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Analysis of large scale sequencing cohorts does not support the role of variants in *UCP2* as a cause of hyperinsulinaemic hypoglycaemia

Thomas W Laver[1], **Michael N Weedon**[1], **Richard Caswell**[1], **Khalid Hussain**[2], **Sian Ellard**[1], **Sarah E Flanagan**[1]

[1]Institute of Biomedical and Clinical Science, University of Exeter, Exeter, UK

[2]Sidra Medical & Research Center, Doha, Qatar

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Congenital hyperinsulinaemic hypoglycaemia (HH) is a rare disorder where episodes of hypoglycaemia are caused by unregulated insulin secretion. Variants in *UCP2* (NM_003355.2) have been reported to cause HH (González-Barroso, et al., 2008) however, since this publication large scale population data have become available which provide important information on the prevalence of rare variants within the population. One such resource is the Genome Aggregation Database (gnomAD) (Lek, et al., 2016) which provides sequencing data from 138,632 individuals. Datasets such as this have revolutionised our ability to interpret variants, as rarity is an important criterion for pathogenicity – the frequency of a variant in a population not selected for the condition should not exceed the prevalence of the condition.

Three studies have reported *UCP2* variants in 9 HH patients (table 1). The original paper (González-Barroso, et al., 2008) describes variants in two probands, Snider *et al.* identified variants in 2 patients out of 417 HH patients screened (Snider, et al., 2013), and Ferrara *et al.* presented 5 patients with *UCP2* variants in a cohort of 211 patients with HH (Ferrara, et al., 2016). Whilst functional studies on the c.803C>G/p.Ala268Gly variant demonstrated a reduction in *UCP2* protein activity (Vozza, et al., 2014), these findings alone are insufficient to assert a causal role of the variant in the aetiology of this disease.

Corresponding author: Thomas W Laver, University of Exeter Medical School, RILD building Level 3, Royal Devon & Exeter Hospital, Barrack Road, Exeter, EX2 5DW, twl207@exeter.ac.uk.

Potential conflicts of Interest

The authors have no conflicts of interest to disclose.

Contribution statement

KH, SE and SEF recruited the cohort. RC designed the targeted capture assay. TWL and MNW carried out the analysis. TWL, SE and SEF wrote the manuscript. All authors were involved in discussion of the manuscript and gave final approval of the version to be published.

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All variants in the original study were inherited from a parent without congenital HH and although Ferrara *et al.* reported that 4 relatives with a *UCP2* variant had unusual glucose and insulin responses to oral glucose, none of the family members had received a clinical diagnosis of HH prior to the study. In these cases the lack of co-segregation could be attributed to age dependent expressivity or incomplete penetrance, as is seen with some other dominantly acting HH mutations (Glaser, et al., 1998).

We investigated the role of *UCP2* in the aetiology of this disorder by analysing data from the gnomAD database and by sequencing the gene in a cohort of 206 individuals with HH by targeted next generation sequencing (Ellard, et al., 2013). Informed consent was obtained from the probands or their parents/guardians and the study was approved by the North Wales Research Ethics Committee, UK.

Whilst no protein truncating variants in *UCP2* were found in our HH cohort, a single missense change (c.836G>A/p.Arg279His – see Supp. Table S1 for *in silico* predictions) was identified in one patient (1/206 (0.5%)). The frequency of rare *UCP2* missense variants was not enriched in patients with HH in our cohort compared to their frequency in gnomAD (OR 0.4, 95% CI 0.009 – 2, P=0.5). The patient with a *UCP2* missense variant was of Indian ethnicity and as the variant is present at 1 in 1274 in the gnomAD South Asian population (estimated HH prevalence in outbred populations is 1 in 50,000 (Bruining, 1990)), our interpretation is that this is a relatively common polymorphism in the Indian population and not causative of the patient's HH. The likelihood that this is a benign variant is further supported by the presence of the variant in the proband's clinically unaffected mother.

