

LETTERS TO THE EDITORS

What is the contribution of a kozak snp in the CD40 gene to graves' disease?

The study by Heward *et al.*¹ is the largest association study of the CD40 gene in Graves' disease (GD), reported so far. They tested 800 Caucasian UK Graves' patients and 785 ethnically matched controls for the frequencies of the C and T alleles and the three corresponding genotypes of a CD40 Kozak single nucleotide polymorphism (SNP) we had previously identified. They report that their analyses showed an increased frequency of the C allele in GD patients that did not reach statistical significance ($P = 0.087$), and no difference in the distribution of the three genotypes (CC, CT, TT) between patients and controls ($P = 0.145$). Previously, we² and others³ have shown that GD patients had a significantly increased frequency of the CC genotype of the CD40 Kozak SNP when compared to the combined frequencies of the non-CC genotypes, that is, the CT and TT genotypes. The test that Heward *et al.* performed on their data was different from the test that we reported, that is, a comparison of the frequencies of the CC genotype *vs.* CT + TT in patients and controls.

Examining their data (Heward *et al.* 2004) shows that the CC genotype was present in 60.1% (481/800) of GD patients and 55.3% (434/785) of controls and, correspondingly, the CT + TT genotypes were present in 39.9% (319/800) of patients and 44.7% (351/785) of controls, a statistically significant difference [$\chi^2 = 3.80$, $P = 0.05$, odds ratio (OR) = 1.22]. Thus, the data reported by Heward *et al.* replicate our previous findings of an association between the CC genotype of the CD40 Kozak SNP and Graves' disease.

Their data also show, as we have previously reported, that when testing unselected GD patients this association is weak. What could be the reason for this weak association in a locus giving a significant LOD score? We believe that this reflects the fact that CD40 contributes to the genetic risk for GD only in a subset of patients, and when this subgroup is diluted by testing all GD patients, the association is weakened. Indeed, in our study the frequency of the CC genotype increased significantly (from 65 to 85%) when testing only the probands of the linked families (i.e. families showing positive evidence for linkage with the GD-2 locus).² In addition, the study by Kim *et al.* has shown that the stimulating TSHR antibody activity was significantly higher in patients with the CC genotype compared to patients with non-CC genotypes, suggesting that the CC genotype is associated with a subset of GD patients with high stimulating TSHR antibody activity.³

Very recently, another group examined the CD40 Kozak SNP in GD patients and controls⁴ showing no significant association. However, we performed a meta-analysis pooling all the data from the four studies published so far (a total of 1537 patients and 1513 controls), and our meta-analysis showed a significant increase in the frequency of the CC genotype in GD patients when compared to controls (58.4% *vs.* 52.7%, $\chi^2 = 9.75$, $P = 0.0018$, OR = 1.26).

In summary, our data,² as well as the data in Table 1 reported by Heward *et al.*,¹ demonstrate a weak but significant association of the CC genotype of the CD40 Kozak SNP with Graves' disease. It remains to be determined by which mechanisms this SNP regulates CD40 expression and/or function.

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DHEA levels in treated Cushing's disease may contribute to low quality of life

In their analysis of quality of life in successfully treated Cushing's disease patients, Heald *et al.* do not mention dehydroepiandrosterone (DHEA) levels.¹ Previous work has shown that for certain periods during life DHEA has a circadian rhythm closely related to that of the secretion of ACTH.² In addition, it has been shown that, after transsphenoidal surgery for Cushing's disease, DHEA levels dropped after surgery and remained low during the follow-up period.³ There is also now ample evidence to show that hypoadrenal individuals have a lower quality of life than healthy controls,⁴ with DHEA replacement being associated with significant improvements in mood, well-being and quality of life.⁵ Thus it may be that the psychologically detrimental effect of curing Cushing's disease may be as a result of long-term adrenal suppression, with subsequent DHEA deficiency. There are several lines of evidence suggesting the biological plausibility for this.⁶ Taken together, these results suggest that studies of longer duration are needed to assess psychological well-being in those individuals in whom normal adrenal function returns after pituitary surgery.

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Pre-operative medical therapy with rosiglitazone in two patients with newly diagnosed pituitary-dependent Cushing's syndrome

We write regarding our experience of rosiglitazone treatment in two patients with newly diagnosed pituitary-dependent Cushing's syndrome. Our data show a possible modest effect of rosiglitazone in Cushing's disease. Further carefully designed studies of both dose and duration are required to confirm and further define its possible role.

