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Molecular evolution of growth hormone and insulin-like growth factor 1 receptors in long-lived, small-bodied mammals

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ABSTRACT

Mammals typically display a robust positive relationship between lifespan and body size. Two groups that deviate markedly from this pattern are bats and African mole-rats, with members of both groups being extremely long-lived given their body size, with the maximum documented lifespan for many species exceeding 20 years. A recent genomics study of the exceptionally long-lived Brandt's bat, *Myotis brandtii* (41 years), suggested that its longevity and small body size may be at least partly attributed to key amino acid substitutions in the transmembrane domains of the receptors of growth hormone (GH) and insulin-like growth factor 1 (IGF1). However, whereas elevated longevity is likely to be common across all 19 bat families, the reported amino acid substitutions were only observed in two closely related bat families. To test the hypothesis that an altered GH/IGF1 axis relates to the longevity of African mole-rats and bats, we compared and analysed the homologous coding gene sequences in genomic and transcriptomic data from 26 bat species, five mole-rats and 38 outgroup species. Phylogenetic analyses of both genes recovered the majority of nodes in the currently accepted species tree with high support. Compared to other clades, such as primates and carnivores, the bats and rodents had longer branch lengths. The single 24 amino acid transmembrane domain of IGF1R was found to be more conserved across mammals compared to that of GHR. Within bats, considerable variation in the transmembrane domain of GHR was found, including a previously unreported deletion in Emballonuridae. The transmembrane domains of rodents were found to be more conserved, with mole-rats lacking uniquely conserved amino acid substitutions. Molecular evolutionary analyses showed that both genes were under purifying selection in bats and mole-rats. Our findings suggest that while the previously documented mutations may confer some additional lifespan to *Myotis* bats, other, as yet unknown, genetic differences are likely to account for the long lifespans observed in many bat and mole-rat species.

1. Introduction

Understanding the genetic basis of ageing and longevity is of exceptional interest. Typically two main sources of information have shed light on this field; first, the manipulation of specific genes in model organisms that can lead to increased lifespan [e.g. as reviewed in (Kenyon, 2010)], and second, attempts to identify the genetic mutations that have led to increased longevity in natural populations [e.g. (Kim et al., 2011)]. Due to their exceptionally long lifespans, high metabolic rates and small body sizes, bats have been proposed as potentially underexploited models for ageing studies [e.g. as reviewed in (Brunet-Rossinni and Austad, 2004; Wilkinson and South, 2002)]. Although little is known about senescence in bats, it appears that they do not undergo the same typical ageing processes as humans (Brunet-Rossinni and Wilkinson, 2009). For example, studies suggest that bats may be able to generate new hair cells within certain regions of the inner ear after birth (Kirkegaard and Jørgensen, 2000), although the functional impact of this on their sensory perception remains unclear. The particular diets of bat species may be either high in fats or sugars, yet bats appear to avoid the associated health implications such as atherosclerosis or hyperglycaemia (Brunet-Rossinni and Austad, 2004; Mqokeli and Downs, 2012; Widmaier et al., 1996) which are frequently seen in ageing human populations.

Hypotheses previously put forward to explain bats' long lives include several relating to hibernation, such as altered metabolism and increased predator avoidance [for review see (Brunet-Rossinni and

Abbreviations: *GH*, growth hormone; *GHR*, growth hormone receptor; *IGF1*, insulin-like growth factor 1; *IGF1R*, insulin-like growth factor 1 receptor; SNP, single nucleotide polymorphism.

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Austad, 2004; Wilkinson and South, 2002)]. However, hibernation alone is unlikely to account for the increased lifespan of bats, since not all bats hibernate and hibernation is associated with increased survival across mammals generally (Turbill et al., 2011). The ability to fly has been proposed as leading to greater predator avoidance in bats and volant birds, and thus may at least partly explain their longevity compared to similarly sized non-volant species; in this case extrinsic mortality is reduced which simultaneously drives an increase in lifespan (Healy et al., 2014). Reductions in extrinsic mortality are expected to result in evolutionary adaptation to enhance survival at later life stages (Williams, 1957).

Currently, little is known regarding the genetic basis behind the increased longevity displayed across the ~1300 currently known species of bat. A recent study by Seim et al. (2013) carried out a genomic analysis of Brandt's bat, *Myotis brandtii*, which holds the longevity record for bats, with one male documented to live for 41 years (Podlutsky et al., 2005). This exceptional longevity, coupled with a small body mass (7 g) implies that this species represents the most extreme mammal species outlier in the proposed lifespan body mass relationship [see Fig. 2 from (Podlutsky et al., 2005)]. Seim et al. (2013) found that members of two bat families (Vespertilionidae and Molossidae) shared unique mutations in the transmembrane domains of two genes thought to play crucial roles in growth and ageing: the growth hormone receptor gene (*GHR*) and insulin-like growth factor 1 receptor gene (*IGF1R*). The protein products of these genes are transmembrane receptors found on the surface of mammalian cells, with a single transmembrane region each. GHR regulates the cellular effects of growth hormone and IGF1R the effects of insulin-like growth factor 1.

