5 Polygenic Obesity

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Key Words: Candidate gene, genome-wide association, melanocortin, insulin-induced gene 2 (INSIG2), FTO (fat mass and obesity-associated gene)

INTRODUCTION

A small number of major genes for human obesity have been identified by molecular genetic analyses; the responsible mutations are rare and are therefore only of minor clinical importance. The genetic mechanisms involved in the predisposition to obesity in most affected people are more likely polygenic *(1,2)*; detection of the first such polygenes has just recently been initiated. Each single polygene makes only a small contribution, in the magnitude of a few hundred grams or less, to the development of obesity. A number of such predisposing gene variants (alleles) should be found in most obese subjects; however, the same alleles would, although at a lower frequency, also be found in normal weight and even lean individuals. Evidently, these alleles can only be identified and validated as obesity risk alleles by statistical analyses *(2)*. Currently, combined genome-wide association (GWAS) analyses on more than 100,000 population-based individuals are under way. Functional in vitro and in vivo studies will ensue and lead to a better understanding of the molecular genetic mechanisms involved in body weight regulation. Prevention and therapeutic approaches will presumably benefit from this knowledge.

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POLYGENES FOR BODY WEIGHT

The term polygene is used for a gene known to harbor inter-individual sequence variation and to account for a fraction of the variation of a specific quantitative trait. Each polygene contains one allele predisposing to higher and the other to lower body weight. It is presumed that more than 100 polygenes play a role in body weight regulation. Based on the thrifty genotype hypothesis *(3)*, those alleles that predispose to a higher body weight are hypothesized to be more common than alleles giving rise to lower body weight. This is because, in evolutionary terms, it was important to both survive and reproduce during periods of food scarcity; as a consequence, mutations enabling a more efficient storage of energy became common in both animals and humans. Obesity is the result of the interaction of several or many of these polygenic variants and their combined interaction with environmental factors. Inter-individual heterogeneity is most likely pronounced and implies that the specific set of polygenes predisposing to obesity in any one individual is unlikely to be the same in another randomly selected obese subject *(1)*.

In contrast to most of the initially detected genetic influences on obesity, which are conferred by a single gene with either a recessive or a dominant mode of inheritance, polygenic effects are small. Polygenic obesity can only ensue if an individual harbors many such variants and lives in an obesogenic environment. Associations between genetic variants and obesity could have implications for diagnosis and risk prediction, prevention, and treatment. So far, 17 gene variants with small but replicable effects on body weight have been identified and validated *(4)*.

CANDIDATE GENE ANALYSIS AND GENOME-WIDE APPROACHES

Two major approaches have been used for the detection of genes involved in body weight regulation.

Candidate gene analyses: Genes considered "candidates" for BMI variance are analyzed because prior research (biochemical, physiological, and/or clinical) implicates their roles in central or peripheral pathways controlling energy intake and expenditure. Pharmacological findings or the location of a gene within a linkage region can also entail its classification as a candidate gene. A large number of association studies for obesity involving cases and controls or, less frequently, families comprising one or more affected children and both parents have been performed. For a limited number of genes, meta-analyses are available.

Genome-wide approaches: Genome-wide linkage studies aim to identify chromosomal regions harboring one (or more) genes relevant for the respective phenotype. For candidate gene analyses, those regions underlying linkage peaks are narrowed down by fine mapping. Over 40 microsatellite-based genome-wide linkage scans have been performed for obesity. As in other complex disorders, the success of linkage studies has been very limited. Interestingly, the region on chromosome 16 harboring the currently most relevant polygene, the 'fat mass and obesity-associated' gene (*FTO*), proved to be the strongest but nevertheless nonsignificant signal in a meta-analysis of 37 genome-wide linkage studies comprising data on more than 31,000 individuals from over 10,000 families *(5)*. This most likely indicates that the effect sizes of genes influencing obesity are small and additionally suggests substantial genetic heterogeneity and variable dependence on environmental factors.

Genome-wide association studies (GWAS) have far greater power to detect polygenes *(6)*. Current advances in DNA chip technology have made high-density single nucleotide polymorphism (SNP)-based GWAS feasible and led recently to the identification of a number of confirmed genes for different disorders (http://www.genome.gov/26525384). Within a brief period of time, they have revolutionized the molecular genetic analyses of complex disorders.

