Impact of genetic factors on outcome from brain injury

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Most human phenotypic characteristics are determined by the interplay of environmental factors (whether external, or related to the internal milieu) with the unique genetic attributes of the individual. The same is true for predisposition to and outcome from most disease states, with acute brain injury being no exception. A greater understanding of this interplay is likely to allow improved risk stratification of patients, the development of new preventative and therapeutic modalities, and the possibility of ‘individualizing’ patient management based upon their genetic inheritance.

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Head injury is the most common cause of trauma-related death. Each year, between 0.5 and 1 million people present to Emergency Departments across the UK with traumatic brain injury (TBI), of whom seven in every one hundred thousand will die. A great many more fail to achieve full functional recovery, carrying with them a substantial economic and social burden. Mortality and morbidity related to brain injury also derives from an array of other insults, including the prevalent vascular causes such as cerebrovascular occlusion or subarachnoid haemorrhage. Understanding the physiological responses to brain injury, whether injurious or protective, offers the prospect of developing new therapeutic modalities which may have generic application. One means of exploring such physiological and pathophysiological pathways is through the use of genetic tools.

Humans all share the same 20 000-or-so genes and it is this common inheritance that defines us as a species. However, this genetic inheritance, our DNA, also varies between individuals. Sections vary in ‘copy number’. Alternatively, common variants, called polymorphisms, may exist when small ‘extra lengths’ of DNA may be present (‘insertion’ variants) or removed (‘deletion’ variants), or the genetic code may vary at a single nucleotide ‘code letter’ (the so-called ‘single nucleotide polymorphisms’ or ‘SNPs’). Some variants may alter the function of the gene (‘functional polymorphism’), either by changing the amount of protein it makes in response to a stimulus or by changing the functional characteristics of that protein.

Human phenotypes (‘the way we are’) are determined by the interaction of environmental stimuli with this genetic inheritance. As, an example, angiotensin-converting enzyme (ACE) plays an axial role in the endocrine renin–angiotensin system (RAS): it yields pressor angiotensin II (Ang II) from angiotensin I and degrades vasodilator kinins. However, such systems are also present within a wide variety of cells and tissues including human myocardium. A polymorphic variant of the human ACE gene has been identified in which the absence (deletion, D) rather than the presence (insertion, I) of a 284 bp fragment is associated with raised ACE activity in circulating inflammatory cells and also in the myocardium. When individuals are exposed to exercise training as a uniform myocardial hypertrophic stimulus, the D-allele is found to be associated with a far greater myocardial growth response. Further studies have demonstrated an association of the ACE D-allele (and hence increased myocardial ACE) with impaired outcome in the context of impaired cardiac systolic performance. Studies such as this confirm the impact of genetic factors on both the aetiology and the outcome from diverse disease processes, and offer the prospect of identifying mechanistic pathways and therapeutic targets which might otherwise have remained concealed.

Genetic factors can influence the risk of developing some conditions which lead to brain injury, such as cerebral arterial aneurysms, and also influence the outcome after such injury. However, the identification of these genetic elements is not as easy as it is in other, simpler, phenotypes because a variety of environmental factors may be at work in both disease pathogenesis and outcome determination. Many of these issues are likely to remain unidentified, and even those that are known will vary in both duration and intensity, often in unquantifiable ways. For example, the
magnitude of a blow to a skull may vary, as may the nature of that blow, its location and the nature and extent of associated injuries. The ultimate injury to the brain will also depend on the thickness of the skin, subcutaneous tissue and the skull, and blood vessel friability. These factors, and a great many more, will therefore determine the actual physical extent and location of the cerebral injury. The level of neuronal injury will also be influenced by factors much less readily quantified, such as intracranial pressure and cerebral blood volume, cytokine and inflammatory response, tissue oxygen tension, temperature, drug therapies, and so forth. Each of these factors may interact with a variety of different genetic factors to influence ultimate outcome. The identification of genetic elements of interest is therefore much more difficult in multi-factorial diseases.

To date, the majority of genetic studies in brain injury have been ‘candidate gene association studies’, most often seeking a difference in allele frequency between different groups such as cases vs controls, or ‘worst affected and best affected’. However, care should be taken in the interpretation of such data, as association does not necessarily infer causation of the measure being assessed. For example, an allele may be found with excess frequency among cases not because it is causal for the disease state, but because the alternative allele is associated with early mortality from that disease (the so-called ‘survivor bias’). In addition, unless expression of a specific gene is known to occur solely in cerebral tissue, an allelic association may be attributed to either local or systemic effects, and this cannot be readily inferred. Finally, a genetic variant may be in close proximity to a neighbouring gene with which it is ‘carried’—the two exhibit ‘linkage’. The association of an allele with an effect may therefore be causally attributable to unidentified functional variation in a neighbouring gene, and not to the gene in which the recognized variation lies.

