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GM Mice: How the principles of Charles Darwin's theory of evolution can be applied for disease research

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Abstract

Charles Darwin's 19th century proposal of evolution by means of natural selection and the resulting Standard Evolution Theory (SET), which also incorporated Mendel's Laws of Heredity and Weismann's Germplasm Theory, unequivocally enable us to understand how species, including humans, have evolved and developed. Despite this, a new framework, Extended Evolutionary Synthesis[1] (EES), has been put forward. It criticises elements of Darwin's work for being antiquated and inapplicable to new evolutionary discoveries; a call-to-arms to update SET. However, I strongly feel that the principles outlined in the *Origin of Species* still bear great precedence for novel science. In particular I will attempt to show that by using Genetically-modified (GM) mice in robustly designed assays, Darwin's theories can be readily and powerfully applied for medical research into genetics and disease.

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[1] Pigliucci, M., G.B. Evolution: The Extended Synthesis MIT Press, 2010

Producing GM mice

Genes are specific sections of DNA that code for the amino acid sequence in the proteins produced by cells. These proteins play important roles in both pathogenesis and the immune response, causing and preventing human diseases. However, in some instances variant proteins are formed due to inherited DNA mutations. Thus a major emphasis of molecular research is to pinpoint these altered genes and to understand their effect on disease. Using model species amenable to genetic analysis is vital for such work to identify hereditable influences on disease. Mice are extremely preferable model organisms, not only as they can be easily bred in laboratory settings, but also 95%[2] of their protein-coding (exon) genome is similar to our own.

CRISPR

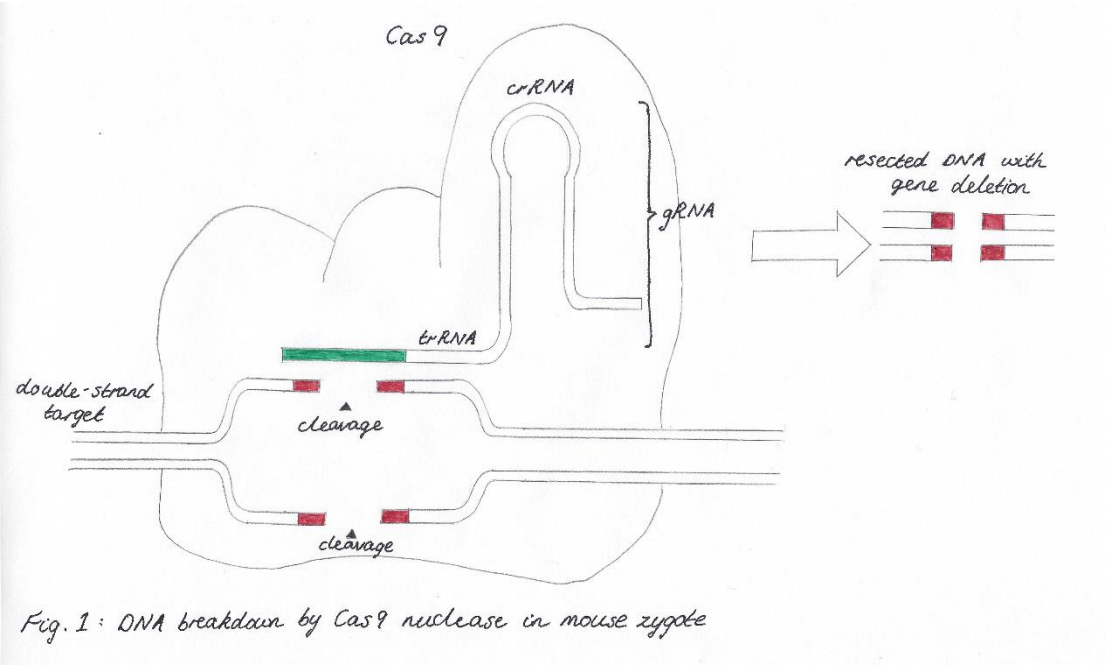
Researching how genetic variation effects human diseases necessitates the ability to readily change the DNA of a model organism such as a mouse. Expensive, cumbersome tools, including RNA interference and zinc-finger nuclei, have previously manipulated mice's DNA composition and gene function. However, in 2012[3] revolutionary and reliable technology to make accurately targeted alterations to living cells' genomes was developed: CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). CRISPR-associated (Cas) genes naturally elicit adaptive immunity in bacteria by eliminating any invasive genetic material. In this mechanism the intrusive viral or plasmid nucleic acid is segmented and placed into a CRIPSR locus on the bacterial cell's genome. Transcription of the loci occurs to form CRISPR RNA (crRNA) and trans-activating (trRNA). These guide an endonuclease enzyme (Cas9) to cleave a specific site on the invading DNA complementary to the RNA's sequence. This causes a double strand break (DSB) in the DNA. Such bacterial systems have been effectively utilised

[2] Batzoglou, S., et al. 2000 Genome Res., 10: 950-958

[3] Jinek, M., et al. 2012 Science, 337, 816-821

for biomedical lab work. A simplified two-component method,[4] where a synthetic guide RNA (gRNA) molecule carries out the role of both crRNA and trRNA, has been successfully implemented during the early development of mice zygotes.[5]

Fig.1[6]



Such technology has proved to be so simple yet powerful, as by just changing the complementary nitrogenous base sequence of the gRNA, Cas9 can home in on a huge range of targets.

