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Age Matching Animal Models to Humans - Theoretical Considerations

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Abstract: Biomedical animal models predict clinical efficacy with varying degrees of success. An important feature of *in vivo* modeling is matching the age of the animals used in preclinical research to the age of peak incidence for a disease state in humans. However, growth and development are highly variable between mammalian species, and age matching is always based on assumptions about the nature of development. We propose that researchers commonly make the assumption that developmental sequences are highly conserved between mammalian species – an assumption that we argue is often incorrect. We instead argue that development is often a modular process. Consideration of the modular nature of development highlights the difficulty in matching animal ages to human ages in a one-to-one scalar manner. We illustrate this with a discussion of the problem of age matching rodents to humans for neuroprotection experiments, and argue that researchers should pay deliberate attention to the modularity of developmental processes in order to optimally match ages between species in biomedical research.

Keywords: Age matching, animal model, clinical translation, development, modularity.

ANIMAL MODELS, BIOLOGY, AND DRUG DISCOVERY

Animal research has played a critical role in drug discovery, particularly when it has been used as an adjuvant to clinical experimentation [1]. Animal experimentation has been of central importance for the production of background knowledge for clinical investigations and for the optimization of procedures prior to human trial. Less clear, however, is whether mass exploratory research using animal models has been a main driver in medical discovery. In many fields translation from preclinical animal research to efficacious clinical treatment has been minimal. For example, in; neuroprotection [2], pain pharmacology [3], and cancer research. In pain research, opioids and NSAIDS - originally bequests from nature and history - remain by far the most important pain relievers. In cancer research, serendipity has been almost the only source of new treatments since the "war on cancer" was initiated by Richard Nixon in 1971 [4-6]. As for neuroprotection, over a thousand drugs have been found to be effective in animal experiments, but none have worked in humans [2]. Each failure to translate success with animal models into success in treating the human condition represents a considerable cost. Despite these failures there seems to have been minimal reassessment of the fundamental concepts involved in using animals to model human disease.

Nevertheless, in some fields, animal models have proven to be very useful at identifying successful clinical treatments – e.g., antibiotics, antihypertensive drugs, and anti-clotting agents. However, the "hit or miss" nature of animal modelling shows that the suite of animal models available today have been produced in an *ad hoc* manner, often with minimal input from fundamental biological theory. For example, many animal models seem to have been produced in disregard of evolution. True, it is recognised that primates provide better human models than rats, and that rats provide better models than reptiles. But the assumption that a similarity between an animal and humans in a pathophysiological process is enough to establish that the animal is a good model betrays a kind of thinking that is divorced from evolutionary reasoning. In evolutionary theory, similarities between species are of three types: similarity by descent (homology); similarity by convergent adaptive evolution (analogy); and the parallel development of similar adaptive forms from similar ancestral states. To this may now be added a new type of convergence, similarity by human manipulation. This means that functional similarity may be underpinned by divergent biochemical and physiological processes, and also that homologous biochemistries can play different functional roles in different species. These complexities are often ignored in current animal modelling of human disease. Although it is conceivable that some types of evolutionary convergence might be sufficiently strong to model one species with another, these are most likely to be superficial with respect to medical research. We argue that a good animal model will be based on (i.) evolutionary homology or parallel evolution with humans, (ii.) minimal functional divergence from a common ancestral state (i.e., insufficient divergence to invalidate the underlying biochemistry of the animal system as a good model for humans.) In this article we focus on an even more specific issue, the degree to which similarities between species reflect fundamentally similar biological process at a particular stage of development or

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age. We refer to this as the age matching problem. Animals respond to many drugs differently over the course of development and aging. Therefore, valid age matching is critical for the successful use of animal modelling in biomedical research in general and drug discovery in particular.

The Importance of Valid Age Matching for the Success of Animal Models

Many conditions that are a target for drug treatment are age dependent. Both the incidence and severity of pathologies, as well as responsiveness to treatment, change with age. In addition, changes in age often bring different co-morbidities which complicate drug treatment and testing. It is clear then that good animal models must match humans at a similar stage of life as possible in order to be used as successful platforms for clinical translation. Several examples are described below.

Cancer is extremely age dependent. Most cancers increase in incidence with age, though some cancers have a higher incidence during childhood. Therefore, cancers are conditions that are intimately connected with the stage of development that an individual has reached. Childhood cancers usually affect the developing hemopoeitic systems, and cancers of the aged often originate in cells that are involved in maintenance and repair. Age related changes to these systems are important for both the etiology of cancer and for the possibilities for successful treatment. However, xenograft cancer models often take no consideration of the animals age and its relationship to the age of peak incidence in humans [7].