For these reasons, the current literature relating genetic variation to cerebral injury is not extensive, although it is likely to expand enormously in the coming years. This review discusses the current knowledge of genes that are believed to be involved with the development of brain injury and those believed to be associated with outcome after head injury.

**Genes and causation of disease**

The genetic origin of some diseases affecting the brain is well recognized. Examples include the autosomal dominant Huntington’s disease and autosomal recessive Hurler’s syndrome, both of which are related to alterations in genes on chromosome 4. However, evidence is now accumulating that genetic variation can also play a role in the pathogenesis and outcome of other ‘complex’ cerebral disease states.

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**Interleukin-6**

Interleukin (IL)-6 is a pro-inflammatory cytokine, orchestrating the synthesis of acute-phase proteins and mediating chemokine and adhesion molecule release from endothelium. The transcranial IL-6 gradient, as measured from arterial and jugular samples, correlates well with the severity of traumatic brain injury (TBI), and peak plasma IL-6 level with brain infarct volume, stroke severity, and outcome after cerebral ischaemic events. Increasing evidence suggests that IL-6 plays a cerebral neuropathic role, and it is therefore likely that IL-6 levels are not mere markers of the extent of cerebral injury but causative in mediating ongoing damage.

Two common ‘single nucleotide’ polymorphisms exist in the promoter region, where a cytosine (C) may be substituted for a guanine (G) at positions −572 and −174. The −174C and −572C alleles are associated with greater IL-6 synthesis in an inflammatory state.

Increasing evidence suggests that the development of cerebral arterial aneurysms may be partly driven by local inflammation within the vessel wall, to which IL-6 may be a key contributor. Morgan and colleagues investigated the prevalence of the two common functional polymorphisms of the IL-6 gene promoter in 91 Caucasian patients with ruptured aneurysms compared with 2720 controls patients. When compared with controls, cases had a higher C-allele frequency at position −572 and position −174; C-allele homozygotes were also more prevalent among controls than cases. The combination of a −572C allele with a −174G allele was associated with an increased relative risk of aneurysms of 1.89, whereas the −572G/174C combination had a reduced relative risk of 0.58. Such data suggest that IL-6 genotype influences either the development of aneurysms themselves or their predisposition to rupture. However, IL-6 genotype may also influence outcome once rupture has taken place. IL-6 is a potent vasoconstrictor of the canine cerebral artery and raised IL-6 levels are strongly associated with a poor outcome after subarachnoid haemorrhage.

In keeping with such observations, premature birth is associated with a significant risk of neurological impairment in which IL-6 may play a causative role. Since the C-allele at position −174 is associated with greater IL-6 production, Harding and colleagues postulated that neurological outcome in preterm infants born before 32 weeks gestation might be worse in those with CC genotype. One hundred and forty-eight Caucasian preterm infants, with a median gestational age of 31 weeks, were successfully genotyped. When compared with those carrying one or more G-alleles, those with CC genotype had an increased incidence of haemorrhagic brain insults (19% vs 6%), ventriculomegaly or white matter damage (26% vs 7%), and disability (31% vs 13%). In addition, carriage of one or more C-alleles at position −572 was associated with impaired cognitive development.
Meanwhile, and of interest, the IL-6 –174 GG genotype is associated with an increased prevalence of Alzheimer’s disease.16

**Haeme-oxygenase**

Haeme-oxygenase-1 (HO-1) is an anti-inflammatory factor with a polymorphic variant in its gene’s promotor region comprising GT dinucleotide repeats. Long (>36 GT) repeats are associated with reduced HO-1 production. Glial HO-1 expression is up-regulated in response to stimuli such as ischaemia and heat3,45 and may limit brain injury after experimental intracerebral haemorrhage.40 Continuing the hypothesis that inflammation plays a role in aneurysm formation, it would be reasonable to hypothesize also that a genetic predisposition for reduced production of anti-inflammatory factors may in turn predispose to aneurysm formation. In a relatively small study, Morgan and colleagues31 demonstrated that patients who have ruptured aneurysms tend to have longer repeats than a control population. However, as in all such studies, an element of survivor bias cannot be excluded; having longer repeats, and presumably a reduced inflammatory response to aneurysm rupture, might in some way improve early (i.e. pre-hospital) survival and thus entry into the study group.

