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Innate immunogenetics: a tool for exploring new frontiers of host defence

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The discovery of innate immune genes, such as those encoding Toll-like receptors (TLRs), nucleotide-binding oligomerisation domain-like receptors (NLRs), and related signal-transducing molecules, has led to a substantial improvement of our understanding of innate immunity. Recent immunogenetic studies have associated polymorphisms of the genes encoding TLRs, NLRs, and key signal-transducing molecules, such as interleukin-1 receptor-associated kinase 4 (IRAK4), with increased susceptibility to, or outcome of, infectious diseases. With the availability of high-throughput genotyping techniques, it is becoming increasingly evident that analyses of genetic polymorphisms of innate immune genes will further improve our knowledge of the host antimicrobial defence response and help in identifying individuals who are at increased risk of life-threatening infections. This is likely to open new perspectives for the development of diagnostic, predictive, and preventive management strategies to combat infectious diseases.

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Introduction

Environmental and host factors are important determinants of susceptibility to infection. In recent years, a rapidly growing body of evidence has underscored the importance of host genetic factors. The effect of genetic and environmental factors on the risk of death was assessed in a study of 960 adoptees.[1] Death of a biological parent (but not of an adoptive parent) from infection before the age of 50 years resulted in a six times increase in the relative risk of dying from infection in the adoptee, strongly suggesting that susceptibility to infection aggregates in families. Individuals who are heterozygous for haemoglobin S are known to be protected against malaria, whereas homozygous individuals have sickle-cell anaemia.[2] The high frequency of sickle-cell anaemia and other red blood cell disorders in regions where malaria is highly prevalent suggests that infectious agents (eg, *Plasmodium falciparum*) can exert quite substantial selective pressure on human populations.[3] Although natural immunity ensures survival of the species as a whole, individuals themselves are not likely to be immunocompetent to all pathogens, and individual differences in susceptibility to specific pathogens are quite common.[4] The development of the Human Genome Project in 1990 propelled the scientific community into a new era, allowing genetic mapping and the development of large-scale gene identification that has greatly facilitated the study of gene polymorphisms.

We review recent advances in the field of innate immunogenetics of host defences and show how an interdisciplinary approach of combining genetic epidemiology, genetics, genomics, and molecular and cellular biology will improve our understanding of the pathogenetic basis of infectious diseases, and help the development of new preventive and therapeutic treatment strategies.

Genetic variation and human diseases

Little inter-individual variation exists within the human genome. In fact, all genetic differences between individuals are estimated to be caused by variability in 3 million bp, which represent about 0·01% of the human genome.[5] Since the mutation rate in mammalian genomes is low (10^{-9} per bp per year), most inter-individual variations are inherited. The most frequent variation is the single nucleotide polymorphism (SNP), which occurs on average every 1300 bp. Another type of genetic mutation is the variable number of tandem repeat (VNTR); VNTRs consist of repeats of sequences ranging from a single basepair to thousands of basepairs.[6] The term microsatellite is used for repeats of one to six nucleotides, whereas repeats of longer units are called minisatellites (seven to 100 nucleotides) or, in the extreme case, satellite DNA (more than 100 nucleotides). Since the number of repeats varies among individuals, VNTRs have been widely used as genetic markers.

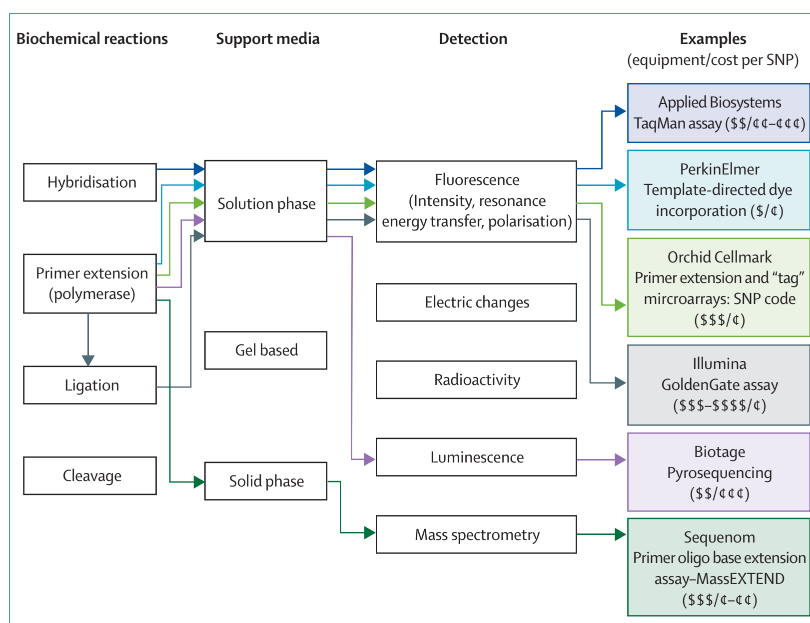


***Figure 1*: Overview of some available genotyping techniques**
The technologies are based on four main principles. The experiments can be run on different support media and different read-out methods can be used to reveal single nucleotide polymorphisms (SNPs). Equipment costs range from a few thousand US$ ($) to over a million US$ ($$$$). Costs per SNP and per sample range from a few cents (¢) to a few dollars (¢¢¢), and tend to be lower for pricey equipments. Cost ranges indicated are purely indicative and may vary. Adapted by permission from Macmillan Publishers Ltd, reference 8, copyright (2001).