DSB repair

DSBs often arise from normal processes in both prokaryotic and eukaryotic cells, particularly due to by-products of cellular metabolism like highly reactive oxygen species. These DNA

[4] Mali, P., et al. 2013 Science, 339, 823-826

[5] Qin, W., 2015 Genetics, 200, 423-430

[6] Loosely based on figure 2 from Reis, A., 2014 NEB Expressions Issue

lesions can cause problematic translocations, deletions or fusions in genetic material. Therefore, cells have checkpoint double strand break repair (DSBR) pathways to prevent mutations during DNA replication in interphase. Although Cas9 can easily remove DNA sections, a useful tool to assess the impact of a gene's absence, research also requires recombinant DNA containing precise additions in nucleotide sequence. To achieve this, lab work can take advantage of and modify natural DSBR systems, especially the Homology Directed Repair (HDR) mechanism. Laboratory HDR has 4 main steps (see figure 2) to achieve nucleotide alterations in genomic DNA:

1. Downstream from the DSB a section of DNA is cut in the 3 prime (3') direction of each antiparallel strand. The resulting 3' overhang is crucial for interaction with other DNA molecules.
2. A repair template containing the new desired base sequence, synthetically produced in vitro, must be present. This invasive component displaces one strand of the original DNA, pairing with the other strand.
3. Smaller fragments of the DNA template join and complete the intermediate molecule, forming a hybrid displacement loop (D-loop).
4. Sites where strands crossover in this hybrid are known as Holliday Junctions (HJ). HJs are resolved by either cuts on the crossing strands (horizontally at green arrows in Fig. 2) or along the non-crossing strands (vertically at purple arrows). Cleavage of both junctions in the same direction produces fully conserved DNA molecules with new modifications.

Fig.2[7]

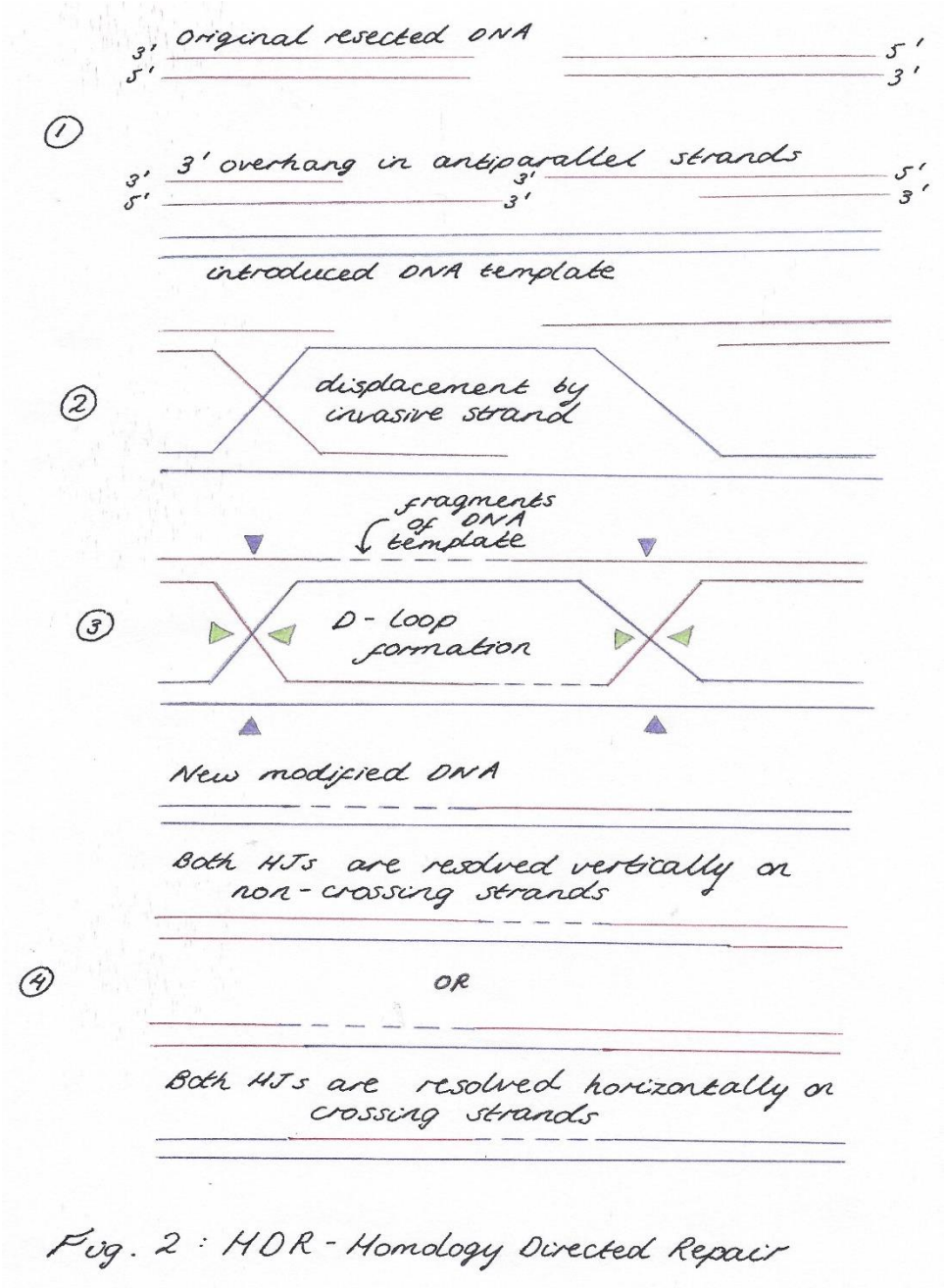


Fig. 2 : HDR - Homology Directed Repair

Over recent years the simplicity and effectiveness of CRISPR gene-editing technology, combining Cas9 and HDR mechanisms, has revolutionised genetic research with GM mice and it has made experimental applications of Darwin's theory of evolution possible.