**ACE genotype**

Tissue RAS (discussed earlier) play diverse roles, including those in the regulation of tissue metabolic, inflammatory, and growth responses. Although Harding and colleagues13 were unable to identify any association between ACE genotype and motor and cognitive development after preterm birth, the ACE DD genotype does appear to be associated with the development of white matter lesions (WMLs) in essential hypertension.15,41 Such effects seem to be mediated, at least in part, through the increased generation of angiotensin II and its action at the angiotensin II type 1 receptor. Polymorphisms in the gene encoding this receptor also appear to be associated with WML development.16

**Genes associated with outcome from brain injury**

**Apolipoprotein E**

The major lipid transport lipoprotein in plasma is apolipoprotein B (APOB) (containing low-density lipoprotein). In cerebrospinal fluid, apolipoprotein E (APOE) performs this role, while also contributing to the maintenance of microtubules within neurones, and to neural transmission itself. APOE is synthesized by astrocytes, combined with cholesterol and phospholipids, and released into the extracellular space. Surface APOE receptors on neurones bind these complexes and internalize them. The cholesterol and phospholipids are then used for cell membrane repair and growth. APOE production from macrophages has been shown to promote nerve regeneration after sciatic nerve transection in rats.5 The genetic regulation of APOE expression has thus been the subject of substantial interest of late.

APOE is a 34 000 Da, 299 amino-acid protein. It is encoded by a single gene (comprising four exons) on chromosome 19, and three allelic variants (ε2, ε3, and ε4) which yield three distinct isoforms (APOE 2, APOE 3, and APOE 4, respectively). Allele frequencies are 7%, 78%, and 15%, respectively, in Caucasians.38 There are therefore three homozygous phenotypes (APOE 2/2, 3/3, and 4/4) and three heterozygous phenotypes (APOE 2/3, 3/4, and 2/4).

The association of the ε4 variant with an increased risk of Alzheimer’s disease in Caucasian populations is now well established.40 However, accumulating evidence also suggests an association with poorer outcome after TBI47,48 and an increased risk of chronic brain injury in boxers.19 There also appears to be an element of synergy between these factors. Mayeux and colleagues27 demonstrated a 10-fold increase in the risk of Alzheimer’s disease in the presence of both APOE 4 and a history of head injury, compared with a two-fold increase with APOE 4 alone.

In general, the bulk of studies are concordant and suggest a detrimental effect of the ε4 variant on outcome after head injury.2,5,9,48 Although Teasdale and colleagues17 failed to demonstrate an overall worse outcome in ε4 carriers when investigating 1094 patients, they did demonstrate an interaction between age and APOE 4 genotype on outcome. Children and young adults bearing at least one ε4 allele had a less favourable outcome with APOE 4 (P=0.007). The inability of some studies to detect this association7 may relate to using gross outcome measures, such as Glasgow Outcome Score, as opposed to finer cognitive and behavioural assessments. Ariza and colleagues carried out fine neuropsychological and neurobehavioural assessments on 77 patients at least 6 months after TBI. Those carrying the ε4 allele had impaired verbal memory, motor speed, fine motor flexibility, visual scanning, attention, and mental flexibility, and considerably more neurobehavioural disturbances than non-ε4 patients.2

Support for an impact of genetic variation in APOE expression on outcome in cerebral disease comes from the recent demonstration that G-219T promoter variation (which influences APOE expression) increases the risk of Alzheimer’s disease20 and worsens outcome after head injury.22

The detrimental impact of the ε4 allele may be mediated through a number of mechanisms. Its presence has been associated with larger intracerebral haematomas,23 worse contusions, and ischaemic brain damage after TBI.43 Meanwhile, APOE 4 drives the deposition of amyloid B protein, recorded in approximately one-third of patients dying shortly after a head injury.34 Indeed, TBI patients have an increased risk of cerebral amyloid angiopathy.21
Interleukin 1 (IL1A, IL1B, and IL1RN)

Until recently, the central nervous system (CNS) has been considered to be immunologically inert. Now there is a great deal of evidence that resident CNS cells produce many types of cytokines in response to injury.\textsuperscript{25} IL-1α, IL-1β, and IL-1RA (receptor antagonist) are cytokines encoded by IL1A, IL1B, and IL1RN genes, respectively, on the long arm of chromosome 2. IL-1 receptor genes are similarly located. IL-1β is produced as an inactive precursor pro-IL-1β which is activated by IL-1β-converting enzyme. When IL-1RA binds to IL-1 receptors, it inhibits the actions of IL-1α and IL-1β. This is a physiological way of regulating the inflammatory response. The IL-1 system has been implicated in a number of acute, chronic, and psychiatric CNS disorders.