Within a coding region of a gene, an SNP can either induce an aminoacid change (non-synonymous SNPs) or not (synonymous SNPs). SNPs may be located in the promoter region of a gene and therefore influence gene expression or splicing. Similarly, different lengths of VNTR regions have been associated with differential gene expression.[7] Certain SNPs or VNTR alleles, or both, may be linked together so that non-functional polymorphisms can be used as genetic markers of functionally important mutations. Only 1·5% of SNPs are thought to be located in a coding region of a gene. The functions of nearly all SNPs that are located outside gene-coding or regulatory regions are unknown.

Genotyping techniques

In recent years, SNP genotyping technologies with high throughput and affordable costs have become available. These technologies are based on a few basic biochemical reactions (hybridisation, PCR with differential primer extension, specific ligation, and differential cleavage), which are used on different support media and can be detected by different methods (figure 1).[8] Recent high-throughput technologies allow genotyping at low cost (ie, a few cents per SNP per sample).[9]

Haplotypes and minimum haplotype tagging SNPs

Once markers have been typed, two main approaches can be used to analyse them: single marker analysis or haplotype analysis. A haplotype refers to the arrangement of two or more alleles on the same chromosome. Currently, there is much debate about which approach is the most appropriate. Studies have proposed that the underlying structure of the human genome can be described by use of a relatively simple framework in which the data are parsed into a series of discrete haplotype blocks.[10,11] This observation has led to the development of haplotype tagging methods that aim to capture the haplotype structure in a candidate region.[11] Haplotype tagging refers to the concept that most of the haplotypic structure in a particular chromosomal region can be captured by genotyping a smaller number of markers than all of those that constitute the haplotypes. The crucial markers to type would be the minimum set of markers that unambiguously identify each possible haplotype.

	Association studies	Linkage studies
Description	Associates a given allele (or set of alleles) with a disease in a population or in families	Determines the approximate chromosomal location of a gene by looking at its cosegregation with markers of known location within families
Participants	Population or families	Families only
Appropriateness for infectious diseases	Usually the most appropriate design	Families most informative for linkage (ie, with multiple affected individuals) may be very difficult and costly to collect
Markers	SNPs, microsatellites	Microsatellites, SNPs
Power to detect a small effect	High	Low
Population stratification bias		
Relevance	Potentially important (debated)	Not an issue
Controlling	Can be controlled for by genotyping a set of unlinked loci or by transmission disequilibrium tests in family-based studies	Not an issue

SNPs=single nucleotide polymorphisms.

Table 1: **Characteristics of association and linkage studies**

Linkage versus association studies

The detection and estimation of familial aggregation is usually the first step in the genetic analysis of a trait. Once familial aggregation has been documented, the traditional approach has been to narrow down the genetic region of interest by use of linkage analysis, followed by fine mapping and association studies (table 1). Linkage and association studies are based on the same underlying principle: once a mutation occurs on a particular chromosome, it is subsequently transmitted to offspring together with nearby loci. This association is broken down at each successive generation by recombination (ie, homologous chromosomes pair during the meiotic cell division and exchange genetic material). When two loci are close enough on the same chromosome that their alleles cosegregate when passed on to the next generation, we say that the two loci are linked.[12] Linkage disequilibrium refers to allelic association that is caused by linkage, or in other words, that has not yet been broken up by recombination.[12] An association between two loci, such as the non-independence of alleles at these loci, may be caused not only by linkage, but also to factors such as population stratification or chance. Population stratification refers to the situation in which study participants are selected from genetically different subpopulations. Population stratification will only lead to a spurious association (and hence be a confounder) if both the allele and disease frequencies differ across subpopulations.[13] Some researchers have argued that too much emphasis has been put on this issue and surprisingly few examples can be found that unequivocally show that population stratification has led to a spurious association.[14,15]

Whereas linkage and association studies can be done in families, only association studies can be done in unrelated cases and controls (table 1). The main difference between related and unrelated cases is the number of meiotic events that separate them, so that unrelated cases share a much shorter chromosomal segment around a particular causative mutation than related cases. Linkage and association can be obscured by incomplete penetrance (ie, there is no one-to-one correspondence between genotype and phenotype), misdiagnoses, genetic heterogeneity (several genes can produce a similar phenotype), phenocopies (ie, environmental factors mimicking the effect of certain genes), and disease

heterogeneity (ie, several subgroups with different genetic causes exist within a specific disease).

An important advance toward enabling efficient whole-genome-scan association studies is the determination of linkage disequilibrium patterns on a genome-wide scale through the HapMap project.[16] Because most diseases are likely to be influenced by several genes and environmental factors, the analysis of gene–gene interactions (epistasis) and gene–environment interactions will represent an important task in the future, but this is, and will remain, a challenging issue for the years to come.