Cardiovascular disease is also extremely age dependent. Hypertension tends to increase with age, along with its consequences. Atherosclerosis, deep vein thrombosis, myocardial infarction, and ischemic stroke all become more common with age, as do co-morbidities such as type 2 diabetes and renal dysfunction. Drugs for treating cardiovascular disease should therefore be tested preferentially in older animals, ideally those with relevant co-morbidities [8]. The development and senescence of the cardiovascular and associated systems is also highly important for the outcome of studies into neuroprotection (aspects of developmental that are important for neuroprotection are discussed in detail below). Other examples where good age matching is clearly required for model validity include pathologies involving the immune system, neurological development, and age related conditions such as neurodegenerative conditions (e.g., Alzheimer's disease) and retinal disorders such as macular degeneration.

Age Matching Decisions from Theories of Biological Development

Given that it is highly important that animal models are appropriately age matched with humans for convincing construct validity, it is not clear how this should be done. Aging consists of pre-natal and post-natal development, a period of reproductive maturity, and senescence – but the trajectory that an organism follows in passing through these stages varies between species depending upon the genetically encoded maturation process of the organism, itself a product of evolution to a particular ecological context. The simplest approach, which could be called *naïve age matching* (or *linear scaling* as it is called in the discussion below) involves compressing the stages of human development into the time span available for a test species (e.g., approximately 2 years for a rat). Another approach involves finding anatomical and/or functional markers for relevant physiological or behavioral systems. In fact, all age matching decisions depend upon the theory of development that a researcher explicitly or (in many cases) implicitly follows. There are many theories of development; arising from classical developmental biology, from the field of "evolution of development" ("evo devo"; a genetical approach to development) [9, 10], and from another approach to the role of development in evolution termed "developmental systems theory" [11]. For the purposes of age matching in biomedical animals models, we present four general theories of development:

1. *Linear scaling*; in this naïve approach to age matching, mammalian development is assumed to be a highly evolutionarily conserved sequence (i.e., is very similar between mammalian species) such that stages of development for a particular species that correspond to a human stage can be found by multiplying the percentage of the average life span for humans at a given stage of development by the average life span of the model species. It seems unlikely that this unrealistic theory has been followed by many researchers.

2. *Nonlinear scaling*: in this theory, it is still assumed that mammalian development follows a relatively consistent sequence of steps, but now it is recognized that the relative duration and speed of each stage varies markedly for each species. One popular theory of development that is of this type is neoteny theory, where one critical difference between related species is the relative duration of the pre-reproductive (i.e., adult) stages of development [12]. For example, humans are considered to be neotenous by comparison with chimpanzees, retaining juvenile features into reproductive maturity [12]. Arguably, nonlinear scaling is one of the default theories that researchers use when trying to age match animals to humans.

3. *Structural modularity*: scaling theories make a kind of intuitive sense, because they are based upon a theory of development based upon branching causation. This is intuitively plausible because development can been seen ultimately as a sequence of binary cell division beginning with the zygote, followed by differentiation and cell death. Indeed some influential accounts of evolution have used such a branching causation model of development [13]. In contrast to this, Williams [14] argued that development consists the timed growth of modules of tissues. Modular development means that not only may some stages of development differ between mammalian species, but also that even the sequence of some of the stages may differ. Indeed, the very concept of a *stage* of development breaks down, with various bodily subsystems developing at different rates. Using this theory of development, age matching does not take place according to matched sequence of developmental stages between humans and the model species, but is focused more narrowly on the state of development of bodily

subsystems considered partly in isolation from the rest of the body. When anatomical markers are the primary criteria used then this could be called structural modularity.

4. *Functional modularity*: a somewhat more complex theory of modular development requires that developmental modules are defined by functional criteria above and beyond structural criteria. In this view, development is not just a process wherein systems progress through immature to mature stages, but one in which functions may shift within bodily systems during development according to the needs of the organism at that particular point. Development – in this view – can be seen as following a set of physiological and ecological priorities, with some functions appearing early (e.g., vascular circulation, which carries out a function which is required early in an organisms development) and some late (e.g., reproductive systems are often the last to become fully developed in many species). A simple example of differing functional priorities between species comes from avian biology; "precocial" species hatch with functioning legs, whereas "altricial" species hatch with rudimentary limbs and develop locomotor functions according to a slower schedule [15].

