Molecular screening of the human Melanocortin 4 Receptor (*MC4R*) gene in obese Maltese Type 2 Diabetic patients

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INTRODUCTION

Obesity is a complex trait that arises from the interaction between lifestyle and a number of genetic factors. It is a risk factor for cardio-metabolic diseases, including type 2 diabetes (T2DM). GWAS have identified associations between around 50 individual SNVs and non-syndromic obesity, as defined by the BMI, waist circumference and waist-hip ratio. The first gene shown to have unequivocal association with obesity was *FTO*. Subsequently, investigations into early onset/severe obesity have identified variants in genes acting on the central regulation of appetite. Of particular interest is the melanocortin 4 receptor (MC4R). This is the hypothalamic receptor for melanocyte stimulating hormone, and blockade of this signaling pathway leads to hyperphagia and reduced energy expenditure. A large number of studies have investigated the role of genetic variation in *MC4R*, and mutations in this gene represent the most frequent cause of early-onset non-syndromic obesity.

OBJECTIVES

The aim of this investigation was to investigate the prevalence of *MC4R* genomic variants in a cohort of obese T2DM patients.

METHODS

- We sequenced the *MC4R* exon in 192 overweight or obese T2DM patients of Maltese ethnicity.
- The single 1000bp exon was amplified by PCR using ATC AAT TCA GGG GGA CAC TG and TGC ATG TTC CTA TAT TGC GTG primers.
- The purified amplicon was subsequently sequenced at GATC Biotech, Germany, followed by bioinformatic sequence analysis.

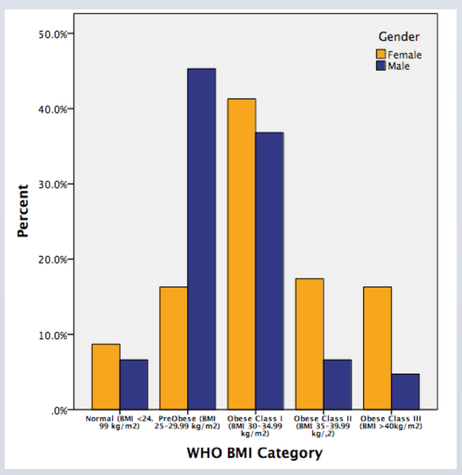
CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

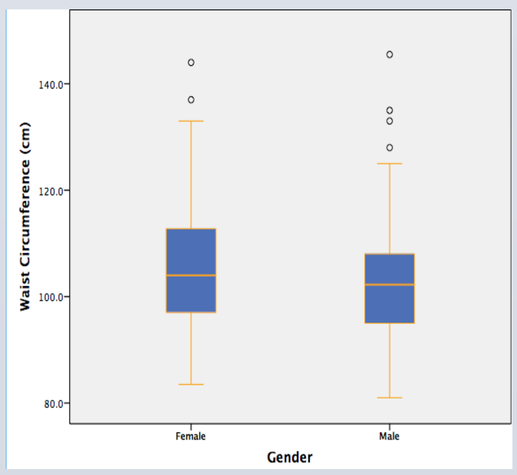
192 unrelated T2DM cases were recruited

91 female and 101 male subjects

Age distribution:
- Females: mean 67 ± 10 years (range 42-96)
- Males: mean 65 ± 11 years (32-88)

BMI distribution:
- Females: mean 33.7 ± 5.9 kg/m^2
- Males: mean 30.4 ± 4.5 kg/m^2





RESULTS 1 – Homozygous SNPs and Insertion-Deletion Variants

Sequence electropherograms were screened for homozygous single nucleotide polymorphisms (SNPs) and insertion-deletion variants (InDels) by aligning the Sanger reads to the reference sequence.

From the best alignment, SNP and InDel candidates were screened, taking into account the quality value of the bases with variation as well as the quality values in the neighboring bases, using neighborhood quality standard. Ssaha2 was used for the alignment and ssaha2SNP was used for detecting polymorphisms.

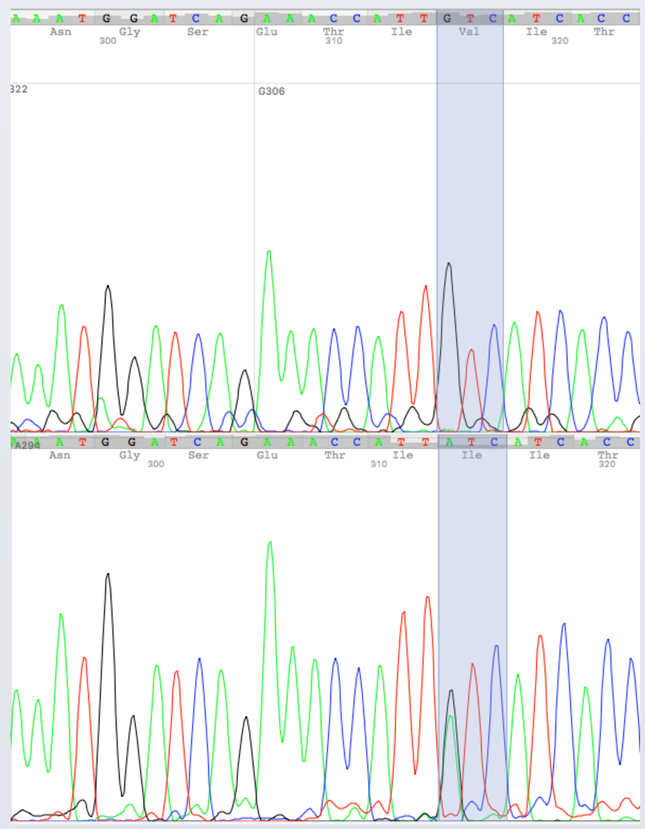
No homozygous SNPs or insertion-deletion variants were detected in the study cohort.

RESULTS 2 – Heterozygous SNPs

Individual sequence electropherograms were inspected to identify heterozygous variants in the *MC4R* exon.

Five cases (3 Males, 2 Females) were identified carrying the missense rs2229616 variant in the heterozygous state.

rs2229616 is a C/T polymorphism that results in a Valine to Isoleucine substitution at codon 103



Right:
Trace electropherograms showing normal sequence flanking *MC4R* codon 103 (top) and the heterozygous rs2229616 missense variant (Val103Ile) (bottom) identified in 5 patients.

CONCLUSIONS

- The Val103Ile missense variant produces a substitution in the second transmembrane domain in MC4R .
- No significant difference was observed in clinical and biochemical parameters between rs2229616 C/C wild-type and C/T heterozygote patients.
- Two large studies have identified a range of *MC4R* variants in severe forms of early-onset non-syndromic obesity characterized by hyperphagia [1,2].
- In contrast, our data shows that *MC4R* mutations are rare, and do not contribute to adult obesity associated with insulin resistance in T2DM.
- These findings are in keeping with the study by E. Miraglia del Giudice *et al*, who showed that obesity causing mutations in MC4R have a very low prevalence (1/208 patients, 0.5%) in an obese cohort from Southern Italy [3].
- The V103I polymorphism identified in the Maltese cohort is the commonest *MC4R* variant, and has been shown to be negatively associated with obesity in a metaanalysis by Heid *et al* [4].

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