Innate immunity

The innate immune system assumes an essential role in the natural host defences against microbes. The recognition of microbial pathogens, either in tissue in contact with the host's environment or in the systemic circulation after invasion of the bloodstream, is done by macrophages, dendritic cells, natural killer cells, granulocytes, and monocytes, which act as sentinels of the innate immune system (figure 2). This process involves coordinated action of several families of proteins, such as Toll-like receptors (TLRs),[17] nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs),[18,19] RNA helicase-containing proteins,[20] and the C-type lectins.[21]

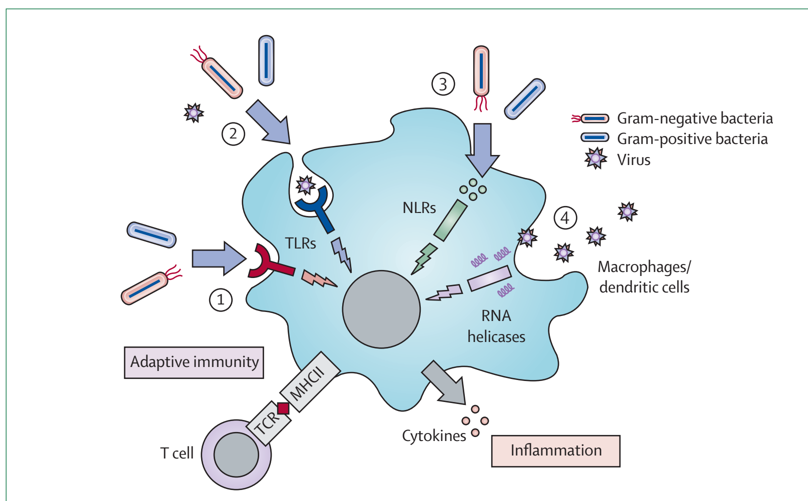


Figure 2: **Recognition of microbial pathogens by the innate immune system**
Microbial-associated molecular patterns are recognised by transmembrane receptors (1: eg, Toll-like receptors [TLRs]), which trigger the activation of several signal-transducing pathways, leading to the production of cytokines and expression of costimulatory molecules. Cytokines induce and regulate the inflammatory response and orchestrate the adaptive immune response. By contrast with other TLRs, TLR3, TLR7, TLR8, and TLR9 are expressed mainly in the endosomal compartment (2), where local acidification is required for recognition of microbial products by their cognate receptors. Intracellular pathogens or microbial products released intracellularly after lysis of ingested microorganisms may also interact with intracytoplasmic receptors, such as nucleotide-binding oligomerisation domain-like (NLR) proteins (3), or the RNA helicase-containing molecules (4: RIG-I or MDA5). TCR=T-cell receptor.

TLRs

TLRs are essential components of the innate immune system.[17,22,23] TLRs are type I transmembrane proteins that function as homodimers or heterodimers. The extracellular domain comprises multiple leucine-rich repeat structures that vary among different TLRs and are implicated in the selective recognition of a vast range of microbial-associated molecular patterns (MAMPs).[24] So far, 12 members of the TLR family have been identified in mammals. Several molecules, including CD14,[25] CD36,[26] and MD2,[25] have also been shown to participate in the sensing of microbial products and are therefore integral components of these receptor complexes. Binding of microbial products to microbial-recognition molecules activates signal transduction pathways and the transcription of immune genes that code for costimulatory molecules expressed at the cell surface or for immunoregulatory effector molecules (including cytokines and chemokines) released in the extracellular milieu that orchestrate the host innate immune defence response.[23,27]

In addition to lipopolysaccharide of Gram-negative bacteria,[28,29] TLR4 detects other MAMPs that are structurally unrelated to lipopolysaccharide, such as mannan (*Candida albicans*) or the fusion protein of respiratory syncytial virus (figure 3). Other endogenous ligands, including fibrinogen, fibronectin, hyaluronic acid, heparin sulphate, beta-defensins, or heat-shock proteins, have been reported to activate TLR4.[17] However, endotoxin contamination has been argued to account for