[7] Simplification of "HR Schematic" by Emw2012, (2009).

Variation under nature and survival - Tuberculosis

"No one supposes that all the individuals of the same species are cast in the very same mould. These individual differences are highly important for us, as they afford materials for natural selection to accumulate."[8]

The *Origin of Species* heavily emphasised the importance of variation in understanding how evolution functions. Illustrating the differences between individuals is key to show that species are not independently created and are not immutable over time.

The presence of great variety between the individuals of a population is a fundamental precondition for the theory of natural selection. In order for an organism in a natural environment to be preferentially chosen over another, based on its advantages and adaptations, they undoubtedly have to vary from each other in a particular aspect, such as physiology, anatomy, behaviour or morphology. Thus, throughout all of Darwin's work, he goes to great lengths to establish the existence of variation. In my opinion he undeniably achieves this by his sound "vera causa" argument, exhaustively listing examples as evidence that variation is a natural phenomenon. The different distribution in nerves close to the central ganglion in insects of the same *Coccus* species, the two distinct forms of the Tanaidacea (a Brazilian crustacean), one has pincers, the other smelling-hairs, trimorphic Malay butterflies and many more examples[9] are used by Darwin to show the magnitude of phenotype variety. Such variation is also presented as an integral part of species development because the progeny of different members of a species inherit varying characteristics. Variation is the method by which a species originated. Species are depicted as the product of a former variant from an entirely separate

[8] Page 59, Chapter II, Variation Under Nature, *Origin of Species*.

[9] Page 60-62, Chapter II, Variation Under Nature, *Origin of Species*

species. This hereditable variation directly links to modern genetics. Random mutations in genes, which, when expressed, cause variations in phenotype, are passed down generations along with environmental and epigenetic changes to the genome, such as methylation.

Darwin embodied the transition in 19th century views: the shift from typological to population thinking. His extensive work into variation in nature overthrew the thesis that all organisms of a species conform to a specific norm (typology). He championed the populationist idea[10] that variation is not an abnormality and that all individuals of a species have distinct physical properties. Population thinking is an essential and crucial basis for all genetic research. Efforts to identify the genes that influence differences in phenotype have been unquestionably driven by Darwin's clear proof of natural variation. In particular, forward genetic[11] techniques have been specifically designed to dissect the genetics of individual disease phenotypes.

The ever-present struggle for existence between organisms, "battle within battle must be continually recurring,"[12] further sets the background for the role of variation in evolution theory. Variation is an on-going process, seeking to advantage a species as it struggles with other competing organisms, predators and the environment. The population size of any species is always trying to increase geometrically, yet life's inevitable competition keeps numbers in a population constant. Darwin himself noted that on a bare, three by two foot patch of ground, 295 of 357[13] new weed seedlings were destroyed over four weeks. This 83% mortality rate, solely due to competition for resources and herbivory, conveys the immensely destructive impact that the struggle for survival has and so emphasises the importance of variation for a species' longevity. Only those species that develop or inherit advantageous variations, benefitting them in changing surroundings, will be able to thrive amongst the competition.

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[10] Mayer, E., Typological versus Population Thinking, 1959

[11] Forward and Reverse Genetics, http://bio.lmu.de/~parsch/evogen/ForRev Gen.pdf , May 2014

[12] Page 87, Chapter III, Struggle for Existence, *Origin of Species*

[13] Page 82, Chapter III, Struggle for Existence, *Origin of Species*

The impact that random, natural variation has on an organism's struggle to survive can be clearly appreciated through dissimilar disease phenotypes within just one species: mice. Individuals differ in their susceptibility to diseases, especially Tuberculosis.

Tuberculosis is a deadly bacterial disease caused by infective droplets of *Mycobacterium tuberculosis* (MTB) that most commonly affects the lungs. (Even Anne Darwin, Charles' beloved daughter, died from tuberculosis in 1851). When an immunocompetent host is infected, MTB in the alveoli is recognised and attacked by macrophages during an immune response, engulfing and surrounding the bacteria in a granuloma barrier. Stable granulomas are a hallmark of latent TB infection (LTBI) because in this condition the bacilli are contained and controlled. However, this status quo can be abolished as TB bacteria hijack leukocytes, multiplying inside immune cells causing them to burst. This erodes the protective granuloma walls and so MTB can infect more pulmonary tissue, causing the characteristic necrotised lesions of blood and sputum in the lungs, as well as attacking other parts of the body (extrapulmonary tuberculosis).