MELANOCORTIN-4 RECEPTOR GENE (MC4R)

The melanocortin-4 receptor gene (*MC4R*) has been a focus of intense investigation in obesity research. Reduced melanocortinergic tone leads to obesity. More than 130 different infrequent non-synonymous, nonsense, and frameshift *MC4R* mutations have been described thus far; most of these mutations were identified in (extremely) obese individuals *(7–11)*. In vitro assays showed that most of these mutations lead to total or partial loss of MCR4 function. Among *extremely* obese individuals, combined frequencies for all functionally relevant mutations range from 2 to 5%.

Interestingly, *MC4R* can also be considered a polygene. The minor alleles of two *MC4R* polymorphisms (Val103Ile, Ile251Leu) are negatively associated with obesity *(12–15)*. Large study groups had to be screened to detect these polymorphisms because they are relatively uncommon and have small effect sizes.

Val103Ile polymorphism. Heterozygosity for the 103Ile variant (Val103Ile) of MC4R is found in 2–9% of people *(12)*. A family-based association test (TDT; *(16)*) in 520 trios (ascertained via a young obese offspring) revealed a reduced transmission of the Ile103 variant. A subsequent meta-analysis, comprising a total of 7,713 individuals (3,631 obese cases and 4,082 controls), confirmed the negative association of the Ile103 variant with obesity (odds ratio 0.69; 95% confidence interval 0.59–0.99). An effect estimate of –0.48 kg/m^2 was calculated for Ile103 carriers, which is approximately equivalent to a reduction of 1.5 kg in a 1.8 m tall individual *(12)*. The negative association of 103Ile with obesity was subsequently confirmed in a single large epidemiological study group of approximately 8,000 individuals *(13)*. Recently, two additional meta-analyses, encompassing a total of up to 29,563 individuals, also have confirmed the initial finding *(15)*.

A recent study showed that the Ile103 polymorphism increased the response to agouti-related peptide, which impairs melanocortin signaling and increases food intake, and reduced the response to proopiomelanocortin-derived agonists, which increase melanocortin signaling and reduce food intake *(17)*. Hence, the polymorphism is associated with increased MC4R function, which could explain its weight-reducing effect.

Ile251Leu polymorphism. Heterozygosity for the Ile251Leu variant of MC4R is found in 0.41–1.21% of people. Its contribution to obesity was analyzed in 16,797 individuals of European origin. In eight of nine studies, a consistent negative association of the 251Leu variant with both childhood and adult extreme obesity (odds ratios 0.25–0.76) was detected; the variant was also associated with reduced BMI in population-based samples. A meta-analysis provided strong evidence of the obesity protective effect of MC4R-251Leu (odds ratio 0.52) *(14)*.

rs17782313 downstream of MC4R. Recently, a large-scale international cooperation encompassing DNA samples of over 90,000 individuals detected a SNP in the vicinity of the *MC4R* by a genome-wide association study (GWAS; *(18)*). Initially, GWAS data from seven studies (a total of 16,876 Europeans) have been analyzed jointly. The second strongest association signal, after *FTO* (see below), mapped 188 kb downstream of the *MC4R* (rs17782313). The location of rs17782313 suggests that its effect on weight regulation may be mediated through effects on *MC4R* expression and may be exerted in concert with variations in *FTO (18)*. The association result was confirmed in an additional 60,352 adults and 5,988 children and 660 nuclear German families encompassing one or more obese offspring and both parents. Among the adults, each copy of the rs17782313 obesity risk allele (C) was associated with a difference in BMI of ~0.22 kg/m^2. A copy of the allele resulted in 8 and 12% increased risks for overweight and obesity, respectively. Interestingly, a copy of the C-allele also resulted in a higher mean height (0.21 cm), suggesting that this SNP (or the functionally relevant SNP(s) in linkage disequilibrium) influences overall adult size.

The association of the rs17782313 SNP with obesity was recently confirmed in a GWAS comprising a total of more than 150,000 individuals (see *(4)*). Interestingly, genotyping of nearly 6,000 children

of the Avon Longitudinal Study of Children and Parents revealed that the effect of the C-allele was not detectable in children prior to age 7. However, in children aged 7–11, the effect size of a copy of the C-allele was twice the amount observed in adults; no effect was observed for body height. The effect on weight was disproportionately due to fat mass.