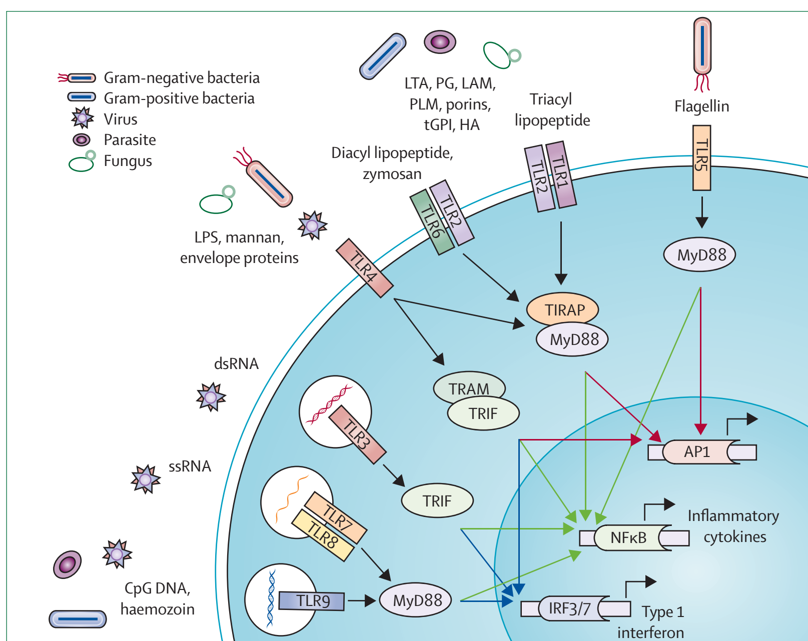


Figure 3: **Toll-like receptors (TLRs), cognate ligands, and the main signalling pathways**
TLR4 detects lipopolysaccharide (LPS), mannan (*Candida albicans*), and the fusion protein of the respiratory syncytial virus. TLR2 forms a heterodimer with either TLR1 to detect triacyl lipopeptide or TLR6 to detect diacyl lipopeptide and zymosan. TLR2 is also involved in the recognition of lipoteichoic acid (LTA), peptidoglycan (PG), lipoarabinomannan (LAM), porins (*Neisseria* spp), glycosylphosphatidylinositol mucin (*Trypanosoma* spp; tGPI), and the haemaglutinnin protein (HA, measles virus). TLR3, TLR7, TLR8, and TLR9 are located in the endosomal compartment and detect nucleic acids and/or haemozoin (*Plasmodium* spp, TLR9). Through their intracellular domain, TLRs interact with specific adaptor proteins, including the myeloid differentiation primary response protein 88 (MyD88), the TIR domain-containing adaptor protein (TIRAP), the TIR domain-containing adapter inducing interferon (TRIF), and the TRIF-related adapter molecule (TRAM). These adaptors lead to the activation of several transcription factors such the nuclear factor κB (NFκB), the activating-protein 1 (AP1), and/or the interferon regulatory factors 3 and 7 (IRF3/7) that ultimately induce the production of pro-inflammatory mediators. ss=single-stranded. ds=double-stranded.

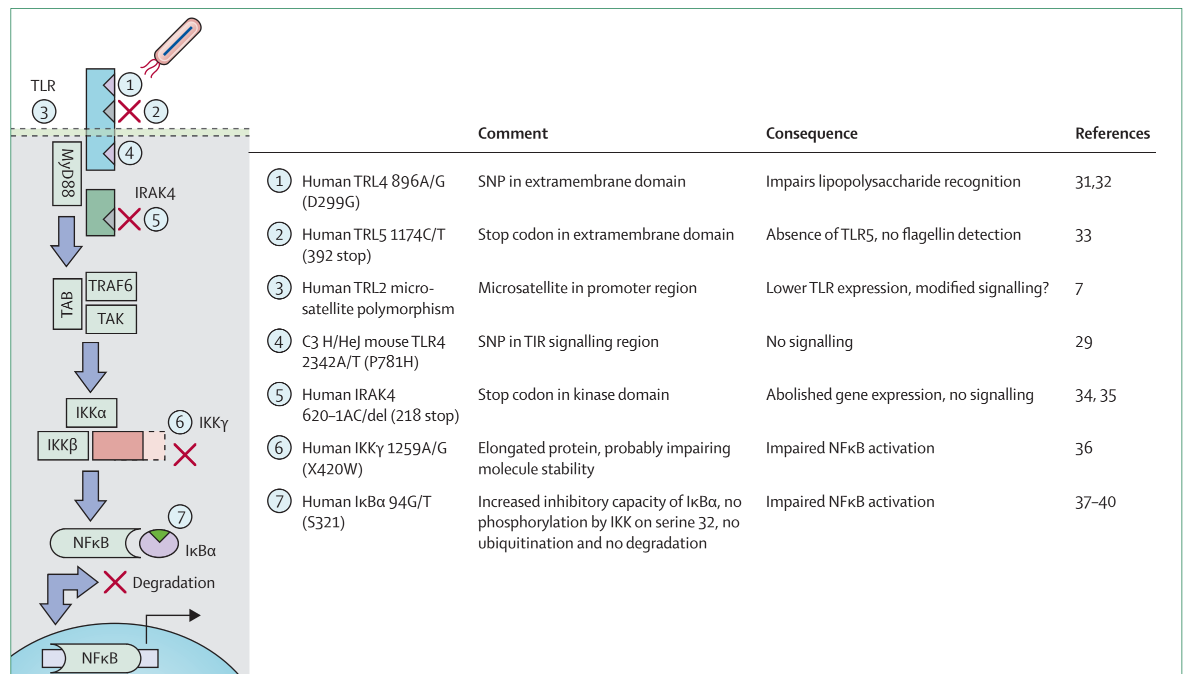
the TLR4 specificity of some of these putative TLR ligands.[30] TLR2 and TLR6 heterodimers detect diacyl lipopeptides, whereas TLR2 and TLR1 heterodimers recognise triacyl lipopeptides.[17] TLR2 has also been proposed to sense lipoteichoic acid, peptidoglygan, lipoarabinomannan, phospholipomannan (*C albicans*), zymosan (*Saccharomyces cerevisiae*), porins (*Neisseria* spp), glycosylphosphatidylinositol mucin (*Trypanosoma* spp), and the haemagluttinin protein of the measles virus.[17] TLR3, TLR7, TLR8, and TLR9, which are mainly expressed in endosomes, serve to detect viral or bacterial nucleic acids. TLR3 detects double-stranded RNA and TLR8 detects single-stranded RNA.[17] TLR9 senses DNA containing the unmethylated CpG motifs found in bacteria and viruses and the malaria pigment haemozoin.[17] Compartmentalisation of TLR3, TLR7, TLR8, and TLR9 thus allows the detection of pathogenic DNA and RNA within the endosomal compartment, while avoiding the detection of self-DNA and mRNA.[23]

