XTREME EVEREST PROJECT GRANT

Project Reference 05/15 [E-mail Ref: MEF/Y]

Project Summary to end December 2009

BACKGROUND

The Xtreme Everest programme of research (later renamed Caudwell Xtreme Everest, CXE) was designed to explore the mechanisms through which human hypoxic adaptation occurs. It was centred around a large, healthy volunteer field study on the approach to (and up to the summit of) Mount Everest. We believe this to be the largest ever programme of field physiology ever performed at altitude. A wide array of studies was performed. Central to this programme of work was not just the description of physiological change, but the investigation of the underlying processes regulating this change. Such investigation cannot be performed solely through observing associated biochemical alterations. A genetic strategy was thus deployed- and this was generously supported by the MEF.

THE CXE GENETICS STRATEGY

All humans share the same 18,000 (or so) genes: it is this common inheritance which defines the species. However, common functional variants exist in all these genes, which determine the response to any given homogeneous environmental stimulus. By associating these genetic variants with differences in such responses, one can infer causality of any given genetic locus with the phenotypic response itself. In gene-environment studies of this sort, one can utilize a number of different methodologies. We proposed to use two:

a. A candidate gene approach. Here, one uses existing scientific knowledge to propose a candidate system thought likely to be fundamental to the physiological (or pathophysiological) response under investigation. A key component of this system is chosen, and its gene (the candidate gene) studied. Common functional variants of that gene (so-called functional polymorphisms) are chosen, and differences in response associated with these.

b. Genome-wide association studies. New technologies have now appeared which have become cost-effective in examining functional variation across the human genome. The Illumina Chip Platform, for instance, offers the chance to interrogate 1.1 million genetic variants, spaced evenly across the whole human genome.

The CXE genetic strategy used *both* these approaches, in a variety of studies. These have been developed over time, and partly due to the generosity of the MEF:

(i) Candidate gene studies , to date, on a common genetic variant of the human angiotensin converting enzyme (ACE) gene. Here, the presence (insertion, I allele) of a 287bp sequence is associated with higher tissue ACE levels than is its absence (Deletion, D variant).

   a. Studies of the likelihood of successful rapid ascent to 5,895m on Kilimanjaro.

   b. Studies of the likelihood of successful ascent to beyond 8000m.

   c. Candidate gene studies related to the Xtreme Everest phenotypes derived on the approaches to, and slopes of, Mt. Everest. During April and May 2007, more than 200 individuals were studied during their trek to Everest Base Camp at 5,300 m. Most were healthy volunteers from the general public. The remainder was comprised of doctors and scientists, 15 of whom continued the studies as they ascended up to 8,000 m. Eight of these investigators reached the summit of Everest and on their descent took the first

measurement of arterial oxygen levels above 8,000 m. A vast array of phenotypes were studied, covering a number of selected themes:

i. Neurocognitive function was assessed before and after the expedition, together with magnetic resonance imaging. Saccadometry was also performed. Taste/ smell changes were also defined and characterized.

ii. Body mass index and body composition changes.

iii. Human physical performance. Central to this work was the conduct of exercise bicycle ergometry / cardiopulmonary exercise testing up to the South Col. Delta-efficiency (a measure of muscle metabolic and mechanical efficiency) was measured, along with oxygen cost, heart rate response, maximal oxygen consumption, and anaerobic threshold. Magnetic resonance spectroscopy before and after the expedition elucidated changes in cardiac metabolism.

iv. Other studies. These included assessment of microvascular flow changes in the sublingual circulation.