[14]Around one third of the world's population is infected with MTB, yet in 2013 just 1.14 million HIV-negative people died from the disease globally. The sheer prevalence of LTBI in contrast to the comparatively small, but still shocking, number of deaths amongst immunocompetent individuals has suggested that a variant genetic factor could predispose people to TB resistance. Although the TB death rate has dropped by 45% since 1990, there has been very little decrease over the past decade. This alarmingly implies that current modes of treatment and prevention are no longer effective, especially as multidrug-resistant tuberculosis (MDR-TB) is on the rise. Thus novel research with GM mice into hereditable variation in TB resistance could shed much needed light on the disease, for both curing it and understanding even more about the pathogen's infection pathway.

[14] All statistics in this paragraph from WHO Global Tuberculosis Report 2014 unless specified otherwise

As aforementioned, forward-genetics is an invaluable technique to analyse specific genes that cause variability in mice's TB susceptibility. A mouse strain (C3HeB) is extremely susceptible to TB, dying within 28 days[15] of intravenous infection, where as other strains, including the highly resistant C57BL, can survive for up to 40 weeks. To locate potential genes causing such phenotypes these two strains can be subjected to IV injection with MTB and then a whole genome scan using markers to identify DNA activity differences between the two strains. This shows that all mice highly susceptible to TB are homozygous for a variant segment, called the sst1 locus (for supersusceptibilty to tuberculosis), on chromosome 1. The implications here are very important; there seems to be a dominant allele within the sst1 locus that provides resistance against tuberculosis.

Sst1-susceptible ($sst1^S$) mice develop distinctive, characteristic necrotic lesions[16] very similar to MTB's pathogenic attack on lung tissue in humans. However, the lung cells of sst1-resistant ($sst1^R$) mice seem to go through an apoptotic rather than necrotic cell death pathway following TB infection. This can be determined by TUNEL technology,[17] which detects fragmentation and nicks in DNA caused by caspase enzymes involved in apoptosis. Macrophage nuclei of $sst1^R$ mice are TUNEL-positive but $sst1^S$ are not, therefore confirming apoptotic death in just the resistant mice. Being able to control and exploit the method and timing of death of host cells is crucial for successful microbial infections. Necrosis, the unprogrammed death of cells and living tissues by an external agent, is the usual pathway for TB bacteria to destroy macrophages. This facilitates further dissemination of the bacteria as only the host cell's plasma membrane is lysed, rupturing it and releasing its bacterial contents. In contrast the $sst1^R$ genetic variant appears to enable mice to survive post MTB infection via programmed cell

[15] Kramnik, I., Genetic dissection of host resistance to Mycobacterium tuberculosis: the sst1 locus and the Ipr1 gene, PubMed, 2008

[16] Pichugin, A., Dominant Role of the *sst1* Locus in Pathogenesis of Necrotizing Lung Granulomas during Chronic Tuberculosis Infection and Reactivation in Genetically Resistant Hosts, American Journal of Pathology, 2009

[17] Kyrylkova, K., Detection of Apoptosis by TUNEL assay, PubMed, 2012

death, which completely destroys the macrophage and all of the pathogenic material within. This genetic protection against TB is so effective as the apoptotic pathway it sparks, following latent infection, is bactericidal and prevents disease progression.

Some of the principles laid out by Darwin have clearly been applied to develop tuberculosis research. The natural phenotype variation between TB resistance and susceptibility in mice colonies has been taken advantage of to reveal the responsible genetic basis; the sst1 locus. In fact, positional cloning has gone further in identifying an individual sst1-encoded candidate gene known as Ipr1 (intracellular pathogen resistance 1). A human homolog, SP110, to this mouse gene has even been identified as well. Just as C3HeB mice struggle to survive amongst the competition of other TB resistant strains, Darwin-based genetic research has potentially shown that the 0.063% of people with LTBI in 2013 died because they lacked a variant TB resistant gene. With MDR-TB becoming an ever-growing problem, such discoveries have opened new doors and have led to the development of new drugs that mimic the action of Ipr1 by inducing apoptosis of infected body cells. Applying Darwin's theories about natural struggles for life and variation have definitely made the world health target for 2030 (reduce TB deaths by 90%)[18] a possibility.

Variation under domestication – Atherosclerosis

"The key is man's power of accumulative selection."[19]

In the opening chapter of the *Origin of Species* it is made very clear that Darwin did not just theorise that variation is a naturally occurring and unpredictable process but that variation can also be formed within a species in domestic conditions. Variation between individuals is an

[18] WHO, The End TB Strategy, 2015.

[19] Page 22, Chapter I, Variation Under Domestication, *Origin of Species*.

inevitable result from the selective actions of breeders and farmers. It is essentially presented by Darwin as the hereditable development of a particular species due to limitations, stimuli and surroundings enforced by scrupulous breeders. He observed how breeders would increase and augment a desired characteristic in their plant and animal stocks by selective mating of suitable individuals. He challenged the view that domestic creatures had been "suddenly produced as perfect and as useful as we now see them." [19] He instead proposed that breeders' accumulative selection of individuals over many generations has produced the perfect and ideal organisms for a domestic demand. Furthermore, he noticed that this was sometimes a methodical process but that it could also be carried out unconsciously, selectively breeding the best, most athletic dogs without intending to produce a new breed of pointer for example. Darwin's main focus as evidence for variation under domestication was the "astonishing"[20] variety of breeds of domestic pigeon. He marvelled at the fantail's marvellous plumage of 40 tail-feathers or the distinctive coos of the trumpeter and laugher. The very fact that he stuck to the theory that all these marvellous breeds had descended from just one species, the rock-pigeon, via breeders' accumulative selection, despite their striking differences, reflects just how powerful and applicable an idea it was and still is. Darwin's stance on artificial selection greatly ties in with modern approaches; random genetic mutations can cause variations in nature but selective and meticulous breeding of individuals can lead to increased frequency of a desired allele in a domestic environment as well.