These four general theories of development all give different answers to the problem of age matching animal models to humans. It is important then that researchers are conscious of which theory that they are explicitly or implicitly following when age matching. To illustrate this, a detailed discussion of an example is given below - age matching rats to humans in cerebral ischemia experiments. This is followed by a discussion of this example in light of the four theories listed above.

Case Study: Age Matching Rats to Humans for Cerebral Ischemia Research

Stroke and other types of cerebral ischemia represent an area of drug development that has failed at the point of clinical translation from apparently successful preclinical animal research [2]. One feature of preclinical research in stroke that has been criticized has been the adequacy of animal models, particularly rodent models [2, 8]. Although much of this discussion has focused on the need to test in aged animals, perinatal asphyxia and stroke are also areas of active research, and we restrict this discussion to the problem of age matching rats to humans in early development

In rodent studies of perinatal hypoxia-ischemia (HI), 7 day old (P7) rats have been conventionally used to model newborn humans. There appears to be a consensus that the brains of P7 rats are morphologically and anatomically the most similar to humans at birth. However, we argue there is no simple or conclusive method to determine at what stage in human development corresponds with a specific age of the rat, and that any decisions in this regard depend heavily upon theoretical considerations of the type discussed above.

The human gestation period is approximately 280 days, whereas the rat gestation period is approximately 22 days, and there are clear differences in the way both species develop. Nevertheless, there have been many attempts to propose equivalent ages between the two species. These ages vary greatly depending on the criteria used to assess age equivalences. Bayer *et al*. [16] used neurogenesis, cellular structure, and ultra-structure formation as markers for relative age for each species. In doing so Bayer *et al.* [16] found that the last cells to migrate and form in both species were the granule cells in the cerebellum, dentate gyrus, and the olfactory bulb, as well as the periglomerular cells in the olfactory bulb. In the human these cells finish migrating and begin to mature around the time of birth, 36-40 weeks after fertilization. In the rat this occurs 16-19 days after birth. Therefore, on these criteria (which closely match with the *structural modularity* theory discussed above) P16-19 rats would appear to model newborn humans. However, in HI research (for example) where the aim is to find neuroprotective interventions these criteria in isolation are insufficient for age matching – also important are the development of the cardiovascular system and endogenous antioxidant systems (which tend to be at an optimal level around the time of actual birth) as well as the particular functional roles of various neurotransmitter systems (see below). Considered from a *functional* standpoint then, it is much harder to find a single age at which rats adequately model all relevant human functions. Indeed, at P16-19 a rat can ascend and descend a wire mesh, rear on two paws without support, avoid a cliff and swim – locomotor functions unavailable to humans until advanced childhood.

It is apparent then that rat age equivalents to humans at birth will differ greatly depending on which histological or behavioral assessments are used [17]. Using a different approach to that used by Bayer and colleagues, Barone *et al.* [18] noted that P21 rats had the same weight of brain tissue in various regions as mature rats. On this criterion P16-19 rats correspond to humans with brains that are close to maturity. Taking yet another approach Wiggans [19] investigated rat age equivalents for humans using myelination as a marker of development, and found that at birth humans are at the highest point of myelination for the peripheral sciatic nerve. At this point myelination of the brain has only just started and myelination of the corpus callosum is yet to start. This pattern of myelination is most similar to P6-P10 rats.

The diversity of age matching results yielded by the different criteria discussed above suggests that development of the brain is highly modular, both in a structural and functional sense. Different aspects of the brain develop at different rates and according to different sets of functional priorities in the rat and human. Consider the example of P26 rats, the human equivalent of which is difficult to assess. Looking at brain weights and behavior these animals are most analogous to the adult human. Using neurogenesis as a marker of age, the stage of human development corresponding to P26 rats appears to be early childhood. When comparing myelination, P26 rats have completed sciatic nerve myelination, have completed much of the myelination of the brain, and are close to the peak of myelination of the corpus collosum, this is most similar to a human 6-18 months after birth. When locomotor functions and the relative complexity of human behavior are considered, along with differences in the size of areas of the cortex, it becomes very difficult to specify the range of human ages that a P26 rat would correspond

to. One approach is to focus on specific aspects of a biomedical process under consideration. For instance, if the aim of an investigation was to study seizure activity, then myelination and brain region size would be two important factors to consider. On this functional consideration, P26 rats would equate to humans between 6 months and 18 years old.