INSULIN-INDUCED GENE 2 (INSIG2)

A total of 694 individuals from 288 families of the Framingham Heart Study were screened by a dense, whole-genome scan (100k Affymetrix). In both children and adults, homozygosity for a common SNP (rs7566605) in the vicinity of the insulin-induced gene 2 (*INSIG2)* was found to be associated with obesity. Confirmation of the initial finding was shown in four of five separate samples comprising individuals of Western European ancestry, African-Americans, and German children and adolescents, respectively. Approximately 10% of the subjects harbored the CC-genotype that, according to this study, predisposes to obesity *(19)*.

Several attempts to replicate the *INSIG2* finding have been or are currently being undertaken. Both confirmations *(20)* and negative findings *(21–23)* have been reported. Currently, data are being compiled for a large-scale meta-analysis, which in the near future will help to determine if *INSIG2* is an obesity polygene. However, *INSIG2* was not detected in any of the GWAS for obesity or body weight regulation either from large population-based samples or meta-analyses or in case–control approaches on individuals at the extremes of the body weight distribution *(24–26)*.

FAT MASS AND OBESITY-ASSOCIATED GENE (FTO)

FTO was first identified in GWAS for type 2 diabetes mellitus (T2DM; *(27,28)*). By adjustment for BMI, Frayling et al. *(27)* found that its association with T2DM was actually due to the higher BMI of diabetic cases in comparison to non-diabetic controls. Confirmation of the BMI effect was obtained in 13 study groups comprising 38,759 adults. A meta-analysis showed that the A-allele of the variant rs9939609 (intron 1 of *FTO*) was associated with a 31% increased risk for developing obesity. The 16% of adults who were homozygous for the risk allele weighed on average about 3 kg more and had a 1.67-fold increased odds for obesity when compared to individuals without risk allele.

Variations in *FTO* were also detected in the first GWAS for early-onset obesity, which was performed in 487 extremely obese German children and adolescents and 442 lean controls (case–control study). Because only individuals at the opposite ends of the BMI distribution were included, this study was well powered to detect obesity polygenes despite the comparatively small sample size. Six intronic SNPs in *FTO* showed the strongest evidence for association with obesity (best SNP: rs1121980, odds ratios for obesity for heterozygosity and homozygosity for the T-allele were 1.67 and 2.76, respectively; *(29)*). Eleven SNPs (including two *FTO* SNPs) were subsequently genotyped in 644 independent obesity families based on at least one young obese index patient. The association with early-onset obesity was confirmed only for the two *FTO* SNPs (rs9939609 and rs1121980).

FTO rs9939609 was genotyped in a total of 17,508 middle-aged Danes. Again, the A-allele was associated with overweight and obesity. Obesity-related quantitative traits such as body weight, waist circumference, fat mass, and fasting serum leptin levels were significantly increased in A-allele carriers. There was an interaction between the *FTO* rs9939609 genotype and physical activity; physically inactive homozygous risk A-allele carriers had an increased BMI (1.95 ± 0.3 kg/m^2) compared with homozygotes for the T-allele. Low physical activity thus seemingly accentuates the effect of *FTO* rs9939609 on body fat accumulation *(30)*.

The obesity risk variant of *FTO* at rs8050136 was associated with a reduced insulin effect on beta activity measured by magnetoencephalography, which implicates a lower cerebrocortical response to

insulin. Since the *FTO* gene is expressed in hypothalamic centers controlling appetite (see below), this might be a mechanism by which variation in *FTO* contributes to the pathogenesis of obesity *(31)*. Wåhlén et al. *(32)* suggested that *FTO* may also play a role in fat cell lipolysis, providing a functional link to body weight regulation.

Detailed computational analysis of the sequence and predicted structure of the protein encoded by *FTO* has been performed. Human FTO is apparently a member of the non-heme dioxygenase (Fe(II)- and 2-oxoglutarate-dependent dioxygenases) superfamily *(33,34)*. Both 2-oxoglutarate and iron should therefore be important for FTO function *(34)*.

Very recently, the first individuals with a non-synonymous mutation (Arg316Gln) leading to inactivation of FTO were described *(35)*. The subjects were members of a large Palestinian Arab consanguineous multiplex family. All affected individuals had postnatal growth retardation, microcephaly, severe psychomotor delay, functional brain deficits, and characteristic facial dysmorphic features. Structural brain malformations, cardiac defects, genital anomalies, and cleft palate were described in some of the affected individuals. Death occurred at 1–30 months of age; it was caused by intercurrent infection or unidentified causes.