A common polymorphism (a C to a T transition at position −889) exists in the gene regulating IL-1α synthesis. The latter allele is associated with the development of juvenile rheumatoid arthritis and Alzheimer’s disease. However, it does not appear to influence outcome after TBI as measured by the Glasgow Outcome Score.\textsuperscript{25} IL-1B polymorphisms are also associated with Alzheimer’s. Polymorphisms at +3953 and −511 have been shown to be significantly associated with outcome in a small study of 69 patients.\textsuperscript{31} Meanwhile, polymorphic variation at the IL1RN gene (consisting of 86 bp tandem repeats) has been shown to correlate with higher incidence of cerebral haemorrhage after TBI.\textsuperscript{11}

Angiotensin-converting enzyme

In a study of 73 subjects, the ACE ‘D’-allele (discussed earlier) has been found to be associated with impaired attention and processing speed after moderate or severe TBI.\textsuperscript{1} There is also evidence from animal studies that ACE inhibition may be neuroprotective against ischaemic insult.\textsuperscript{39}

P53

P53 is a transcription factor that regulates the cell cycle and functions, among other things, as a tumour-suppressor gene. It has important roles in DNA repair and apoptosis. The TP53 gene that codes for P53 is located on chromosome 17 (17p13.1). Martinez-Lucas and colleagues\textsuperscript{26} examined the association of the Arg72Pro polymorphism in the p53 gene with outcome after TBI in 90 Caucasian subjects. Glasgow Outcome Score was recorded at discharge and at 6 months. Argine/argine genotype was significantly more common in those with a poor outcome (69\% vs 31\%), and conferred a 2.9-fold greater risk of a poor outcome at the time of discharge. Although such figures seem very convincing, it is important to remember that p53 may be exerting its influence through physiological mechanisms other than by direct neurological effects.

Dopaminergic pathways

Dopamine D2 receptor allele

McAllister and colleagues have stated that ‘dopaminergic tone’ helps modulate memory, attention, and frontal-executive functions after TBI. They therefore hypothesized that polymorphisms in the dopamine D2 receptor may correlate with cognitive function after TBI. A common polymorphism, the ‘TaqIA’ polymorphism, is a single C/T nucleotide polymorphism 10 kb centromeric to the stop codon of DRD2 on chromosome 11q23. The T-allele is associated with a 40\% reduction in expression of D2 receptors in the striatum without affecting affinity.\textsuperscript{49} In a study of just 39 TBI patients, the T-allele was associated with poorer performance on a California Verbal Learning Test recognition task.\textsuperscript{26}

Catechol-O-methyltransferase

Catechol-O-methyltransferase (COMT) modulates dopaminergic neurones and hence is thought to influence frontal-executive function. A polymorphism in exon 4 of the COMT gene results in a high-activity enzyme (COMT Val) or a low-activity enzyme (COMT Met), respectively. Reduced cortical dopamine (from a more active enzyme) was hypothesized to result in poorer executive functioning. Lipsky and colleagues\textsuperscript{24} studied 113 patients with TBI and found COMT Val homozygotes to give more perseverative responses, that is, more difficulty with cognitive flexibility or shifting mental set.

Future prospects

Understanding how polymorphic variants affect disease development and outcome after brain injury of any aetiology offers a number of potential benefits. First, it may allow risk stratification. If a patient is known to have an increased risk of a disease (such as aneurysm formation) because of a genetic predisposition, they could be advised to avoid other environmental factors that could amplify this risk. Such risk stratification might also be applied to outcome after brain injury, with genetic information complementing that from more conventional sources, such as history, initial GCS, and imaging, in predicting prognosis.

Specific ‘individually targeted’ prophylactic or therapeutic strategies might also be applied. For example, Alzheimer’s patients who lack the APOE 4 allele respond better to cholinesterase inhibitors\textsuperscript{17} and it might well be that genetic variations in those with brain injuries could also influence pharmacological management.

Conclusion

Genetic variation interacts with environmental factors to determine the risk of developing some cerebral pathologies and in determining outcome from them. Studying such genetic elements offers hope for the development of new management and therapeutic strategies.
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