Table 1. Comparison of Quality of life (QoL) scores and measured variables between genotype groups in treated and asymptomatic patients ($n = 173$)

	GHR genotype		
	fl/fl	*d3/fl* or *d3/d3*	*P* value
Measured variables			
Frequency			
On rhGH	72	59	NS
Asymptomatic	23	19	NS
Peak GH @diagnosis (mU/l)			
On rhGH	2·7 (3.4)	2.4 (2.2)	NS
Asymptomatic	1·7 (2.0)	2.0 (1.8)	NS
rhGH Dose (mg) On rhGH	0·52 (0.6)	0.36 (0.2)	NS
Serum IGF-1 @diagnosis (nmol/l)			
On rhGH	13.3 (5)	11.1 (4.1)	NS
Asymptomatic	10.3 (4)	11.4 (5.1)	NS
Serum IGF-1 (nmol/l)			
On rhGH	25.5 (12)	25.5 (11.5)	NS
Asymptomatic	11.4 (5)	12.7 (5.3)	NS
QoL			
AGHDA			
On rhGH	9 (6)	10 (7)	NS
Asymptomatic	5 (4)	5 (5)	NS
HADS-depression			
On rhGH	6 (5)	8 (5)	NS
Asymptomatic	5 (4)	2 (2)	0.02
Measurements			
Height (SDS)			
On rhGH	−0.51 (1.4)	−0.24 (1.2)	NS
Asymptomatic	−0.31 (1.5)	−0.73 (1.3)	NS
BMI (SDS)			
On rhGH	2.1 (1.1)	2.2 (1.0)	NS
Asymptomatic	2.2 (1.2)	2.3 (0.9)	NS
WHR			
On rhGH	0.92 (0.1)	0.93 (0.1)	NS
Asymptomatic	0.98 (0.1)	0.95 (0.1)	NS
% body fat			
On rhGH	34.2 (8.8)	35.7 (10.2)	NS
Asymptomatic	30.9 (11.4)	32.7 (9.4)	NS

Significance $P<0.05$; NS, not significant; AGHDA, Growth Hormone Deficiency Assessment; HADS, Hospital Anxiety and Depression. Data are presented as mean (SD).

rhGH continue to have suboptimal QoL. This has been reported in previous studies showing that the QoL scores initially improve and then plateau after a few years on treatment[5]. In conclusion, we have demonstrated that the deletion of exon 3 in the GHR gene does not influence QoL, energy levels and body composition in GHD adults.

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References

1 Dos Santos, C., Essioux, L., Teinturier, C. *et al.* (2004) common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nature Genetics* **36**, 720–724.

2 Pilotta, A., Mella, P., Filisetti, M. *et al.* (2006) Common polymorphisms of the growth hormone (GH) receptor do not correlate with the growth response to exogenous recombinant human GH in GH-deficient children. *Journal of Clinical Endocrinology and Metabolism* **91**, 1178–1180.

3 Blum, W.F., Machinis, K., Shavrikova, E.P. *et al.* (2006) The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. *Journal of Clinical Endocrinology and Metabolism* **91**, 4171–4174.

4 Leong, K.S., Walker, A.B., Martin, I. *et al.* (2001) An audit of 500 subcutaneous glucagon stimulation tests to assess growth hormone and ACTH secretion in patients with hypothalamic-pituitary disease. *Clinical Endocrinology* **54**, 463–468.

5 Wallymahmed, M.E., Foy, P., Shaw, D. *et al.* (1997) Quality of life, body composition and muscle strength in adult growth hormone deficiency: the influence of growth hormone replacement therapy for up to 3 years. *Clinical Endocrinology* **47**, 439–446.

Effect of rimonabant and metformin on glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 in obese women with polycystic ovary syndrome

Rimonabant, a canabonoid receptor-1 blocker, has been shown to reduce weight, free androgen index (FAI) and insulin resistance in obese patients with polycystic ovary syndrome (PCOS) compared with metformin during a 12-week period.[1] Subsequent treatment with metformin in these subjects for another span of 3 months maintained their weight loss with further improvement in the metabolic and biochemical parameters, compared with 6 months of metformin treatment alone.[2] Metformin's actions appear to be mediated by activation of AMP kinase activity protein kinase.

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) have been

reported to play an important role in not only insulin sensitivity but also energy balance. Recently, exenatide, which shares similar glucoregulatory properties to GLP-1, has been reported to improve both FAI and insulin sensitivity in women with PCOS, through weight PCOS.[3] Therefore, we aimed to determine whether rimonabant may potentially have an effect on the incretin system thereby augmenting its weight reduction effect.

Subjects and methods

A randomized, open-labelled, parallel study of metformin and rimonabant for 12 weeks in 20 patients with PCOS with a body mass index ≥30 kg/m^2 was undertaken followed by an extension arm with the addition of metformin for another span of 12 weeks.[2] All the patients who were on rimonabant were switched over to metformin 500 mg three times daily for 3 months, whereas all the patients who were on metformin were continued on metformin for another span of 3 months.