DISCUSSION

Functional Sequences in Development and Age Matching

When attempting to model human cerebral ischemia in early development, it is clear that scaling theories of development are inadequate for age matching rats to humans. Various measurable factors in the brain and associated systems develop according to different schedules in the rat and human, and in a way that cannot be captured even by nonlinear scaling. Therefore, there is no one-to-one relationship between any given rat age and a corresponding human age for these kinds of studies. Indeed it is hard to find any species that closely matches the developmental pattern of humans. In humans there is a pause in development immediately before puberty, and then accelerated growth during puberty. This is in contrast to the smooth and continuous pattern of growth that most mammals follow [20].

The development of the biological systems relevant to cerebral ischemia appears to be strongly modular, and some of these systems undergo functional shifts during development. For example, mechanisms of cell death change with development, with a shift from programmed cell death early in development to necrosis in the mature brain [21]. Finding equivalent points for pharmacological intervention in rats and humans then requires an understanding of the type of cell death targeted [22]. Also, excitatory neurotransmitter functions change during development; over-activation of *N*-methyl-D-aspartate (NMDA) receptors causes excitotoxicity in acute injury [23] but the same receptors have an important role in regulating synaptic plasticity during recovery from injury. NMDA receptors undergo changes in function in the developing brain; in the early stages they facilitate neural plasticity, but with heightened susceptibility to excitotoxicity compared to latter stages [24]. In related fashion, the ability of astrocytes to absorb excitatory neurotransmitters is relatively low in the immature brain [25]. A third example is the even more dramatic functional shift in gamma-aminobutyric acid (GABA) receptors during development, shifting from initially modulating excitatory currents to later modulating inhibitory currents. Early excitatory activity has been hypothesized to be important for neuronal growth and synapse formation [26]. GABA receptors are important targets in stroke research [27]. Fourth, the developing brain varies in sensitivity to increases in reactive oxygen species. At birth the brain is partially protected from hypoxia; adaptive mechanisms help the newborn adapt to the high oxygen environment outside the womb [28]. But generally the developing brain has lower levels of antioxidants than more mature brains [29] .

Shifts in neuronal function during the process of myelination are particularly instructive. From one standpoint unmyelinated neurons are simply neurons on the way to becoming functional neurons following myelination. But from another standpoint, myelination does not only increase speed and fidelity of neuronal transmission but also marks the end of one phase of a particular type of neuronal plasticity. Therefore, myelination can be seen as a shift from one function to another, depending upon the changing needs of the organism during development, with each functional module forming the basis for the emergence of other functional modules in an orchestrated procedure.

CONCLUSION: EVOLUTION AND AGE MATCHING

Bogin [20] argued that even within the primates there is a tendency to think of animals as miniature or imperfect *Homo sapiens*, and that this is due to residual adherence to the long discredited philosophy of the *scala naturae*, in which all species can be placed on a scale of perfection with humans at the apex. Scalar theories of development hark back to this mistaken view of biology, whereas modular theories recognize that biological lineages that are most evolvable have been favored by natural selection, and that modular development is readily evolvable. Bogin [20] further writes "…growth spurts [are] modular and highly evolvable features of ontogeny. Natural selection…can effectively put spurts where (anatomically) and when they are needed to increase fitness…the universal process is modularity and evolvability of growth spurts (or decelerations), and … this process need not produce a uniform pattern between any two species…"

We argue then that careful consideration has to be given to precisely what aspect of human biology an animal model can reasonably be considered to mimic, and whether other aspects of the animals biology are sufficiently similar to those that are important in the human condition to justify strong predictions about clinical translation. One way in which progress could be made is if functional elements of the genome were considered in animal model design [30]. If projects like the Encyclopedia of DNA elements (ENCODE) project included detailed data for animal species used in biomedical research, then animal models could be based more closely on functional as well as genetic similarity when functionally equivalent elements of the genome could be identified. With respect to the arguments developed in this article, we argue that it is important for researchers to move beyond scaling theories of development, and to consider development not only from the standpoint of modularity, but also to think of the functional challenges that the developing organism faces in each species. It is also important to realize that accelerated or decelerated development of any given physiological system does not imply that other systems that may be important for a biomedical model are similarly accelerated or decelerated. Indeed, tradeoffs in the economy of development means that development of various anatomical modules is often competitive and is hence negatively correlated [31]. Considered as sequences of interacting functional priorities, the development of a given species can be seen as following its own particular logic. We argue that this kind of understanding is critical for making rational decisions about age matching, and thus for the construct validity of biomedical animal models.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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