INVITED REVIEW

Insights into obesity and insulin resistance from the study of extreme human phenotypes

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Abstract

The detailed study of rare, extreme human phenotypes has a long and distinguished history in endocrinology. Such individuals have often acted as ‘experiments of nature’ providing important novel information regarding endocrine physiology and mechanistic insights relevant to the study of more common endocrine disorders. This review presents a personal experience of the study of two such extreme phenotypes, obesity and severe insulin resistance.

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Extreme human phenotypes as a guide to endocrine physiology

The study of rare individuals with extreme disturbances of their physiology has long had an impact in terms of scientific understanding grossly disproportionate to the infrequency of the particular disease being investigated. In these days when research funding is increasingly targeted at common diseases of public health importance, it is necessary to formally state the rationale for spending time and effort on such human rarities. In my view there are three reasons for the importance of this type of research: (i) the diseases, while rare, frequently cause severe morbidity and early mortality for those unlucky enough to suffer from them and there is an often an urgent requirement to improve therapeutic strategies; (ii) the study of such individuals can illuminate normal biological control mechanisms; and (iii) the understanding gleaned from such cases can inform our studies of the more common diseases.

There are numerous examples of this in the endocrine literature. Korach and colleagues’ studies of an extremely tall young man led to the discovery that the oestrogen and not the androgen receptor was the final mediator of sex steroid-induced epiphyseal closure (1). The study of an extremely short child with severe intellectual retardation by Clark and colleagues led to the finding of a homozygous deletion of the insulin-like growth factor (IGF)-I gene and provided the first information that IGF-I was necessary for cortical development (2). By studying subjects whose blood pressure was severely elevated from a young age, Lifton and colleagues have made a series of discoveries regarding the control of ion resorption in the kidney and the seminal importance of renal sodium resorption in the control of blood pressure (3). The discovery of glucocorticoid interconversion, which is a major focus of contemporary endocrine research, came initially from the study of patients with hypertension and hypokalaemia (4). The discovery of the critical role of the HNF family of transcription factors in pancreatic and hepatic metabolism and development was initially illuminated through the finding in mutations in patients in whom type 2 diabetes developed very early in life (5).

Type 2 diabetes is a complex disorder characterised by both beta cell dysfunction and insulin resistance. More than 85% of subjects with this disease are obese. While there had been considerable success in defining monogenic disorders affecting beta cell function, it has been a much greater challenge to discover genetic factors underlying the insulin resistance, or obesity that predispose to type 2 diabetes. Over the past 10 years we have taken an ‘extreme phenotype’ approach to insulin resistance and obesity, seeing if, by studying individuals in whom the disturbances were severe and/or of very early onset, we might be able to enrich our study cohorts with individuals who had major genetic abnormalities.

Obesity

Genetics of obesity study (GOOS) cohort

Our GOOS cohort was started in earnest in 1996 by Dr Sadaf Farooqi and myself following our reports of the first cases of human leptin deficiency (see below). Entry criteria for the cohort are: (i) severe obesity (body mass index (BMI) standard deviation score
prohormone convertase 1 (PC1)

A patient (YC) presented to my regular clinic at the age of 42 with long-standing symptoms suggestive of reactive hypoglycaemia. She had a complex past medical history involving severe early-onset obesity (40 kg at the age of 4, see Fig. 1) and idiopathic hypogonadotropic hypogonadism. Investigation revealed partial adrenocorticotrophin deficiency. Circulating proinsulin levels were massively elevated but true insulin was undetected, yet the proinsulin gene sequence was normal (6). Pro-opiomelanocortin (POMC) levels were also hugely elevated suggesting a generalised defect in prohormone conversion and, following the characterisation of the genomic structure of the PC1 gene we went on to find two loss of function mutations in the neuroendocrine convertase PC1 (7). Further studies of this patient have illuminated the role of PC1 in endocrine processing events with proinsulin and POMC being highly dependent and parathyroid hormone and procalcitonin being independent whereas proglucagon and progastrin have intermediate levels of dependence on normal PC1 function (S O’Rahilly and RS Jackson, unpublished observations). For several years this patient remained the only known organism who was deficient in PC1. We have recently discovered a neonate who suffered from an ultimately fatal congenital diarrhoeal illness in association with multiple endocrine dysfunctions (Fig. 1). This infant was found to be a compound heterozygote for two, essentially null mutations in PC1 (S O’Rahilly and RS Jackson, unpublished observations). Further evaluation of our original proband YC, showed a severe and previously unnoticed small bowel dysfunction with steathorea, bile salt and vitamin B12 malabsorption. Thus, PC1 has a, previously entirely unsuspected, role in the maintenance of the absorptive ability of the small intestine. As PC1 is involved in the processing of multiple propeptides (8), it is not easy to make a definitive link between any particular hormone deficiency and an aspect of this patient’s phenotype. However, it is notable that mice deficient in the processing enzyme carboxypeptidase E, which acts in concert with the PCs to finalise the processing of prohormones, are also obese and infertile (9).