In the previous section forward-genetics exploited the principles of natural variation to dissect common variants in disease phenotype into their genetic influences. However, the methodology of variation under domestication can be applied in a slightly different way in a laboratory: reverse-genetic techniques. Here instead the genotype is broken down into the variant phenotype that it causes. Mice are genetically modified as desired, the progeny that inherit the modification are artificially selected for breeding and the resulting phenotype is observed. This is an extremely useful tool for researching the molecular basis of atherosclerosis, where

[20] Page 15, Chapter I, Variation Under Domestication, *Origin of Species*.

domestically induced variation can be taken one step further by even altering the diets of the GM mice.

Atherosclerosis is a complex disease. Cholesterol and other fatty substances carried in low-density lipoproteins (LDL) become deposited on artery endothelia, forming streaky plaques. These eventually build up along with dead smooth muscle and dead white blood cells to produce an atheroma. Blood flow becomes restricted, potentially causing angina if the atheroma forms in the coronary artery, and the artery lining can rupture, enabling a thrombus (clot) to form. This can entirely block the blood vessel or break off into emboli that block smaller vessels elsewhere. These blockages result in ischaemia, as blood supply to particular tissues is reduced, and possibly even death.

A lot of effort has been spent trying to identify genetic factors for atherosclerosis and recently mouse models have elucidated potential molecular and genetic mechanisms. Reverse-genetic techniques involve setting up a desired genotype. However, prior to any alterations to mouse genome, a background needs to be established in the mice that enables the symptoms and characteristics of atherosclerosis to be easily identified; this often incorporates protein knockdown and changes to diet. There are two main types of protein knockdown that tend to be used in atherosclerotic research. The first is LDL receptor knockdown ($Ldlr^{-/-}$)[21] and such mice do not have the primary receptor needed for LDL uptake, thus their plasma LDL/cholesterol levels increase. Secondly, mice can have all apolipoprotein E removed ($Apoe^{-/-}$).[22] Apolipoprotein E is a component of lipoproteins and acts as a highly sensitive ligand for hepatic receptors. Without ApoE a mouse's liver cannot take up cholesterol for synthesis into bile, again increasing plasma cholesterol. Both $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice strongly develop atherosclerosis. The progeny of these mice that also inherit the required protein knockdown are

[21] Henninger, D., Low-Density Lipoprotein Receptor Knockout Mice Exhibit Exaggerated Microvascular Responses to Inflammatory Stimuli, Circulation Research, 1997
[22] Meir, K., Atherosclerosis in the Apolipoprotein E-Deficient Mouse, ATVB Journals, 2004
[23] Pellizzon, M., Brief Scientific Literature Review- Diet- Induced Atherosclerosis/Hypercholesterolemia in Rodent Models, Research Diets Inc. Articles, 2008

selected and bred by researchers, thus producing colonies of mice with the desired atherosclerosis phenotype in exactly the same manner as a breeder would have artificially selected his pigeons. Varying the diet that the mice are fed can enhance these increased atherosclerotic affects even more. The Paigen diet is high in fat (15%)[23] and cholesterol (1.25%) thus, along with the Western-type diet (21% lipid), induces increased atheroma formation in mice. A regular laboratory Chow diet, which contains only a moderate amount of fat, is also occasionally used because protein knockdown is sometimes sufficient in itself to create distinctive and noticeable atherosclerosis without a high-fat diet.

Many genome-wide association studies (GWAS) have been carried out amongst ethnically similar groups of people suffering from cardiovascular diseases. GWAS simultaneously examines many common gene variants within a population, identifying if there is any particular genetic factor associated with atherosclerotic traits. Though generally useful, these tests can be problematic because they return extremely large numbers of possible genes and loci, especially for such a complex and all-encompassing disease as atherosclerosis. However, by striking the right balance between protein knockdown and diet in selectively bred mice, the affects of removing specific GWAS-identified genes can be easily, efficiently and effectively observed using mice models. Several human genes and their mouse homologs that control the development of atherosclerosis have been found via these methods (see figure 3) and most revealing of all is the p21-activated kinase 1 gene (Pak1), which seems to have a plethora of functions in managing atherosclerosis.