Existing treatments for pituitary-dependent Cushing's syndrome include pituitary or adrenal surgery, pituitary irradiation and medical treatment with metyrapone, ketoconazole or aminoglutethimide.^{1–3} Pituitary microsurgery is the usual therapy of first choice but is not always successful in achieving early apparent remission and is also associated with a significant incidence of late relapse.^{4,5} The very significant morbidity and mortality associated with Cushing's syndrome and the limitation of current therapies highlight the need for new therapeutic strategies.

Rosiglitazone has recently emerged as a potential therapy for Cushing's disease.⁶ This drug, a thiazolidinedione, is a peroxisome proliferator activated receptor (PPAR)-gamma ligand. There is considerable experience of its use in type 2 diabetes.⁷ The PPAR-gamma receptor is a member of the nuclear receptor superfamily⁸ and functions as a transcription factor. Activation of the receptor by a variety of ligands leads to effects on adipose tissue, glucose homeostasis and insulin action, inflammation, thrombosis and fibrinolysis and

cell growth and migration. PPAR-gamma receptor ligands have been shown to inhibit tumour cell growth in the prostate and colon.^{9,10} Heaney *et al.*⁶ demonstrated that PPAR-gamma receptors are expressed in normal ACTH-secreting human pituitary cells and in all of the six ACTH-secreting human pituitary tumour cells that they tested. PPAR-gamma agonists induced G₀/G₁ cell-cycle arrest and apoptosis in human and murine ACTH-secreting tumour cells, and development of murine ACTH-secreting tumours was prevented in four of five mice treated with rosiglitazone, with ACTH and cortisol secretion suppressed in all treated mice.⁶

Case 1 was a 32-year-old female who presented with a 1-year history of headache, weight gain, muscle weakness, proximal myopathy, bruising, livid striae, amenorrhoea, hirsutism, back pain and hypertension. There was biochemical confirmation of hypercortisolism with 24-h urinary free cortisol levels of 1610 and 1808 nmol/24 h (normal range less than 350) and failure of adequate suppression during a low-dose dexamethasone suppression test (0800 h serum cortisol 571 nmol/l after eight 0.5 mg doses given 6 hourly). A high-dose dexamethasone suppression test (90% suppression), corticotrophin-releasing hormone (CRH) testing (rise in serum cortisol of 46%) and bilateral inferior petrosal sinus sampling (baseline ratio of higher petrosal to peripheral ACTH 12:1 and after CRH 22:1) were in keeping with a pituitary-dependent disease. A magnetic resonance (MR) scan of the pituitary showed a 2.2 × 1.3 × 1.3 cm macroadenoma and after operation this was confirmed histologically as an ACTH-secreting pituitary adenoma. Random glucose was elevated at 9.4 mmol/l, fasting glucose normal at 5.5 mmol/l. Pre-meal glucose readings were normal.

Case 2 was a 56-year-old female who was admitted as an emergency to hospital following a collapse secondary to a chest infection. There was a history of subarachnoid haemorrhage in 1984 treated by right cerebral artery aneurysm clipping. While in hospital, hypokalaemia developed and random glucose was elevated above 11.1 on two occasions confirming a new diagnosis of diabetes mellitus. Clinical features of Cushing's syndrome were noted. Hypercortisolism was confirmed with 24-h urinary free cortisol of 5110 and 3766 nmol/24 h and failure of adequate suppression during a low-dose dexamethasone suppression test with 08:00 h serum cortisol 690 nmol/l after eight 0.5 mg doses 6 hourly. A high-dose dexamethasone suppression test (60% suppression), CRH testing (rise in serum cortisol of 57%) and bilateral inferior petrosal sinus sampling (baseline ratio of higher petrosal to peripheral ACTH 3.5:1 and after CRH 28:1) were in keeping with pituitary-dependent disease. Computed tomography (CT) of the pituitary demonstrated a microadenoma 5 mm in diameter. An MR scan was not carried out because of the previous cerebral aneurysm surgery. Once the investigations were completed, metyrapone, 250 mg four times daily, was commenced to alleviate the significant symptomatology before surgery.

Written informed consent was obtained from both patients prior to commencing treatment with rosiglitazone. The Queen's University of Belfast ethical committee and the UK Drug Licensing Authority approved the study.

Rosiglitazone 8 mg orally per day was given for 33 days in case 1 and for 20 days in case 2. Metyrapone had been administered for 5 days prior to commencing the rosiglitazone in case 2 and was continued during subsequent therapy.