The mutation in this family localizes to an evolutionarily conserved region of FTO and leads to inactivation of its enzymatic activity. Functional data further implied that FTO is essential for normal development of the cardiovascular and central nervous systems in humans. Detailed anthropometric data were unfortunately not available on unaffected family members. However, none of the heterozygous parents were obese; nor did they show any of the clinical features detectable in homozygotes. It was speculated that carriers of loss-of-function mutations in *FTO* might be relatively resistant to develop obesity *(35)*.

A series of studies evaluated the functional role of FTO. Recombinant murine Fto catalyzes the Fe(II)- and 2OG-dependent demethylation of 3-methylthymine in single-stranded DNA. Concomitantly succinate, formaldehyde, and carbon dioxide are produced. Fto localizes to the nucleus in transfected cells, which is consistent with a potential role in nucleic acid demethylation. In wild-type mice, *Fto* mRNA is most abundant in the brain, particularly in hypothalamic nuclei governing energy balance. In fasted mice *Fto* mRNA levels in the arcuate nucleus were reduced by approximately 60%. Future studies will identify the physiologically relevant FTO substrates and determine how nucleic acid methylation status is linked to an elevated fat mass *(33)*. Recently it was shown that complete (homozygous knockout) loss of *Fto* in mice leads to postnatal growth retardation and a significant reduction in adipose tissue and lean body mass. The leanness of *Fto*-deficient mice results from increased energy expenditure and systemic sympathetic activation despite decreased spontaneous locomotor activity and relative hyperphagia. *Fto* expression in heterozygous *Fto*± mice was reduced; this led to reduced weight gain after 12 weeks. These observations suggest that the effects of *Fto* on energy homeostasis are mediated, at least in part, through the control of energy expenditure *(36)*.

In summary, complete *Fto* disruption leads to growth failure, with reductions in adipose tissue and lean body mass. These effects appear to be mediated, at least in part, through central-dependent increases in energy expenditure. Heterozygosity for *FTO* mutations may protect against obesity. The association of obesity with intronic polymorphisms in *FTO* suggests that the polymorphisms may increase FTO activity.

MORE POLYGENES IDENTIFIED IN RECENT GENOME-WIDE ASSOCIATION STUDIES

In 2009 three groups reported novel obesity genes with small effect sizes *(24–26)*; more than 150,000 individuals were analyzed in total.

A meta-analysis of 15 GWAS for body weight regulation ($n = 32{,}387$) was performed by the Genetic Investigation of ANthropometric Traits (GIANT) consortium based on approximately 2.4 million genotyped or imputed SNPs. The top 35 signals were followed up in 14 additional cohorts ($n = 59{,}082$). A strong confirmation was detected for *FTO* and *MC4R*. Additionally, six novel loci were identified near or within the following candidate genes: *TMEM18* (transmembrane protein 18), *KCTD15* (potassium channel tetramerization domain), *GNPDA2* (glucosamine-6-phosphate deaminase 2), *SH2B1* (SH2B adaptor protein 1), *MTCH2* (mitochondrial carrier 2), and *NEGR1* (neuronal growth regulator 1; a 45 kb deletion copy number variation is the candidate variant). Several of these presumptive 'obesity' genes are highly expressed and/or known to play a role in the function of the central nervous system. The effect of the variants on BMI ranged from 0.06 to 0.33 kg/m^2 per allele, which corresponds to 173–954 g of weight per allele in adults who measure ~170 cm in height. Together, the six newly discovered loci account for 0.40%, and in combination with *FTO* and *MC4R* a total of 0.84%, of BMI variance in otherwise normal individuals. The combined impact of these loci on BMI was also estimated: Individuals with 13 or more obesity predisposing alleles across eight loci were on average 1.46 kg/m^2 (equivalent to 3.7–4.7 kg for an adult 160–180 cm in height) heavier than individuals with less than 3 of these alleles *(24)*.

Another GWAS was performed in 25,344 Icelandic, 2,998 Dutch, 1,890 European American, and 1,160 African-American subjects and combined with results published by the Diabetes Genetics Initiative (DGI) based on 3,024 Scandinavians. A total of 19 regions comprising 43 variants were selected for follow-up in 5,586 Danish individuals. The results were compared with results of the obesity GWAS of the GIANT consortium (see above; *(24)*). In 11 chromosomal regions a total of 29 variants reached a genome-wide significance threshold of $p < 1.6 \times 10^{-7}$. In addition to variants at seven loci that had previously not been associated with obesity, both *FTO* and *MC4R* were reconfirmed; furthermore, the two obesity candidate genes *BDNF* and *SH2B1* were reidentified *(25)*.