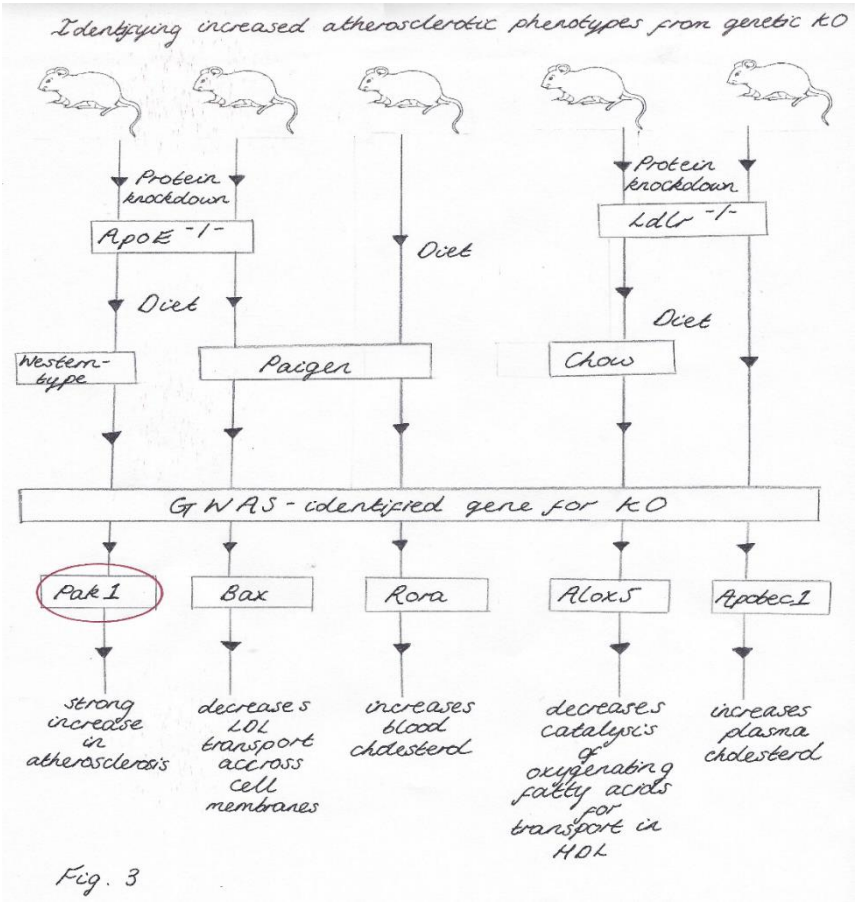


Fig. 3[24]

Using an Apoe$^{-/-}$ and Western-type diet background to easily and rapidly identify any atherosclerotic developments, gene knockout (KO) of Pak1 clearly increases atherogenesis. This has led to the significant discovery that Pak1 has crucial roles in limiting the expression of arterial adhesion molecules, reducing oxidised LDL uptake, restraining cholesterol efflux from dead leukocytes in atheromas and reducing plaque formation. [25]

Just as a farmer chooses the best cows for milk or meat, researchers choose the best mice for analysing the genes involved in atherosclerosis. The mechanisms of variation under domestication as explained by Darwin have evidently been utilised to great effect in finding novel genes such as Pak1. Although atherosclerosis is a very multifactorial disease influenced by lifestyle, age, diet, smoking and much more, understanding its intricate genetic framework

[24] Flow chart utilises data from table 1 in: Stylianou, M., Genetic Basis of Atherosclerosis: Insights from Mice and Humans, Circulation Research, Jan 2012
[25] Singh, N., Disruption of *p21-activated kinase 1* gene diminishes atherosclerosis in apolipoprotein E-deficient mice, Nature, 2014

improves the potential for early diagnosis and future methods of prevention.

Natural selection - Kuru

"This preservation of favourable variations and the rejection of injurious variations, I call natural selection."[26]

The fundamental core of the theory of evolution is natural selection, which is Darwin's amalgamation and conclusion from all his other observations regarding the struggle between different organisms. During his extensive travelling Darwin made three main observations:

- More offspring are produced than required
- Population size remains constant
- Variations in form and physiology are inherited.

From these insights it is clear to conclude that only individuals that inherit beneficial variations will survive, reproduce and thrive. This is natural selection and it clearly shows how species have no immutable essence. The differing phenotypes within a species can often have an advantageous or an injurious impact on individuals. If the variation is beneficial, adapting the organism perfectly to its environment and lending advantage in the competitive struggle to survive, then such an individual will be more likely to develop and reproduce. The resulting offspring will therefore also inherit the advantageous variation; thus it is naturally selected and an entirely new species can even be formed. However, if the variation is adverse or detrimental for the individual's survival then these organisms will be more likely to die and fall out of existence. In this way Darwin powerfully proposed that natural selection is the means by which a variety becomes a whole new species. Furthermore, he observed that natural selection is the acquisition and accumulation of many small hereditable changes over a very long time period,

[26] Page 94, Chapter IV, *Origin of Species*

correlating with the slow rate of change in the world's inhabitants as shown by the geological record.

Natural selection, in which auspicious variations are favoured and disadvantageous ones are rejected, is a very tangible and easily understandable notion. Darwin even provided a very simplistic analogy that I personally feel effortlessly captures the awe-inspiring nature and potential of evolution. His simile describes a great tree. Green, budding leaves represent current species and former leaves are the extinct species. In spring, twigs grow and branch out, trying to outcompete other branches in the same manner as varied species struggle to survive. Only the longest branches gain enough light, and only those with the broadest leaves and most enticing flowers will dominate, just like how individuals with advantageous variations are chosen by nature to thrive. Natural selection's removal of injurious variations from a species is also compared to the twigs that fall down and decay in the muddy soil beneath the tree.

Natural selection can surely promote many different traits, including those associated with disease resistance. This can be put to the test in the case of prion diseases.