On binding of cognate ligands, the intracellular Toll-interleukin-1 receptor (TIR) domain of TLRs recruits and activates different adaptor proteins, including myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adaptor protein, TIR domain-containing adapter-inducing interferon β (TRIF; also known as TICAM), and TRIF-related adapter molecule, ultimately leading to the activation of several specific signal-transducing pathways and transcription factors such as nuclear factor κB (NFκB) and activating protein 1 (AP1; figure 3 and figure 4).[40] MyD88-dependent signalling pathways (NFκB and AP1) are activated by all TLRs, whereas MyD88-independent, TRIF-dependent signalling pathways (interferon regulatory factor [IRF] 3) are activated only by some TLRs (such as TLR3 and TLR4). The observation that different TLRs may activate different signalling pathways with different biological consequences shows that the innate immune system can produce pathogen-specific defensive responses.

NLRs

In addition to the TLRs, the family of proteins comprising NOD proteins and the NALPs (neuronal apoptosis inhibitor [like] proteins), also known collectively as NLRs or NACHT-leucine-rich-repeat-containing proteins, have been shown to have a crucial role in the sensing of microbial products, invasive pathogens, and endogenous host proteins. NLRs are cytosolic proteins composed of three different structural domains, a carboxy-terminal ligand-binding domain consisting of leucine-rich repeats, a nucleotide oligomerisation domain, and an amino-terminal effector domain consisting of various caspase-recruitment domains (CARD), a pyrin domain, or a baculoviral inhibitor-of-apoptosis repeat.[17,41]

NOD1 and NOD2 have been shown to recognise specific bacterial peptidoglycan motifs,[42] and to interact with TLR signalling pathways.[19,42] NOD2 detects muramyl-dipeptide, a peptidoglycan fraction of Gram-positive and Gram-negative bacteria,[43] whereas NOD1 detects



	Comment	Consequence	References
1 Human TRL4 896A/G (D299G)	SNP in extramembrane domain	Impairs lipopolysaccharide recognition	31,32
2 Human TRL5 1174C/T (392 stop)	Stop codon in extramembrane domain	Absence of TLR5, no flagellin detection	33
3 Human TRL2 micro-satellite polymorphism	Microsatellite in promoter region	Lower TLR expression, modified signalling?	7
4 C3 H/HeJ mouse TLR4 2342A/T (P781H)	SNP in TIR signalling region	No signalling	29
5 Human IRAK4 620–1AC/del (218 stop)	Stop codon in kinase domain	Abolished gene expression, no signalling	34, 35
6 Human IKKγ 1259A/G (X420W)	Elongated protein, probably impairing molecule stability	Impaired NFκB activation	36
7 Human IκBα 94G/T (S321)	Increased inhibitory capacity of IκBα, no phosphorylation by IKK on serine 32, no ubiquitination and no degradation	Impaired NFκB activation	37–40

***Figure 4*: Example of genetic variants that impair the host innate immune response**
IκBα=inhibitor of nuclear factor κB (NFκB) α. IKK=inhibitor of NFκB kinase. IRAK4=interleukin-1 receptor-associated kinase 4. MyD88=myeloid differentiation primary response protein 88. TAB=TAB1, TAB2 and TAB3: TAK1-binding proteins 1–3, also known as M3K7-interacting proteins 1–3. TAK=transforming growth factor (TGF)-β-activated kinase 1, also known as mitogen-activated protein (MAP) 3 kinase 7 (M3K7). TIR=Toll-interleukin 1 receptor domain. TLR=Toll-like receptor. TRAF6=tumour necrosis factor-receptor-associated factor 6. SNP=single nucleotide polymorphism.

γ-D-glutamyl-*meso*-diaminopimelic acid, a peptidoglycan fraction found in Gram-negative bacteria and in a few Gram-positive bacteria (*Listeria* and *Bacillus* spp).[44,45] On exposure to microbial products, NODs activate transcription factors, including NFκB and the mitogen-activated protein kinase, and induce the cleavage of pro-interleukin 1β into active interleukin 1β.[46–48] The NALP subfamily of NLR proteins interact with several adaptor molecules, including ASC (apoptosis-associated speck-like protein containing a CARD domain), caspase 1, and caspase 5, and are essential for the activation of interleukin 1β.[49] The NALP-related protein CARD12 (also known as IPAF) is involved in *Salmonella typhimurium*-induced activation of caspase 1.[50] NALP3 is implicated in the detection of ATP,[51] bacterial RNA,[52] and uric acid crystals.[53] However, most NALPs are orphan recognition proteins with no known ligands.