Four of the *UCP2* variants reported as pathogenic in individuals with HH (Ferrara, et al., 2016; González-Barroso, et al., 2008; Snider, et al., 2013) are present at a high frequency in gnomAD – a dataset where individuals with severe paediatric disease have been excluded (see Table 1).These four variants account for eight of the nine patients reported to have HH caused by variants in *UCP2*. Ferrara *et al.* reported five probands with *UCP2* variants; four were of African American ethnicity and had variants that have a frequency of greater than 1 in 2000 in the gnomAD African population. The fifth proband, of Western European ancestry, was heterozygous for the c.816-2A>G/p.? variant which is present in the non-Finnish European gnomAD population at a frequency of 1 in 3824.

The p.Ala268Gly variant reported as causative of HH in three separate studies (Ferrara, et al., 2016; González-Barroso, et al., 2008; Snider, et al., 2013) is present in 227 people in gnomAD, including 188 Africans. If this variant were pathogenic its frequency in the gnomAD African population would equate to an incidence of HH of 1 in 128 which is significantly higher than the known incidence of HH which is 1 in 50,000 in outbred populations (Bruining, 1990)(binomial test, P= $<1x10^{-7}$). Lek *et al.* used ExAC to demonstrate that the presence of a variant at a high frequency in a public database is grounds for re-defining it as non-pathogenic despite supporting functional evidence (Lek, et al., 2016). While there is *in vitro* evidence that the p.Ala268Gly variant causes a reduction in *UCP2* protein function (Ferrara, et al., 2016; Vozza, et al., 2014), this does not mean that it is sufficient to cause a clinical phenotype. We suggest that p.Ala268Gly could be a functional polymorphism and that the role of *UCP2* in the aetiology of HH should be reconsidered.

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In outbred populations estimates of HH range from 1 in 27,000 in Ireland (Glaser, et al., 2000), 1 in 40,000 in Finland (Otonkoski, et al., 1999) to 1 in 50,000 in the Netherlands (Bruining, 1990). While these estimates are likely an underrepresentation of the true prevalence as they likely miss milder cases of HH, all estimates are greatly in excess of the frequency observed for the published variants in *UCP2*. It is possible that variants in *UCP2* could be involved in the aetiology of HH but as low effect risk factors in a similar manner to the role played by the *KCNJ11* c.67G>A/p.E23K variant in type 2 diabetes where it is a known risk factor (OR 1.23) (Gloyn, et al., 2003) despite a frequency of 1 in 1.5 in gnomAD. However, if variants in *UCP2* are acting as low effect risk factors or only cause a very mild phenotype then *UCP2* should still not be screened as part of genetic diagnostic panels as it would not be a monogenic cause of severe HH.

In conclusion, our data does not support the role of variants in *UCP2* as a monogenic cause of HH. We therefore urge caution in the diagnostic testing of *UCP2* until its role in the aetiology of HH is proven.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

***UCP2* (NM_003355.2) variants reported in patients with hyperinsulinaemic hypoglycaemia, and their frequency in the gnomAD database (gnomad.broadinstitute.org).**

Variant	Patients	Published in	gnomAD database		
			Number of individuals n= 138,632	Frequency	Popmax ‡ frequency
p.Ser47Asn	1	Ferrara *et al.*	42	1 in 6592	1 in 616
p.Gly61Ser	3	Ferrara *et al.*, Snider *et al.*	21	1 in 13190	1 in 1601
p.Gly174Asp	1 *	González-Barroso *et al.*	0	0	0
p.Leu175Val	1 *	González-Barroso *et al.*	0	0	0
p.Ala187Asp	1 *	González-Barroso *et al.*	0	0	0
p.Ala268Gly	3	Ferrara *et al.*, González-Barroso *et al.*, Snider *et al.*	227	1 in 1216	1 in 128
p.Arg279His ◊	1	This study ◊	27	1 in 10208	1 in 1274
c.816-2A>G	1	Ferrara *et al.*	33	1 in 8157	1 in 3824

‡ defined by Lek et al 2016 "popmax" is "the highest allele frequency in any one population"

* The 3 variants were all found in the same patient

◊ Variant submitted to LOVD http://databases.lovd.nl/shared/variants/0000163118#00022323