Prion diseases, collectively known as transmissible spongiform encephalopathies, are a group of neurodegenerative diseases that invariably result in death and are caused by the misfolding of prion proteins in various regions of the central nervous system. "Transmissible" indicates their infectious nature and "spongiform encephalopathies" refers to the microscopic destruction of the brain whereby brain tissue takes on the appearance of a sponge. The most common prion disease in humans is Creutzfeldt-Jakob Disease (CJD), occurring in different forms. Sporadic or Classic CJD is the most common, arising from unknown causes, and Variant CJD (associated with Mad Cow Disease) can also occur from contact with cow-BSE contaminated food products.

A prion (abbreviation of Proteinaceous Infectious Particle) is a protein of two types, PrP-sen & PrP-res, unique in its ability to replicate on its own. These two protein isoforms differ in secondary & tertiary structure, which plays a crucial part in PrP-res' ability to cause prion

diseases. Normal, healthy neural & glial cells produce PrP-sen, which is believed to be involved in the transport of messages across synapses in the brain. The second type of prion protein, PrP-res, is the disease-causing form. "res" stands for "resistant" as this PrP form is resistant to being broken down due to its non-helical, pleated sheet structure[27] that results from the misfolding of normal PrP protein. (However, the presence of PrP-res does not cause an immune response as it still has the same antigens as normal neural protein). PrP-res differs drastically from other infectious agents as it contains no genetic material yet can still replicate. Evidence supports the hypothesis of "templating," in which contact between the two isoforms stimulates normal cellular prion protein to fold into PrP-res. This results in a chain reaction of infectious prion self-propagation. The unusual shape of PrP-res proteins causes them to clump together, forming beta-amyloid plaques. It is the accumulation of these long-chain amyloid molecules that disrupts & weakens neurons in the brain, which in turn triggers an inflammatory response of astrocytes[28] that break down damaged nerve cells. This results in the characteristic, sponge-like holes where neurones used to be, that are seen during the post-mortem of CJD sufferers. The damage caused to the brain severely afflicts normal neural functioning & ultimately leads to death.

There is a separate form of prion disease known as Kuru, indigenous to Papua New Guinea. Here the Fore tribe carry out endocannibalism, eating the brains of village elders during ritualistic funerals, and they unwittingly and fatally ingest and transmit misfolded prion proteins. At the pinnacle of the Kuru epidemic up to two per cent[29] of villages' population were dying each year. Thus it cannot be surprising to assume that a random, spontaneous mutation in DNA, which elicited resistance to the disease, was naturally selected amongst the Fore. This theory can be evaluated using a GM mice assay.

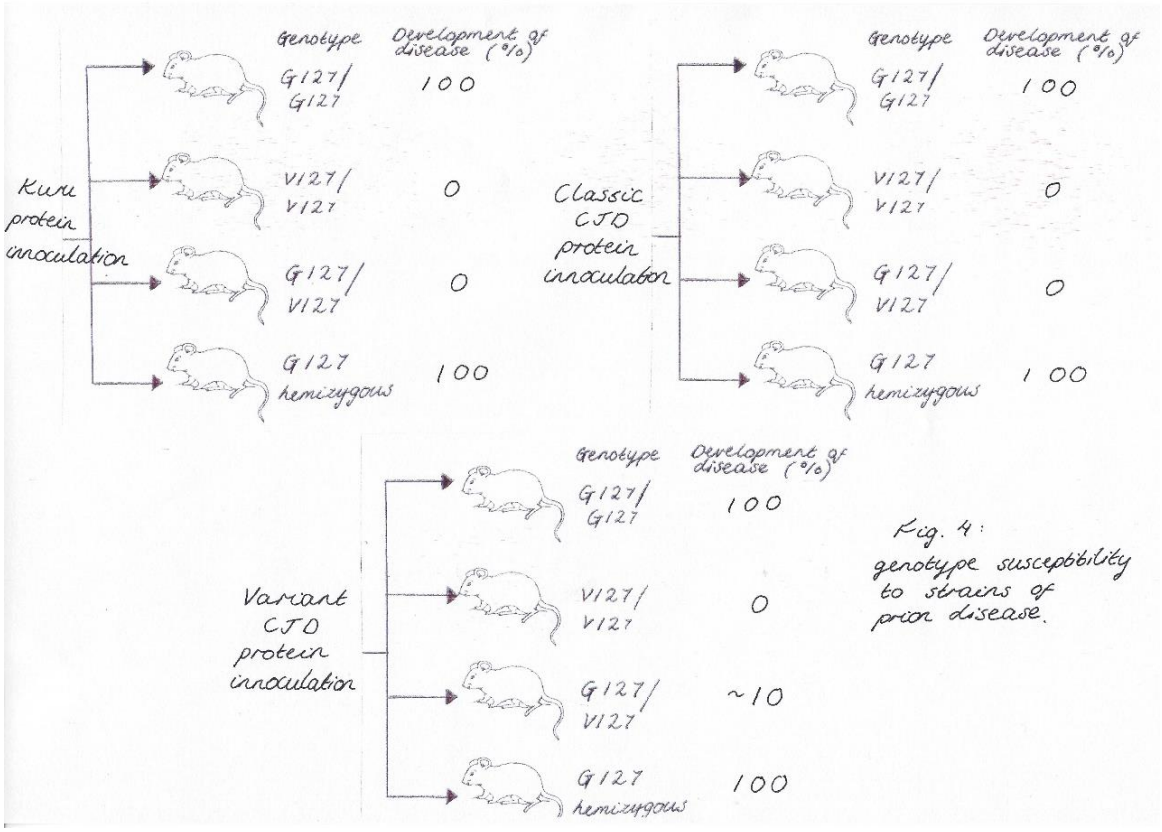
[27] Baker, H., Prion Diseases Hardback, April 1996, Human Press Inc.
[28] Lima, F., Cellular prion protein expression in astrocytes modulates neuronal survival and differentiation, Journal of Neurochemistry, 2007
[29] Alpers, M., The epidemiology of Kuru: monitoring the epidemic from its peak to its end, PubMed, 2008.