RNA helicases

A series of fascinating articles have provided strong evidence implicating the innate immune system in the host defence against viruses. Two intracytoplasmic molecules have been implicated in the detection of viral RNA. Retinoic-acid-inducible protein I (RIG-I)[54] and melanoma differentiation-associated gene 5 (MDA5)[55–57] possess a CARD domain and RNA helicase domains that function as sensors of double-stranded RNA.[58] RIG-I and MDA5 signal through the adaptor molecule MAVS (mitochondrial antiviral signalling protein; also known as CARDIF or VISA)[18,59,60] and interact with several other signal-transducing molecules, including FADD (tumour necrosis factor receptor superfamily member 6 precursor [TNFRSF6, also know as FAS]-associated death domain protein), RIPK1 (receptor-interacting serine/threonine-protein kinase 1; also known as RIP or RIP1), TBK1 (TRAF family member-associated NF-kappa-B activator [TANK]-binding kinase-1), and IKKE (inhibitor of NFκB kinase subunit epsilon; also known as IKK-*i*).[40] These molecules are involved in the production of type I interferons (interferons α and β) in response to infection by RNA viruses. Therefore, RIG-I and MDA5 are able to detect single-stranded RNA present in the cytoplasmic compartment and thus not accessible to endosomal TLR3. Interestingly, RIG-I and MDA5 can discriminate between different types of viruses. RIG-I is essential for the production of interferons in response to paramyxoviruses, influenza virus, and Japanese encephalitis virus, whereas MDA5 is crucial for detection of picornavirus.[20]

Intrinsic immunity

A newly described form of innate immunity, termed intrinsic immunity, ensures protection by providing a constitutive, always-on line of defence, relying on intracellular obstacles to hinder the replication of pathogens.[61] This component of the immune system has gained much attention as a cornerstone of the resistance of mammals against several classes of retroelements and retroviruses.[61] Among the best studied proteins are the family of apolipoprotein B mRNA-editing enzyme catalytic polypeptide 3 (APOBEC3) proteins, which interfere with the viral lifecycle by incorporating themselves into viral particles, leading to viral DNA hypermutation on the next round of infection.[62,63] A series of studies involving infection of human CD4+ T cells and macrophages with wild-type HIV-1 and HIV-1 deficient in the *vif* gene showed that the antiviral effect of ABC3G (also known as CEM15 or APOBEC3G) is counteracted by Vif.[64] Interestingly, in non-human primates, ABC3G orthologues provide antiviral activity against wild-type HIV-1,[65] but not their cognate simian immunodeficiency viruses, suggesting that virus permissiveness in different primates results from species-specific differences within *vif*.[63] One human variant of ABC3G has been associated with rapid HIV-1 disease progression.[66]

The tripartite motif (TRIM) family is a well-conserved family of proteins characterised by a structure comprising a ring-finger domain, one or two B-box motifs, and a predicted coiled-coil region.[67] Additionally, most TRIM proteins have additional carboxy-terminal domains. Members of the TRIM protein family are involved in various cellular processes, including cell proliferation, differentiation, development, oncogenesis, and apoptosis.[68,69] Some TRIM proteins exert antiviral properties. TRIM5α is reported to restrict retroviral infection by specifically recognising the viral capsid and promoting its premature disassembly.[70] Human TRIM5α has limited efficacy against HIV-1, whereas some primate TRIM5α orthologues can potently restrict this particular lentivirus.[68,69] Substantial interspecies sequence diversity characterises TRIM5α and may underlie differences in the pattern and breadth of restriction of multiple lentiviruses. Human TRIM5α variants do not modify susceptibility to HIV-1; however, they change susceptibility to other retroviruses, such as N-tropic murine leukaemia virus.[71] Polymorphisms found in TRIM5α might conceivably have been selected in past epidemics by viruses unrelated to HIV-1.

Comparative innate immunogenetics

The increasing availability of genomic data allows comparative analyses of genetic sequences involved in innate and intrinsic immunity. This approach, also described as evolutionary genomics, identifies the role of adaptive forces on protein-encoding genes by determining signs of positive (diversifying) or negative (purifying) selection. For example, positive selection in the human genome indicates shifts in living conditions experienced by modern human populations, such as different habitats, food sources, population densities, and exposure to pathogens.[72]

Several families of innate immunity genes have been investigated by use of comparative genomics. Vertebrate TLRs are an example of evolutionary conservation that