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenaemia (Ferriman-Gallwey score >8; FAI >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound. Five subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 years prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee.

Clinical and biochemical assessments were performed at baseline, 12 weeks and 24 weeks. Blood samples were processed and analysed. Glucose-dependent insulinotropic polypeptide and GLP-1 were measured using ELISA methods (Linco Research, Missouri, MO, USA) with an intra-assay CV of 7·3% at 4·2 pmol/l and 7% at 28 pmol/l respectively. Data are reported as mean ± SEM.

Statistical analyses were carried out using the paired *t*-test. The biochemical data were normally distributed when tested using the Kolmogorov–Smirnov test. For all analysis, a two-tailed $P \leq 0{\cdot}05$ was considered as indicating statistical significance. Statistical analysis was performed using SPSS for Windows NT, version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

All the 20 subjects completed the study. The compliance was 98% in both groups. There was a significant increase in GIP levels after rimonabant treatment for 3 months (7·78 ± 0·38 *vs.* 21·62 ± 1·96 pmol/l, $P = -0{\cdot}04$) that decreased when switched over to metformin (21·62 ± 1·96 *vs.* 8·94 ± 0·4 pmol/l, $P = -0{\cdot}08$). There were no significant changes in GIP levels either at 3 months (6·88 ± 0·28 *vs.* 6·08 ± 0·2 pmol/l, $P = -0{\cdot}23$) or at 6 months (6·08 ± 0·2 *vs.* 6·22 ± 0·34 pmol/l, $P = -0{\cdot}89$) with metformin (Fig. 1).

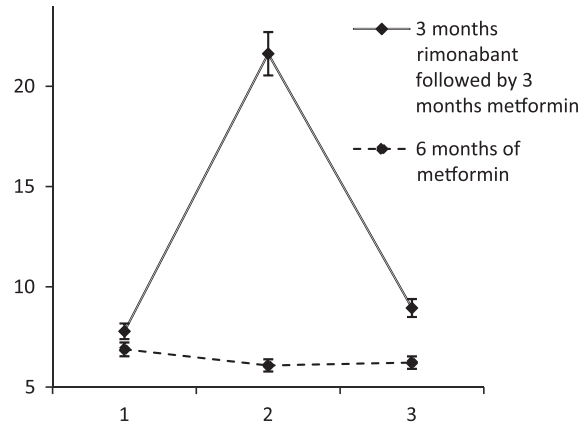

Fig. 1 Glucose-dependent insulinotropic polypeptide levels after 3 months of Rimonabant followed by 3 months of metformin and after 3, 6 months of metformin. *X*-axis – visit 1, visit 2, visit 3. *Y*-axis – Glucose-dependent insulinotropic polypeptide in pmol/l.

There were no significant changes in GLP-1 levels after rimonabant treatment for 3 months (18·6 ± 0·9 *vs.* 21·4 ± 1·2 pmol/l, $P = -0{\cdot}42$) and 6 months (21·4 ± 1·2 *vs.* 21·6 ± 0·9 pmol/l, $P = -0{\cdot}92$) or after metformin treatment at 3 months (22·2 ± 1·5 *vs.* 21·0 ± 1·4 pmol/l, $P = -0{\cdot}72$) and 6 months (21·0 ± 1·4 *vs.* 19·6 ± 1·8 pmol/l, $P = -0{\cdot}54$). There was no significant correlation between the increase in GIP and weight loss with rimonabant ($r = 0{\cdot}12$; $P = 0{\cdot}89$).

Discussion

This study showed a significant (and reversible) increase in GIP levels after 3 months of rimonabant treatment. There were no changes in either GLP-1 or GIP levels with metformin.

The increase in GIP levels could be as a result of rimonabant stimulating incretin hormones rather than secondary to weight loss as there was no correlation between weight loss and increase in GIP levels. However, this latter finding may simply be a consequence of the number of study participants.

Curiously, this study demonstrates that rimonabant affects GIP levels, but not GLP-1 in this group of obese patients with PCOS. GLP-1 is produced by L cells located mainly in the ileum and colon and to a lesser extent by L cells in the duodenum and jejunum, whereas GIP is produced by K cells in the proximal gut.[4] Cannabinoid receptor type 1 (CB1) receptors are also present in the duodenum and jejunum and activation of CB1 receptors depresses gastrointestinal motility by inhibiting contractile transmitter release. Moreover, CB1 receptor activation/agonists inhibit gastric emptying and intestinal transit, delay gastric emptying in humans and rodents and also inhibit gastric acid secretion,[5] functions that precisely mirror those of GIP. In conclusion therefore, it is likely that the increase in GIP by rimonabant in patients with PCOS may contribute to the metabolic changes found with the drug, but that the rise may simply be a compensatory response to maintain gastrointestinal homeostasis.