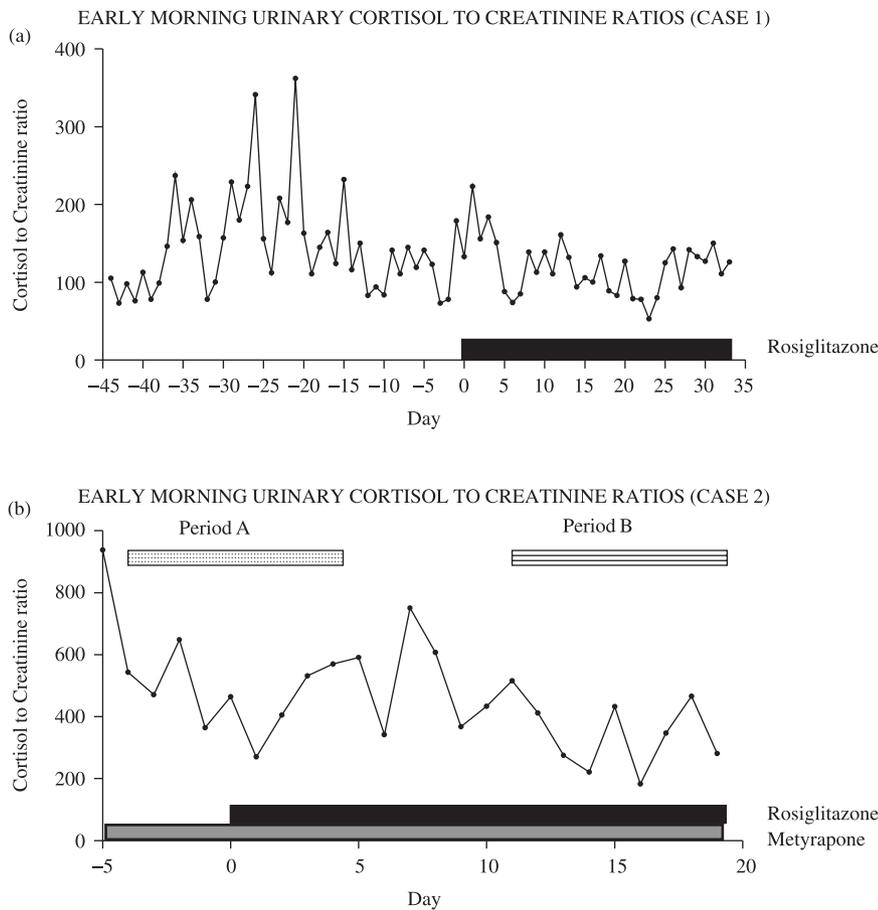


Fig. 1 Early morning urinary cortisol-to-creatinine ratios in (a) case 1 and (b) case 2.

The main biochemical assessment was made using early morning cortisol-to-creatinine ratios, which were measured daily prior to and during treatment in both patients. We have previously shown¹¹ these to correlate highly significantly with 24-h urinary free cortisol values and they therefore allow cortisol production to be studied over an extended period. Values are expressed as the ratio of urinary free cortisol (nmol/l) to urinary creatinine (nmol/l), the normal range being < 30. Results are presented as mean and standard deviation, with an unpaired Student's *t*-test used for statistical comparisons.

No side-effects, hypoglycaemia or symptoms of hypoadrenalism occurred in either patient. In case 1 there was no significant improvement in cushingoid features. An MR scan in the final week of rosiglitazone treatment showed no significant change from the pretreatment scan. Mean early morning urinary cortisol-to-creatinine ratio was 120.9 (36.7) in the week before rosiglitazone and 126.0 (19.1) in the final week of treatment, $n = 7$, $P = \text{ns}$ (Fig. 1a). There was a nadir of 97.9 (33.4), $n = 7$, $P = \text{ns}$, during the fourth week of treatment. Mean ACTH and cortisol values on a day curve 2 hourly from 0800 to 1600 were 81.4 (34.1) ng/l and 605.8 (154.7) nmol/l prior to rosiglitazone treatment and 67.6 (26.0) ng/l and 523.0 (170.0) nmol/l in the final week of rosiglitazone, $P = \text{ns}$. Urinary free cortisol was 1581 and 2144 nmol/24 h prior to commencement of rosiglitazone and 1141 and 1099 nmol/24 h during the final week of treatment, a reduction of 60%. In case 2 there was no significant improvement in cushingoid features. A CT scan in the final week of rosiglitazone treatment showed no significant change from the pretreatment scan.