Leptin

The discovery of leptin in 1994 by Friedman and colleagues represented a landmark in the molecular understanding of the control of mammalian body fat mass (10). However, two years after the discovery of leptin, its role in human biology was uncertain. In general, plasma leptin levels correlated positively with fat mass and no mutations in leptin had been found in humans (11). Given the human appetite and the multiple social and developmental influences on this, was it possible that leptin was now a vestigial hormone in humans and its plasma levels merely a reflector of fat mass? We found two cousins, of Pakistani origin, who were homozygous for frameshift mutations in the leptin gene (12); the mutation resulted in a mutant truncated leptin, with an aberrant C-terminus, which did not appear to be secreted normally from cells (13). The children were extremely obese from a young age and had severe hyperphagia. Subsequent discovery of adults with congenital leptin deficiency by others confirmed that, like the ob/ob mice, leptin-deficient humans also fail to undergo normal puberty due to a central defect in gonadotrophin release (14). We have now identified a total of five leptin-deficient children (S O’Rahilly and IS Farooqi, unpublished observations). While many of their phenotypes are very similar to those seen in the mouse there are key differences between the human and mouse phenotypes indicating interspecies differences in the degree to which leptin controls certain aspects of physiology (Table 1). In particular the profound effects of lack of leptin on linear growth and the hypothalamic–pituitary–adrenal axis that are seen in mice are not seen in humans. Four of the children are now being treated with s.c. recombinant leptin and all have had beneficial
responses (15, and unpublished observations). Unfortunately, the fact that native leptin is a neoantigen for these children does present a problem of the production of neutralising antibodies, which has been a problem for two of the four children. However, the response in the other children has been dramatic with huge benefits in terms of weight, mobility, metabolic disturbance and quality of life. These children therefore represent the first example of a successful, mechanism-based, pharmacotherapy of a form of human morbid obesity.

Could lesser degrees of leptin deficiency predispose to human obesity? This is an important question as there is a wide scatter of plasma leptin levels for any particular BMI and, if subsets of 'common' obese patients are indeed relatively leptin deficient that could have a major therapeutic impact. We studied the heterozygous relatives of the children with congenital leptin deficiency and obtained evidence that (i) these individuals did have subnormal leptin levels, (ii) that they had a higher than expected prevalence of obesity and (iii) that compared with ethnicity-matched controls they had a higher percentage of body fat than would be expected from their height and weight (16). These data raise the possibility that a subgroup of obese subjects with relatively low leptin levels might show a beneficial therapeutic response to leptin supplementation.

### Melanocortin 4 receptor (MC4R)

One of leptin’s major actions in the brain is to activate hypothalamic arcuate neurons expressing POMC (17). POMC-derived melanocortin peptides then act on MC4R to mediate the anorexigenic responses to leptin (18). The key role of the MC4R was revealed by murine genetic experiments and two years later simultaneous reports from Vaisse et al. and our laboratory described the first obese humans who were heterozygous for null mutations in MC4R (19, 20). Since then, we and others have described multiple different mutations in the MC4R associated with obesity, including homozygotes for this condition (21–23). We have, to date, found disease-causing mutations in the MC4R in 25 of 500 of our GOOS subjects thus far studied (unpublished observations). In addition to obesity and hyperphagia, this group of subjects are characterised by early marked growth acceleration, increased bone density and hyperinsulinaemia disproportionate to their degree of obesity. More recently, we have been able to correlate the in vitro functional properties of their mutant receptors with the phenotype with a clear correlation being seen for all phenotypes studied. In summary, MC4R deficiency is by far the commonest single-gene disorder thus far described which presents as early-obesity human obesity. Although, as yet, specific therapy has not been developed by us in this condition, its identification has brought enormous relief and shedding of guilt for many families for whom an explanation of their child’s obesity is now available. We would argue that the determination of the sequence of the MC4R gene should now be a standard part of the assessment of any severely obese child.