	Polymorphisms	Type of infection	Effect of polymorphism on susceptibility in individuals with genetic variant
CD14			
Sutherland et al[83]	–159C/T (untranslated)	Gram-negative sepsis	Increased
Laine et al[84]	–159C/T (untranslated)	Periodontitis	Increased
Lammers et al[85]	–159C/T (untranslated)	Pouchitis	Increased*
Rupp et al[86]	–159C/T (untranslated)	*Chlamydia pneumoniae*	Increased
Ouburg et al[87]	–159C/T (untranslated)	*Chlamydia trachomatis*	No evidence for association
Tal et al[88]	–159C/T (untranslated)	RSV	No evidence for association
Szebeni et al[89]	–159C/T (untranslated)	Necrotising enterocolitis	No evidence for association
LBP			
Hubacek et al[90]	292T/G (C98G); 1306C/T (P436L)	Sepsis	Increased
MBL			
Sutherland et al[83]	X0/0 and 0/0 haplotype pairs	Sepsis	Increased
TLR1			
Kesh et al[91]	239G/C (R80T); 743A/G (N249S)	Aspergillosis†	Increased
TLR2			
Sutherland et al[83]	–16933T/A (untranslated)	Sepsis	Increased
Bochud et al[92]	–15607A/G (untranslated) and haplotypes 2 and 4	Severity of HSV2 infection	Increased
Yim et al[93]	Microsatellite in intron 2	Tuberculosis	Increased
Ogus et al[94]	2258G/A (R753Q)	Tuberculosis	Increased
Lorenz et al[95]	2258G/A (R753Q)	Sepsis	Increased
Moore et al[96]	2258G/A (R753Q)	Sepsis	No evidence for association
Rupp et al[86]	2258G/A (R753Q)	*C pneumoniae*	No evidence for association
Schroder et al[97]	2258G/A (R753Q)	Lyme disease	Decreased
TLR4			
Rezazadeh et al[98]	896A/G(D299G) and 1196C/T(T399I)‡	Brucellosis	Increased
Tal et al[88]	896A/G(D299G) and 1196C/T(T399I)‡	RSV	Increased
Mockenhaupt et al[99]	896A/G(D299G) and 1196C/T(T399I)‡	Severe malaria	Increased
Mockenhaupt et al[100]	896A/G(D299G) and 1196C/T(T399I)‡	Manifestations of malaria	Increased§
Montes et al[101]	896A/G(D299G) and 1196C/T(T399I)‡	Osteomyelitis	Increased
Balistreri et al[102]	896A/G(D299G) and 1196C/T(T399I)‡	Rickettsiosis	Increased
Agnese et al[103] Lorenz et al[104] Barber et al[105]	896A/G(D299G) and 1196C/T(T399I)‡	Sepsis	Increased
Feterowski et al[106] Child et al[107]	896A/G(D299G) and 1196C/T(T399I)‡	Sepsis	No evidence for association
Brett et al[108] Kinane et al[109]	896A/G(D299G) and 1196C/T(T399I)‡	Periodontitis	Increased
Laine et al[84] D'Aiuto et al[110] Folwaczny et al[111]	896A/G(D299G) and 1196C/T(T399I)‡	Periodontitis	No evidence for association
Van der Graaf et al[112]	896A/G(D299G) and 1196C/T(T399I)‡	Candidiasis	Increased
Van der Graaf et al[113] Morre et al[114]	896A/G(D299G) and 1196C/T(T399I)‡	Candidiasis	No evidence for association
Genc et al[115]	896A/G(D299G) and 1196C/T(T399I)‡	Bacterial vaginosis	Increased

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indicate the difficulty for the microbes to mutate genes that encode MAMPs.[73,74] The CD209 (DC-SIGN) proteins, a family of C-type lectins that participate in the recognition of various pathogens, display a complex pattern of evolution. Whereas CD209 has been under a strong selective constraint that prevents accumulation of aminoacid changes, CD209L (also known as DC-SIGN2 or DC-SIGNR) exhibits greater variation across human populations.[75] Such variations may be tolerated because of the potentially redundant functional activities of the molecules encoded by these genes.[76] The killer-cell immunoglobulin-like receptor (KIR) genes encode a family of receptors expressed by natural killer cells, which participate in early responses against infected or transformed cells through production of cytokines and direct cytotoxicity.[77,78] By contrast with TLRs and CD209, only a small proportion of KIR alleles are conserved among primates, showing a rapid species-specific diversification of the KIR gene family members and a plasticity of the genomic region that parallels that of the MHC loci.[79] Thus, the evolutionary forces driving the genesis of natural killer receptors and their HLA ligands represent a concerted response to pathogens. Finally, a remarkable success of evolutionary genomics in infectious diseases is the identification of protein regions relevant for host–pathogen interactions in HIV-1 infection. Comparative analysis of the primate antiretroviral cellular defence genes encoding for ABC3G and TRIM5α have revealed the powerful selective pressures emerging from a long-standing battle between retroviruses and their hosts.[80–82] Singular aminoacids or regions (patches) contain key residues that confer primates the ability to combat HIV-1.

Innate immunogenetics

Given that the innate immune system is at the interface between the host and the pathogen, polymorphisms of innate immune genes are very likely to affect the host susceptibility to infections. Since the innate immune system senses only a limited number of highly conserved microbial-associated molecular patterns[23] via a limited number of receptors and signalling molecules, as anticipated, several polymorphisms have been found to confer an increased susceptibility to specific pathogens (table 2, table 3, and figure 4).

Common polymorphisms in TLRs (complex inheritance)

A study from Turkey revealed an association between susceptibility to tuberculosis and an SNP (R753Q) in the *TLR2* gene.[94] 14 (9·3%) of 151 tuberculosis patients were homozygous for the minor allele compared with two (1·7%) of 116 healthy controls (odds ratio 6·0, 95% CI 1·3–3·9, p=0·009). Of note, R753Q was associated with decreased responsiveness to bacterial lipopeptides.[95] The role of a microsatellite polymorphism (GT repeat) in the exon 2 of *TLR2* has been studied in 176 Korean

