Discovery of an Obesity Susceptibility Gene, KSR2, Provides New Insight into Energy Homeostasis Pathways

Anna P. Pilbrow, PhD


Study Hypothesis

Obesity is a heterogeneous condition with considerable heritability. Multiple adiposity genes have now been identified through genome-wide association studies and studies of rare forms obesity caused by single-gene mutations. Several of these monogenic forms of obesity seem to replicate mouse obesity phenotypes, with notable examples including obesity caused by inactivating mutations in leptin, the leptin receptor, and the melanocortin-4 receptor. A large-scale screen for additional obese phenotypes in mice found that targeted deletion of Ksr2 leads to obesity, suggesting a role for Ksr2 in energy homeostasis. Ksr2 encodes the scaffolding protein kinase suppressor of ras 2 (KSR2), which may regulate multiple signaling pathways by binding kinases, such as Raf and AMP-activated protein kinase (AMPK). In this article, Pearce et al.2 hypothesize that genetic variants in KSR2 may contribute to the development of severe, early onset obesity in humans.

How Was the Hypothesis Tested?

Using a combination of Sanger and whole-exome sequencing, the authors sequenced the coding region and intron/exon boundaries of KSR2 to identify rare variants (minor allele frequency, <0.5%) in 2101 individuals of mixed European ancestry who developed severe obesity before 10 years and in 1536 controls from a UK population-based study. They also performed a small replication study (238 cases and 1117 controls) and sequenced KSR2 in family members of severely obese probands to determine whether KSR2 variants cosegregated with overweight/obesity in these families. They then performed a series of mechanistic studies aimed at addressing whether variants located in or near the kinase domain of KSR2 altered its ability to regulate signaling through the Raf-mitogen-activated protein kinase/extracellular signal-regulated kinase (Raf-MEK-ERK) pathway, a key pathway regulating cell metabolism and growth. These studies used confocal microscopy, coimmunoprecipitation, and flow cytometry to characterize interactions between wild-type and mutant forms of KSR2 with endogenous B-Raf, MEK, or ERK. They also used x-ray crystal structure modeling for each of the KSR2 mutations to predict whether these would disrupt interactions between the KSR2 kinase domain and MEK.

Next, the authors characterized the phenotype associated with loss-of-function KSR2 mutations in humans by measuring an extensive range of clinical and metabolic parameters in 18 severely obese probands and family members carrying KSR2 mutations and 26 equally obese volunteers in whom KSR2 mutations were excluded. In parallel, they investigated the influence of loss of KSR2 function on energy homeostasis in Ksr2–/– mice by assessing the contribution of hyperphagia to weight gain from weaning to 18 weeks of age and by examining intolerance to cold temperature (a sensitive measure of impaired fatty acid oxidation in rodents). Finally, they used immunoprecipitation to test whether mutations in KSR2 disrupted binding to AMPK (a cellular energy sensor) and measured markers of glucose and fatty acid oxidation in cells overexpressing wild-type or mutant KSR2. Cells were subsequently pretreated with the antidiabetic drug metformin, which can activate AMPK, to determine whether this could restore levels of fatty acid oxidation in cells expressing mutant forms of KSR2 to that of cells expressing wild-type KSR2.

Principal Findings

The authors identified multiple rare variants in KSR2 in humans that seem to alter the function of KSR2 and strongly predispose affected individuals to obesity and severe insulin resistance. Specifically, the authors found a total of 27 different KSR2 mutations in 21.1% of severely obese individuals and 1.0% of normal weight controls, of which 23 mutations were unique among obese individuals. However, the mutations did not cosegregate with obesity in family members of affected individuals in a strictly Mendelian manner, suggesting that their effect may be modulated by other genetic and environmental factors.
In transfected cells, many of the mutations studied impaired signaling through the Raf-MEK-ERK pathway by reducing the ability of KSR2 to bind to MEK, ERK, and B-Raf. This was strongly consistent with structural modeling analysis, which showed that many KSR2 mutations affect highly conserved residues and disrupt key interactions among KSR2, B-Raf, and MEK. Clinical characterization of obese individuals and family members with KSR2 mutations showed that they have hyperphagia in childhood, lower heart rate and reduced basal metabolic rate when compared with obese controls, and severe insulin resistance. These phenotypes overlapped with phenotypes seen in Ksr2−/− mice, which were hyperphagic, and gained more weight (resulting from an increase in fat mass) than in wild-type littermates when fed the same amount. These findings suggest that both increased energy intake and reduced energy expenditure contribute to obesity in both mice and human KSR2 mutation carriers. In addition, Ksr2−/− mice fed ad libitum became cold intolerant by 10 weeks, indicating that their lower energy expenditure might be explained by impaired fatty oxidation. This finding was corroborated by experiments in transfected cells, which showed that almost all mutant forms of KSR2 impaired glucose oxidation and fatty acid oxidation, even though only a small proportion of mutant KSR2 proteins bound AMPK with reduced affinity. The reduced level of fatty acid oxidation in cells expressing mutant KSR2 protein could be completely restored by metformin to levels in cells expressing wild-type KSR2.

**Implications**

This study confirms KSR2 as a key regulator of energy homeostasis and suggests that disruption of KSR2 strongly predisposes affected individuals to an obesogenic phenotype and severe insulin resistance. The findings of this study are noteworthy because they imply that pathways regulated by KSR2 may represent new therapeutic targets for obesity and type 2 diabetes mellitus. In contrast to other rare forms of obesity, KSR2 mutations were associated with increased energy intake concomitant with reduced energy expenditure (potentially explained by impaired substrate use), suggesting that both energy intake and energy expenditure contribute to the development of obesity in KSR2 mutation carriers. In addition, this study provides a plausible mechanistic explanation for the anecdotal clinical observation that metformin, prescribed for severe insulin resistance in some KSR2 mutation carriers, can lead to weight loss, a finding that clearly warrants further exploration in prospective clinical studies.

However, several questions remain: precisely how is AMPK involved, do KSR2 mutations influence other components of energy expenditure, and what is the effect of partial loss-of-function KSR2 mutations on KSR2 function? Interestingly, family studies linking chromosome 12q24, the region containing KSR2 in humans, to obesity and type 2 diabetes mellitus have subsequently been corroborated by some genome-wide association studies for obesity-related traits, suggesting that common genetic variants in KSR2, not just rare mutations, also contribute to common obesity. The study by Pearce et al brings KSR2 to the forefront of obesity genetics and highlights that rare forms of obesity can yield important insights into key pathways that regulate energy homeostasis in humans.

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**Disclosures**

None.

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