### The severe insulin resistance (SIR) cohort

Unlike obesity, which is clinically obvious, the diagnosis of extreme insulin resistance is often missed for many

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**Table 1** Monogenic obesity syndromes in humans. Not included in this table are a range of monogenic disorders, including the syndromes of Bardet–Biedl, Alstrom, Prader–Willi and Cohen and pseudohypoparathyroidism, where juvenile obesity is usually found against a background of a complex phenotype including multiple developmental abnormalities.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Major phenotypic features</th>
<th>Mode of inheritance</th>
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<tbody>
<tr>
<td>Leptin</td>
<td>Severe early obesity, hyperphagia</td>
<td>Recessive</td>
</tr>
<tr>
<td>Leptin receptor</td>
<td>Severe early obesity, hyperphagia</td>
<td>Recessive</td>
</tr>
<tr>
<td>PC1</td>
<td>Severe early obesity, hyperphagia</td>
<td>Recessive</td>
</tr>
<tr>
<td>MC4R</td>
<td>Severe early obesity, hyperphagia, Tall stature</td>
<td>Co-dominant</td>
</tr>
<tr>
<td>POMC</td>
<td>Severe early obesity, hyperphagia, Increased bone density</td>
<td>Recessive</td>
</tr>
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years/decades and patients present to a wide variety of specialists including endocrinologists, gynaecologists, dermatologists, diabetologists etc. (24). Although its mechanistic link with insulin resistance is poorly understood, we have taken the skin lesion acanthosis nigricans as the most important entry criterion for our SIR cohort. On a population-wide basis, severe obesity is by far the commonest cause of insulin resistance. However, we wished to use this cohort to find genes that might influence insulin action independently of obesity. Therefore we excluded morbidly obese subjects from the SIR cohort. The criteria for entry into the SIR cohort are shown in Table 2. Table 3 shows the diagnostic categories of patients in the SIR cohort.

Our initial efforts in this cohort were directed at the insulin receptor gene. Approximately 5% of this cohort were found to harbour pathogenic insulin receptor mutations. This included the first case of the null phenotype of the insulin receptor in a child with Donohue’s syndrome who was homozygous for a nonsense mutation truncating the receptor in the proximal part of the alpha subunit (25). This child predated the murine knock out and established that relatively normal intrauterine development, including cognitive development, could occur in the complete absence of the insulin receptor. In this family we also described the first use of molecular genetics in prenatal diagnosis in a disorder of insulin signalling (26). The detailed functional studies of several naturally occurring insulin receptor mutants provided novel insights into insulin signalling (27, 28). Additionally, we also described a specific biochemical defect in insulin-stimulated phosphatidylinositol 3-kinase (PI3 kinase) activity in cells from patients with the pseudoacromegalic variant of SIR (29). In the case of distinct recognisable lipodystrophic syndromes of insulin resistance we collaborated with colleagues undertaking positional cloning approaches that were ultimately successful in identifying mutations in lamin A/C as the cause of familial partial lipodystrophy (30) and seipin as the cause of one form of congenital generalised lipodystrophy (31).

### Industrialising the approach

The complexity of insulin signalling and the capacity of multiple factors to interfere with normal insulin action, make the range of candidate genes for insulin resistance very large indeed. Any attempt at comprehensive coverage is beyond even a well-funded academic laboratory. With that in mind, in 1997 we joined forces with a new biotechnology company, Hexagen (subsequently subsumed into Incyte Genomics), which had developed high-throughput candidate gene analysis suitable for industrial-scale applications and embarked on the study of more than 100 candidate genes in our SIR cohort. Multiple mutations have been found in many candidate genes and much research into the physiological significance of these variants is ongoing. However, two recent discoveries illustrate the power of this approach.

**Peroxisome proliferator-activated receptor-γ (PPARγ)** In two probands we found two different non-conservative, missense mutations in the ligand-binding domain of the nuclear receptor PPARγ (32). Both of these mutant receptor were studied in collaboration with Krishna Chatterjee, were severely deficient in

### Table 2 Criteria for entry to severe insulin resistance cohort.

| Acanthosis nigricans | BMI > 37 kg/m² | Fasting insulin > 150 pmol/l or post-prandial > 1000 pmol/l or insulin requirements > 200 U/day if lean, > 300 U/day if obese |