The third GWAS on 1,380 Europeans with early-onset or late-onset morbid obesity and 1,416 age-matched normal weight controls revealed 38 markers showing strong association. These were further evaluated in 14,186 European subjects *(26)*. In addition to *FTO* and *MC4R*, significant association with obesity was detected for three new risk loci within the endosomal/lysosomal Niemann–Pick C1 gene (*NPC1*), near the transcription factor c-MAF gene (*MAF*) and near the phosphotriesterase-related gene (*PTER*). Additionally, candidate genes were analyzed in the GWAS data set. Interestingly, the association with Val103Ile of the *MC4R* was, among other genes, confirmed. Most of these results were replicated in independent approaches *(37,38)*.

CONCLUSIONS AND PERSPECTIVES

Initially, molecular genetic studies led to the identification of a small number of major genes for human obesity. The relevant mutations have a profound influence on the development of excess body weight, but they are rare and therefore only of minor clinical importance. The majority of confirmed genes involved in the predisposition to obesity are of polygenic nature. The contribution of any single polygene to the development of obesity is small; detection and confirmation of such variants require screening of thousands of individuals.

In 2007 a variation in exon 1 of *FTO* was shown to be associated with obesity. Within a short period of time it became evident that *FTO* represents a major polygene in populations of European, African, and Asian descent. Clinical and experimental observations confirm its importance in energy homeostasis. Sixteen other polygenes for body weight regulation have been reported. The 103Ile variant (minor allele) of the *MC4R* is of interest as it confers protection from obesity. Other *MCR4* variants also contribute to obesity risk. Thus, genetic variation of genes expressed in the CNS plays a prominent role in BMI variation. This is not surprising, given the role of the brain in behavior and energy balance.

If genetic variation accounts for 50–70% of the variance in human BMI, we have far to go; only 1–2% of BMI variance is explained by the currently known polygenes. Realistically, we might assume that the currently detected variants represent the tip of the iceberg; effect sizes of variants detected in the future may be even smaller. Obviously, sample sizes have to be very large to detect these signals and to confirm them independently. If BMI heritability results from the effect of hundreds of alleles, many of which account for less than 50 g, we would have substantial genetic heterogeneity among obese individuals. Assuming this scenario, simplistic ideas of genotype–phenotype correlations would have to be dismissed.

In light of the low BMI variance explained by the polygenes detected in recent GWAS, we can speculate that infrequent alleles with stronger effect sizes, not readily detected in GWAS, may explain a larger part of the variance of BMI. Another disconcerting idea pertains to genotype–environment interactions. These may be rather specific, based on the genotype of an individual. While formal genetic studies have taught us that non-additive factors play a prominent role in BMI heritability estimates, the currently known variants seemingly act in an additive manner only. Future analysis of genetic factors involved in body weight regulation will substantiate our understanding of the mechanisms leading to obesity and hopefully lead to improved therapeutic approaches.

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Editor's Questions and Authors' Response

- **BMI heritability appears to result from the effects of hundreds of alleles with small effects, and there is substantial genetic heterogeneity among obese individuals. Moreover, recent investigations suggest that epigenetic effects on a number of imprinted genes are likely to play important roles in disease risk (Kong et al., 2009). Given this complexity of regulation, is it likely that future genetic analysis of parents or young people will be able to predict reliably the risk of childhood or adult obesity?**

- The predictive value of the detected obesity alleles varies between monogenic gene and polygenic effects. The effect sizes (and thus the explained part of the respective individual's body weight) are in the magnitude of 30–50 kg or more for the monogenic forms. The polygenic variants increase the body weight just by a few hundred grams to 1–2 kg. Hence, the predictive value of these genetic variants is quite different. If a lot of these variants are indeed detected in the future, prediction will potentially become feasible. However, we are currently far from being able to do so because the BMI variance that can be explained currently by polygenic variants is only about 1–2%. Thus, a lot of variance remains to be detected in order to explain the heritability estimates in the range of 0.5.

- **More to the point, would a straightforward but detailed family history provide as much or more predictive power?**

- The answer currently is yes (see above). However, the analysis of a family history cannot give hints to the involvement of specific genetic mechanisms. Hence, genetic tests could provide much more detailed data pertaining to pathways predisposing to obesity within the analyzed family. Currently, obese children and adolescents are in some cases being screened for MC4R mutations. We assume that the obesity of such individuals is largely due to their respective mutation.

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