Mean early morning urinary cortisol-to-creatinine ratio decreased significantly from 474.1 (115.2) during 9 days from after the rapid initial fall with metyrapone had taken place (period A) to 348.1 (115.4) in the final 9 days of treatment (period B), $P = 0.03$ (Fig. 1b).

In summary, therefore, in case 1 there was a suggestion of a reduction in 24-h urinary free cortisol and of a smoothing out of the peaks in early morning urinary cortisol to creatinine ratios and in case 2 there was a statistically significant reduction in early morning urinary cortisol-to-creatinine ratio during the final week of rosiglitazone treatment. No significant clinical improvement was seen. This does not exclude the possibility of a clinical response with longer-term treatment.

A possible confounding factor in the results seen in case 2 is the coexisting metyrapone treatment. This was given in a dose of 250 mg four times a day for 5 days prior to commencement of rosiglitazone. The subject was experiencing severe pain in the right hip and mobility problems, with plain radiographs suggesting a combination of osteoarthritis and avascular necrosis of the femoral head. Metyrapone was considered to be clinically indicated to help to relieve these symptoms. Metyrapone is a rapidly effective therapeutic agent in the control of hypercortisolaemia. There is a decrease in serum cortisol as early as 2 h after administration of 750 mg orally¹². There is also an early effect on cortisol excess with regular treatment.¹² Significant reduction in serum cortisol measured 3 hourly over 24 h was seen early after administration of metyrapone 500 mg three times daily in a group including 57 patients with pituitary-dependent Cushing's

syndrome.¹² In our patient we observed a rapid early fall after commencement of metyrapone and, as can be seen from Fig. 1, early morning ratios were thereafter reasonably stable, allowing us to compare statistically the early and late ratios across the administration of rosiglitazone.

We suggest further studies of longer duration and different doses in patients with milder or recurrent disease. The effect of rosiglitazone in Nelson's syndrome would also be of interest. We were only able to use the drug over a short period of time because of the severity of the disease and it should be noted that in diabetes its onset of action is delayed for 2–8 weeks.⁷ In addition, there are no dose-response studies and we had to use the maximum dose licensed for use in diabetes.

In conclusion, this study shows a possible modest effect of rosiglitazone in Cushing's disease. Further carefully designed studies of both dose and duration are required to confirm and further define its possible role.

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Clinical application of rhTSH in differentiated thyroid cancer: the facts and the questions

Since the first clinical study on recombinant human TSH (rhTSH) was published in 1994,¹ more than a hundred publications have appeared about its application. One of the main fields of clinical application is the follow-up of differentiated thyroid cancer, reviewed extensively by Woodmansee and Haugen.²

Although the introduction of rhTSH has offered new perspectives for diagnostic procedures and therapy in differentiated thyroid carcinoma, with positron emission tomography (PET) scanning following rhTSH as an important example, there are still uncertainties about the clinical application in differentiated thyroid cancer. In this letter we add some arguments to the discussion on this issue.

In a recently published meta-analysis about the diagnostic value of thyroglobulin (Tg) measurements,³ it was concluded that the best accuracy of Tg measurement during follow-up is obtained (if treatment includes remnant ablation) during thyroid hormone withdrawal. Although this study may be hampered by the retrospective character of the analysis and the number of Tg methods, it shows that the circumstances for optimal Tg measurement are not yet clear. In addition, the introduction of highly sensitive Tg assays will probably place the need for TSH-stimulated Tg testing in a new perspective. The first illustration has recently been published by an evaluation of a new chemiluminescent immunoassay that showed equally high sensitivity and specificity both during suppressive therapy and after TSH stimulation.⁴

With the development of these methods of earlier Tg detection, another factor must be taken into account: how to deal with patients with low but measurable Tg levels without proven thyroid cancer. This dilemma has been discussed in a recent European consensus paper,⁵ suggesting that every centre should define an institutional cut-off value for Tg levels. When these cut-off levels are not established, the risk of introducing sensitive strategies is that they may lead to an increase in diagnostic procedures and treatments with uncertain effect on recurrence rate or mortality and, as such, lead to increased costs and patient burden.

Thus several comments can be made on the optimal strategy of the application of rhTSH in the follow-up of differentiated thyroid cancer in terms of diagnostic validity, positioning in the light of the development of new, sensitive, Tg assays and the cost-benefit relationship for the patients. Comparison of long-term results in terms of recurrence rate and survival of different follow-up protocols,

together with cost–benefit analyses, is necessary to develop a well-founded protocol for treatment and follow-up in patients with differentiated thyroid cancer.

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