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References

1 Sathyapalan, T., Cho, L.W., Kilpatrick, E.S. *et al.* (2008) A comparison between rimonabant and metformin in reducing biochemical hyperandrogenaemia and insulin resistance in patients with polycystic ovary syndrome (PCOS): a randomized open-label parallel study. *Clinical Endocrinology*, **69**, 931–935.
2 Sathyapalan, T., Cho, L.W., Kilpatrick, E.S. *et al.* (2009) Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS). *Clinical Endocrinology*, **70**, 124–128.
3 Elkind-Hirsch, K., Marrioneaux, O., Bhushan, M. *et al.* (2008) Comparison of single and combined treatment with exenatide and metformin on menstrual cyclicity in overweight women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **93**, 2670–2678.
4 Drucker, D.J. (2003) Enhancing incretin action for the treatment of type 2 diabetes. *Diabetes Care*, **26**, 2929–2940.
5 Pertwee, R.G. (2001) Cannabinoids and the gastrointestinal tract. *Gut*, **48**, 859–867.

BMP15 and premature ovarian failure: causal mutations, variants, polymorphisms?

Premature ovarian failure (POF) is a frequent pathology leading to female infertility. Clinically, this disorder is characterized by amenorrhoea under 40 years and high plasma gonadotrophins levels. The high incidence of cases considered as idiopathic is mainly due to a poor understanding of the complex interactions between genetic and environmental factors underlying this condition. *BMP15*, as well as its close paralog *GDF9,* have attracted much interest in the field of human reproduction. Since the first report of a pathogenic *BMP15* mutation (p.Tyr235Cys) in a POF case,[1] genetic screenings of large panels of women presenting with non-syndromic POF have revealed further potentially pathogenic variants.[2]

BMP15 and GDF9, as other members of the TGF-β family, are synthesized as precursors, involving a signal peptide, a pro-region and a C-terminal region corresponding to the mature peptide. Post-translational processing includes signal peptide removal, dimerization and a further cleavage in order to release the bioactive mature peptide. Up to now, some *BMP15* mutations located in the propeptide (i.e. p.Tyr235Cys, p.Leu148Pro, p.Arg68Trp, p.Glu211X) have been considered as the best candidates to have pathogenic effects. However, the involvement of some *BMP15* variants in the aetiology of POF is still unclear.[2,3]

A recent publication describes two novel *BMP15* heterozygous mutations (p.Ser5Arg, p.Arg138His) in women with non-syndromic POF as well as four previously known substitutions.[4] This work, using an elegant functional reporter assay of BMP15 activity, helps assess whether these variants are involved in POF or not. Their results provide strong evidence for a functional impact of previously known and novel variants (i.e. p.Leu148Pro, p.Arg68Trp and p.Arg138His). They confirm that BMP15 mutations, involving rather drastic amino acid changes at conserved positions, interfere with normal protein function. This is the case of p.Leu148Pro and p.Arg68Trp that we and others have previously noticed but whose functional effects were to be shown. Interestingly, co-expression of the mutant and wild-type (WT) proteins leads to a decreased bioactivity. This points to a mature-protein concentration insufficiency. However, it is not clear whether this is linked to a dominant negative effect (DNE) or not. Such a possibility would not be unprecedented since BMP15 acts as a dimer, and abnormal dimers containing processed and unprocessed chains have been reported for the mutant p.Tyr235Cys.[1]

Very recently, we have described in *Clinical Endocrinology,* the potentially damaging variant p.Ser5Arg in a POF patient of Tunisian origin,[5] inherited from her mother who had POF at 36 years old. Our *in silico* analyses predicted a quantitative alteration (but not an abolition) of the processing of the signal peptide. In agreement with this prediction, Rossetti *et al.*[4] found a significantly decreased bioactivity of the mutant protein when expressed alone (−15% with respect to the normal protein) even though a decrease in protein processing was not detectable in the Western-blot experiments. However, in their experimental conditions, when BMP15-Ser5Arg was co-expressed with the WT protein the global activity was not different from the WT level. As already said, this was not the case for seemingly more damaging variants such as p.Arg138His, p.Leu148Pro, p.Arg68Trp. This has led the authors to consider p.Ser5Arg as weakly pathogenic (if at all). However, the case of the p.Ser5Arg and of other mutations can be revisited.

Let us first consider the case of p.Leu148Pro, which seems to be one of the worst mutations since the pre-proprotein is hardly processed (according to figure 1 of Rossetti *et al.*[4]). Intriguingly, the virtual absence of mature peptide does not correlate with the retention of 2/3 of the WT activity in the functional assay. This can be explained by assuming that the reporter system, in their experimental conditions, is extremely sensitive to BMP15 and therefore working near saturation. Thus, even a low amount of BMP15-Leu148Pro would be enough to elicit a rather strong signal transduction. In such circumstances, even a DNE induced by this mutation might be overlooked. To confirm or infirm the existence of such a DNE it would be interesting to test this mutation (as well as p.Arg138His, p.Arg68Trp) with the biological assays previously used for the analysis of p.Tyr235Cys.