetic analysis of obesity

Among the difficulties in identifying molecular targets, the most regularly highlighted are the difficulties of analysis (mostly of a statistical nature) and the need to combine several methods in heterogeneous populations. Obesity is indeed characterized by a high phenotype heterogeneity linked to differences in stages of evolution of the disease. Each stage in the development of obesity (weight gain, weight maintenance, the variable response to treatment, the incidence of comorbidities) is probably associated with a different molecular mechanism. At present there are no known biological or molecular predictors (biomarkers) of the transition from one stage to another. The study of gene expression in the tissues involved in the different stages of obesity may contribute to the elucidation of the role of particular signals and provide a better understanding of the mechanisms of energy homeostasis. If obesity is considered as a disease of adaptation to environmental pressures, one hypothesis is that some of the fat mass development could originate within the groups of genes expressed in the peripheral tissues that are implicated in responses to changes in the nutritional environment. This hypothesis is the basis for research that aims to combine various fields (clinical, biochemical, genetic, transcriptomic) and process the mass of information generated, using subjects of diverse phenotypic and genotypic characteristics in different nutritional conditions. It is widely recognized that there is a need to investigate multifactorial diseases by integrating all available sources of information. By using 'omic' analysis strategies it is now possible to simultaneously quantify the expression of a large number of genes and/or proteins in different tissues, as well as metabolites, under different conditions. These strategies complemented by the genetic approach can be used to study the regulation of energy balance. At some stage it will be necessary to adopt an overlapping approach between 'omic' strategies and available information about the susceptibility genes in man and rodents.

### Conclusion

With the compilation of DNA and clinical databanks from a broad range of cohorts of unrelated patients and of families, positive progress in the understanding and

management of obesity can be foreseen. The combined studies of hereditary characteristics, of the genome and the function, regulation and interaction of genes, are modifying the current approach to diseases that have a high impact on public health. The future will reveal whether the choice of new combined strategies has been successful in identifying targets for new pathways and therapies.

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