Contrasting the genetic makeup of the Fore people with global CJD sufferers reveals that there is a slight difference in the gene that codes for the 127th amino acid residue of prion proteins. This residue can be valine (V) in the Fore but is solely glycine (G) in the rest of the world, and V's presence seems to induce protection. PrP null mice are genetically modified to express different homozygous and heterozygous combinations of G127 and V127 in a chromosome pair (see figure 4). Then they are challenged by inoculation with prion proteins extracted from Kuru, Classic CJD and Variant CJD patients. Thereafter all mice are carefully monitored for signs of prion disease including tremors, rigid tail and ears, loss of coordination, abnormal breathing and hindrance of the righting reflux. Kuru or Classic CJD does not affect any mice that are heterozygous or homozygous for V127, thus this experiment undeniably shows that V127 exhibits dominant inhibition for some prion diseases. Using a hemizygous G127 mouse that does indeed develop the early symptoms of neurodegeneration further proves the dominant characteristic of valine. V127/V127 mice interestingly are not impacted by variant CJD inoculation, which is a novel prion strain that the population of Papua New Guinea would not have been exposed to. This demonstrates the extremely powerful protection that V127 can provide.

The prion variant V127 evidently stops disease and must have been under positive evolutionary selection throughout the outbreak of Kuru. This explicitly illustrates that by understanding the process of natural selection, such knowledge can be applied to make miraculous discoveries in preventing a deadly neurological disease. As CJD is currently an incurable human disease, with prion proteins not even being affected by high-level radiation, the identification of a gene that confers protection holds great potential. Developing our understanding of how exactly V127 prevents pathogenic protein propagation may also enhance therapeutic treatments. The role of V127 has even wider significance because the assembly of misfolded host-protein has been noticed in all major human neurodegenerative diseases.[30] Therefore research into the genetic basis of Kuru using GM mice could represent a step further in curing and preventing infamous conditions like Alzheimer's and Parkinson's.

[30] Soto, C., Unfolding the role of protein misfolding in neurodegenerative diseases, Nature reviews, 2003

Fig.4 [31]



Conclusion

I believe that I have shown that the core principles of Charles Darwin's theory of evolution can unequivocally be applied for genetic research in order to assess the hereditary basis of diseases. Variation, interspecies and intraspecies competition and the mechanisms of natural selection were put forward at a time when the dominant ideology and beliefs stated that all creatures were immutable and that they had been independently created by a great pseudo-deity figure. Darwin's work not only revolutionised 19th century views on evolution but it clearly still has great weight and potential for modern science.

[31] Asante, E., A naturally occurring variant of the human prion protein completely prevents prion disease, Nature, Jan 2015

Throughout this article I have related an aspect of the theories in the *Origin of Species* to its application for identifying novel genes that express resistance to a particular disease, namely tuberculosis, atherosclerosis and prions. I have made it evidently clear that inheriting these variant and mutated genes is very beneficial for an individual's survival. However, a concerning major issue is raised; should human embryos be genetically modified to possess this disease-preventing genome? Gene-editing embryos sounds like a marvellous idea but even the targeted approach of CRISPR technology can cause unplanned, unpredicted and unwanted mutations. This can have extremely serious consequences, as the baby produced might develop physical deformities and other abnormalities, with these grave germline mutations also being inherited by and afflicting many generations. If genetic embryo modifications were freely permitted then this could also lead to the inappropriate and unsafe use of such techniques with hideous outcomes. Thus GM human embryos clearly pass too far over an ethical line. I hope that the main conclusion drawn from my article will not be that I am in overwhelming favour of producing GM human embryos, but that any potential reader will instead agree with me that understanding more about the genetic and molecular basis of various diseases can improve treatment, prevention and effective therapy.

There are even more impressive applications of Darwin's theories, which I have not explored. For example, the principle of plasticity can be utilised to prove that the environment has an effect on cancer. Plasticity is the ability of an organism to mould and adapt itself to its environmental surroundings, an effect which can be clearly observed when mice implanted with glioma cells (a type of spine and brain tumour) are placed in an enriched environment, with lots of stimuli such as climbing ladders and seesaws. The well-stimulated mice develop tumours that are much smaller (23.9% reduction[32]) than mice in a standard environment, thus showing how the idea of plasticity can be deployed to slow the progression of malignant tumours and aid recovering cancer patients.

[32] Garofalo, S., Enriched environment reduces glioma growth through immune and non-immune mechanisms in mice, March 2015, Nature

Overall, an updated EES is not required and contemporary science, especially the spectra of medical genetics, owes a lot to SET and the bold, powerful ideas of Charles Darwin. Darwin himself said, "in the distant future I see open fields for far more important researches,"[33] and I similarly feel that modern medicine is only at the very beginning of a whole new era for evolutionary discoveries.

[33] Page 527, Chapter 14, *Origin of Species*

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