RESULTS TO DATE

a. Studies of the likelihood of successful rapid ascent to 5,895m on Kilimanjaro. These have been published (Kalson NS, Thompson J, Davies AJ, Stokes S, Earl MD, Whitehead A, Tyrrell-Marsh I, Frost H, Montgomery H. The effect of angiotensin-converting enzyme genotype on acute mountain sickness and summit success in trekkers attempting the summit of Mt. Kilimanjaro (5,895 m). Eur J Appl Physiol. 2009 Feb;105(3):373-9). In brief, altitude-naïve trekkers were studied. Subjects ascended from 1,860 m to the summit over 4 days (n = 34, 'direct-profile') or 5 days (n = 82, 'slower-profile'). Proportionally more ACE I-allele homozygotes amongst the direct-profile subjects were successful than ID or DD, although the difference was not significant (100% of II subjects, 52% ID and 43% DD, P = 0.09). There was no difference in success amongst subjects on the slower-profile (50% II, 45% ID and 58% DD, P = 0.54). There was a non-significant trend for increasing AMS scores in ID/DD subjects.

b. Studies of the likelihood of successful ascent to beyond 8000m. Results have also been published (Thompson J, Raitt J, Hutchings L, Drenos F, Bjargo E, Loset A, Grocott M, Montgomery H; Caudwell Xtreme Everest Research Group. Angiotensin-converting enzyme genotype and successful ascent to extreme high altitude. High Alt Med Biol. 2007 Winter;8(4):278-85). In brief, 141 mountaineers who had participated in expeditions attempting to climb an 8000-m peak completed a questionnaire and provided a buccal swab for ACE I/D genotyping. ACE genotype was determined in 139 mountaineers. ACE genotype distribution differed significantly between those who had successfully climbed beyond 8000 m and those who had not (p = 0.003), with a relative overrepresentation of the I-allele among the successful group (0.55 vs. 0.36 in successful

vs. unsuccessful, respectively). The I-allele was associated with increased maximum altitudes achieved: 8079 +/- 947 m for DDs, 8107 +/- 653 m for IDs, and 8559 +/- 565 m for IIs ($p = 0.007$). There was no statistical difference in ACE genotype frequency between those who climbed to over 8000 m using supplementary oxygen and those who did not ($p = 0.267$). This study demonstrates an association between the ACE I-allele and successful ascent to over 8000 m.

Together, these data support an association of ACE genotype with high-altitude performance- especially on the highest of peaks.

c. Candidate gene studies related to the Xtreme Everest phenotypes derived on the approaches to, and slopes of, Mt. Everest. To date, we have yet to complete these studies, for good reason:

   (i) It has taken some 36 months to clean all the data, and to load databases and validate these entries.

   (ii) Since the inception of the study, genotyping costs have plummeted. Whereas, in 2006, a single gene variant could be analysed for perhaps $10 per DNA sample, it is now possible to study 1.1 million gene variants (a so-called genome wide association study or GWAS) for some $600. This price is falling fast. However, such analysis brings with it great complexity in the statistical analysis of results.

   For these reasons, a Genetics Strategy Steering Group was established, which took expert advice. This recommended that a series of defined candidate genes be defined for each phenotype, and these analysed. It then recommended a second tier analysis of 'valid' but less likely targets be chosen, and applied to GWAS-derived data. It finally

recommended that a GWAS be performed, with results offering candidates for another study on Everest (planned for 2013).

We thus raised the money for 'metabolic gene variant' analysis- which has studied 56,000 such variants. Analysis of such data are about to begin.

Meanwhile, we have sought to strengthen the validity of any GWAS findings through acquiring other DNA sets. These include mouse DNA across an altitude cline on Hawaii (in progress) and a similar cline-set in Tibet (acquired, with GWAS analysis in progress).

SUMMARY

With the help of the MEF at a crucial moment, we have been able to perform the largest and most detailed series of physiological studies known in field physiology at altitude. We have a validated and extremely rich dataset and, with the unheralded acceleration in genetic technologies, we now have the unique opportunity to dissect the pathways which regulate adaptation to hypoxia at a cellular level. Papers published to data have been largely descriptive, and the genetic papers exploratory in associated datasets. We anticipate genetic analysis of the CXE dataset commencing as soon as chip-funding has been acquired.

We express our sincere gratitude to the MEF or their generosity.