### Table 3 Provisional classification of syndromes of severe insulin resistance.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Causative mechanism</th>
<th>Mode of inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptoropathies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donohue’s syndrome</td>
<td>Insulin receptor mutations</td>
<td>Recessive</td>
</tr>
<tr>
<td>Rabson–Mendenhall syndrome</td>
<td>Insulin receptor mutations</td>
<td>Recessive</td>
</tr>
<tr>
<td>Type A insulin resistance</td>
<td>Insulin receptor mutations in 5–10%</td>
<td>Usually dominant</td>
</tr>
<tr>
<td></td>
<td>One family with PPARγ and PPP1R3 mutations</td>
<td>Digenic</td>
</tr>
<tr>
<td>Lipodystrophic syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital generalised lipodystrophy</td>
<td>At least two loci</td>
<td>Recessive</td>
</tr>
<tr>
<td>(Berardinelli–Seip)</td>
<td>Chromosome 11q (seipin)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chromosome 9q34 (1-aclyglycerol-3-phosphate-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-acetyltransferase 2)</td>
<td></td>
</tr>
<tr>
<td>Face-sparing lipodystrophy</td>
<td>Lamin A/C mutations</td>
<td>Dominant</td>
</tr>
<tr>
<td>Syndromes of uncertain aetiology</td>
<td>Dominant-negative mutations in PPARγ</td>
<td></td>
</tr>
<tr>
<td>Pseudo-acromegalic insulin resistance</td>
<td>Functional defect in P13 kinase signalling</td>
<td>Unknown</td>
</tr>
<tr>
<td>Acquired syndromes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type B insulin resistance</td>
<td>Anti-insulin receptor antibodies</td>
<td>N/A</td>
</tr>
<tr>
<td>Acquired lipodystrophy</td>
<td>Unknown</td>
<td>N/A</td>
</tr>
</tbody>
</table>
their ability to transactivate and, indeed were able to suppress the transcriptional activity of a co-transfected wild-type receptor (32). Thus, they represented classical dominant-negative receptors, analogous to the many such mutations in the thyroid hormone receptor that have been found in patients with thyroid hormone resistance (33). Although pharmacological ligands for PPARγ had been described to act as insulin sensitisers (34), until the discovery of these patients, there was no genetic evidence that PPARγ was involved in carbohydrate metabolism. The SIR that we have now found in all five family members with these mutations (S O’Rahilly and D Savage, unpublished observations) represents the first definitive genetic proof that this receptor is central to the normal control of insulin sensitivity and carbohydrate metabolism. Additionally, our finding that all mutation carriers are hypertensive from an early age reveals a previously unsuspected role for this receptor in the control of blood pressure. In fact, the mutation carriers (see Fig. 2) express a syndrome of hypertension, insulin resistance dyslipidaemia, fatty liver and hyperuricaemia, which is highly reminiscent of syndrome X as well as a stereotyped form of partial lipodystrophy. Recently, we have been able to treat some of these subjects with pharmacological agonists with, in at least one case, a dramatic improvement in clinical status that correlated with the ability of the agonist to overcome dominant negativity in vitro (VK Chatterjee and S O’Rahilly, unpublished observations).

A digenic disorder We recently studied a family in which five members had acanthosis nigricans and severe hyperinsulinaemia. Intriguingly all five subjects, but none of their other relatives, were double heterozygotes for frameshift mutations in two unlinked genes (35). Single heterozygotes had fasting insulin

![Figure 2](https://example.com/figure2.png)

**Figure 2** Acanthosis nigricans (a) and upper limb lipodystrophy (b) in a 50-year-old Caucasian female with a dominant-negative mutation in PPARγ.
concentrations within the normal range. One mutation was a frameshift/premature stop mutation in PPPARγ which produces a truncated PPARγ which is unable to bind DNA and, in contrast to the missense mutation described above, did not act as a dominant-negative. The other mutation was a frameshift/premature stop in a gene encoding a regulatory subunit of phosphatase 1. The protein, PPP1R3A, is expressed exclusively in striated muscle and is involved in the control of glycogen synthesis (36). The mutation truncates the protein, resulting in mistargeting of PPP1R3 within the cell. While the PPARγ mutation appears to be private to this pedigree, the PPP1R3 mutation is more widespread in Caucasian populations (between – 0.5 and 2%) and preliminary data suggest that it may increase the risk of type 2 diabetes. While the precise reasons for the combination of defects to lead to such SIR is as yet unclear, the fact that a combination of two defects, one in skeletal muscle and one in adipose tissue, that by themselves cause at most subtle functional consequences, can severely impair insulin action may provide a model for the sorts of gene–gene interaction that disturb metabolism in common metabolic disorders such as type 2 diabetes.

Conclusions

Our studies of rare extreme human phenotypes of obesity and insulin resistance have demonstrated the ability of this approach to illuminate certain aspects of human molecular physiology and has, thus far, led to the effective targeted therapy in two of these rare disorders. As illustrated by the PPP1R3 mutation, populations of subjects with extreme phenotypes may be enriched in genetic mutations that may play a broader role in susceptibility to more common genetic diseases.

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References

21. Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, Butler G et al. Dominant and recessive inheritance of morbid obesity associated...


27 Krook A, Moller DE, Dib K & O'Rahilly S. Two naturally-occurring mutant receptors phosphorylate insulin receptor substrate-1 (IRS-1) but fail to mediate the biological effects of insulin. *Journal of Biological Chemistry* 1996 271 7134–7140.


33 Collingwood TN, Adams M, Tone Y & Chatterjee VK. Spectrum of transcriptional, dimerization and dominant negative properties of twenty different mutant thyroid hormone beta-receptors in thyroid hormone resistance syndrome. *Molecular Endocrinology* 1994 8 1262–1277.


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