Mutations in the genes related to these two hormones and their associated receptors have been linked to several clinical disorders including dwarfism in humans and a long-lived dwarf phenotype in mice (Flurkey et al., 2001; Godowski et al., 1989). Furthermore, genomic evidence from domestic dogs suggests that allelic variation in *IGF1* is responsible for nearly all of the variation in body size found across breeds (Sutter et al., 2007), which in turn is inversely related to breed lifespan (Greer et al., 2007). Additional evidence suggests that a non-synonymous single nucleotide polymorphism (SNP) in *IGF1R* may further contribute to the body size of 'tiny' dog breeds (Hoopes et al., 2012). Single nucleotide polymorphisms in the coding sequence of *IGF1R* in Angus cattle have also been shown to be associated with significant body mass differences in calves (Szewczuk et al., 2013). Such evidence led Seim et al. (2013) to propose that the observed amino acid substitutions in the Vespertilionidae bat transmembrane regions of GHR and IGF1R, together with traits such as hibernation and low reproductive rate, may contribute to the unusually long lifespan of *Myotis* bat species.

On average bats have a maximum recorded lifespan that is 3.5 times longer than expected given their body size (Wilkinson and South, 2002). Since great longevity is a trait shared by most bats, it is interesting that the documented amino acid changes in bat GHR and IGF1R were not found in all bat species examined. In particular, the transmembrane domains of larger-bodied fruit-eating bats from the Phyllostomidae and Pteropodidae families were not found to share the same amino acid substitutions seen in *Myotis* bats (Seim et al., 2013). While there is good evidence that the mutations are conserved across *Myotis* bats and closely related species from the same suborder (Yangochiroptera), it is currently unknown whether other long-lived, small-bodied bat species from the other suborder (Yinpterochiroptera), e.g. *Rhinolophus ferrumequinum* — 30.5 years [references within (Wilkinson and South, 2002)], share these mutations.

In addition to bats, African mole-rats (family Bathyergidae) have also been shown to be long-lived for their body size. This is classically illustrated in the naked mole-rat, *Heterocephalus glaber*, which can achieve a maximum lifespan of 31 years with a body mass of 35 g [The AnAge Database: (Tacutu et al., 2013)]. Naked mole-rats have been cited as an example of a mammal that displays negligible senescence [as reviewed in (Buffenstein, 2008)]. They do not undergo age-related mortality until very late in their lives and breeding females remain fertile and physiologically do not show the typical signs of ageing; for example, decline of vascular system function and increased tumorigenesis (Buffenstein, 2008; Csiszar et al., 2007; Liang et al., 2010). Less is known about the maximum lifespan of many of the other mole-rat species; however, the recorded maximum age of captive *Georychus capensis* is ~5 years (Bennett et al., 2006), while reproductive queens of *Fukomys damarensis* may live >8.5 years (Schmidt et al., 2013). Similarly to bats, reduced extrinsic mortality through predator avoidance has recently been suggested as one possible route to increased longevity in mole-rats, although in this case this is attributed to their fossorial lifestyles (Healy et al., 2014). A high quality genome is available for *H. glaber* (Kim et al., 2011), and through this and related resources, several possible underlying molecular mechanisms underpinning its exceptional lifespan have begun to be documented (Edrey et al., 2012; Kim et al., 2011; Morgan et al., 2013; Yu et al., 2011). However, to date little is known about the molecular evolution of the GH and IGF1 receptors in the naked mole-rat and closely related rodent species.

To gain further insights into the molecular evolution of these two receptors relating to the genetic control of longevity and body size in bats and mole-rats, we performed phylogenetic analysis of sequence data from each clade, combined with outgroup mammal species. We compared overall substitution rates and selection pressures acting on both of these genes in clades of interest compared to other mammals, and also examined the specific amino acid substitutions that have occurred in the transmembrane region of each gene. Finally, we examined levels of parallel sequence evolution across all pair-wise branch comparisons within the tree (excluding tips) for each gene to test for evidence of molecular convergence between mole-rats and bats.

2. Materials and methods

2.1. Species representation and datasets

We surveyed *GHR* and *IGF1R* nucleotide sequences in 75 mammals, including 26 bats and five mole-rats, generating a total of 64 and 49 sequences for *GHR* and *IGF1R*, respectively. Bat and mole-rat sequences were obtained from a range of sources, including 10 published genomes and five transcriptomes, as well as 17 RNA-seq assemblies of short-read Illumina data assembled with the default parameters of Trinity v. 2013-02-25 (Grabherr et al., 2011). We obtained wide representation of bat species from both the Yinpterochiroptera and Yangochiroptera suborders, including non-echolocating Old World fruit bats and laryngeal echolocating species. From the mole-rats, we obtained sequences from five species which display a range of ecologies and diverse social structures. In addition, to increase the taxonomic sampling we obtained sequences for other divergent subterranean mammals for both genes from RNA-seq assemblies of short-read Illumina data from the East African root rat, *Tachyoryctes splendens*, and *IGF1R* from the golden mole, *Amblysomus hottentotus*.

2.2. Identification of homologous sequences

Complete and partial coding sequences were obtained from genomes and transcriptomes using a BLAST approach (Altschul et al., 1997). For genomic datasets, scaffolds putatively containing genes of interest were initially identified with TBLASTX against the query transcript with an e-value cutoff of $1e^{-6}$ and only keeping hits recovered with >75% identity and with the query sequence in the correct reading frame. Identities were then confirmed by best reciprocal BLAST hits with the same parameters as above. Coding sequences were subsequently extracted from the genomic sequences using BL2SEQ with the query coding sequence. In each case multiple queries were used; human coding sequences (Ensembl IDs: ENSG00000112964 and ENSG00000140443) were initially used in all cases. Additionally, sequences from *Pteropus vampyrus* (Ensembl IDs: ENSPVAG00000005609