patients with tuberculosis and 196 healthy controls.[7] A shorter GT repeat was found more frequently in tuberculosis patients than healthy individuals (49·4% *vs* 37·7%, p=0·02), and was associated with weaker promoter activities and lower TLR2 expression in CD14-positive peripheral blood monocytes. Two non-synonymous SNPs in the extracellular domain of *TLR4* found to be in linkage disequilibrium (D299G and T399I) have been associated with an increased susceptibility to infections caused by Gram-negative bacteria,[103,104] *Brucella* spp,[98] respiratory syncytial virus,[88] and *P falciparum*.[99] Individuals heterozygous for the D299G and T399I SNPs were hyporesponsive to lipopolysaccharide as measured by bronchospastic response after inhalation of endotoxin.[31] Furthermore, airway epithelial cells isolated from heterozygous individuals had deficient response to lipopolysaccharide, suggesting that D299G and T399I acted in a dominant fashion with respect to the wild-type allele.[31] However, monocytes and whole blood isolated from heterozygous individuals did not show abnormal responses to lipopolysaccharide,[127,128] suggesting that the effects of these mutations may vary between cell types. Although two studies had shown that D299G,[103] or D299G and T399I,[104] were associated with an increased risk of Gram-negative infections or septic shock, three subsequent studies did not find an association in patients with meningococcal sepsis.[120–122] However, in one study, rare heterozygous missense mutations of *TLR4* were linked with the development of meningococcal disease.[120] Unexpectedly, D299G and T399I were associated with decreased rather than increased susceptibility to *Legionella pneumophila* infection.[32] A stop codon polymorphism in *TLR5* (R392stop), shown to abolish the ability of TLR5 to detect bacterial flagellin, has been associated with increased susceptibility to pneumonia caused by *L pneumophila*.[123]

Mendelian disorders in TLR adaptors (monogenic inheritance)

Several studies have shown associations between mutations in genes encoding several proteins of the TLR signalling pathways (*IRAK4*,[33,34] *IKBKG*,[36–38] and *IκBα*[35,129]) and rare inherited immunodeficiencies. Complete recessive interleukin-1 receptor-associated kinase 4 (IRAK4) deficiency is characterised by recurrent infections with pyogenic bacteria at an early age that tend to disappear over time. By contrast, mutations affecting the other genes result in X-linked (*IKBKG*) or autosomal-dominant (*IκBα*) anhydrotic ectodermal dysplasia, which is characterised by increased susceptibility to a broader range of pathogens, such as atypical mycobacteria or *Pneumocystis jirovecii* and a complex disorder involving impaired development of skin appendages, conical teeth, and hypotrichosis.[36–38]

Taken together, these data clearly show that mutations in the genes encoding TLRs and downstream signal-transducing molecules influence innate immune responses and increase susceptibility to many infectious diseases. Similarly, polymorphism of cytokines and cytokine receptor genes, which are key effector molecules, have also been associated with altered susceptibility to invasive pathogens.[130]

NLRs

Polymorphisms in genes encoding NLRs have been shown to influence susceptibility to inflammatory diseases. Polymorphisms in NOD2 have been associated with susceptibility to Crohn's disease,[131,132]

(Continued from previous page)

Goepfert et al[116]	896A/G(D299G) and 1196C/T(T399I)‡	Bacterial vaginosis	No evidence for association
Newport et al[117]	896A/G(D299G) and 1196C/T(T399I)‡	Tuberculosis	No evidence for association
Szebeni et al[89]	896A/G(D299G) and 1196C/T(T399I)‡	Necrotising enterocolitis	No evidence for association
Rivera-Chavez et al[118]	896A/G(D299G) and 1196C/T(T399I)‡	Acute appendicitis	No evidence for association
Morre et al[119]	896A/G(D299G) and 1196C/T(T399I)‡	*C trachomatis*	No evidence for association
Smirnova et al,[120] Allen et al,[121] Read et al[122]	896A/G(D299G) and 1196C/T(T399I)‡	Meningococcal sepsis	No evidence for association
Hawn et al[32]	896A/G(D299G) and 1196C/T(T399I)‡	Legionellosis	Decreased
Smirnova et al[120]	Rare mutations	Meningococcal sepsis	Increased
TLR5			
Hawn et al[123]	1174C/T(392stop)	Legionellosis	Increased
Dunstan et al[124]	1174C/T(392stop)	Typhoid fever	No evidence for association
TLR6			
Kesh et al[91]	745C/T(S249P)	Aspergillosis†	Increased
TLR9			
Bochud et al[125]	1635A/G (P545P) and 1174G/A (untranslated)§	CD4+ cells decline in HIV-1 infection	Increased
Mockenhaupt et al[100]	−1486T/C	Manifestations of malaria	Increased¶
Lammers et al[85]	−1237T/C (untranslated)	Pouchitis	Increased*
Mockenhaupt et al[100]	−1237T/C (untranslated)	Manifestations of malaria	No evidence for association¶
NOD2			
Meier et al[126]	3020C/ins (1007ins)	Pouchitis	Increased
Szebeni et al[89]	3020C/ins (1007ins) 2104C/T (R702W) 2722G/C (G908R)	Necrotising enterocolitis	No evidence for association

*Combined carriage of the CD14 −159C/T and TLR9 −1237C are associated with pouchitis; CD4 −159C/T is also known as −260C/T. †TLR1 239G/C alone and combined carriage of TLR1 743A/G and TLR6 745C/T were associated with the occurrence of invasive aspergillosis. ‡TLR4 896A/G (D299G) is in strong linkage disequilibrium with 1196C/T (T399I); most studies analysed both 896A/G and 1196C/T with similar results. §TLR9 1635A/G is in strong linkage disequilibrium with TLR9 1174G/A. ¶In pregnant women with malaria, TLR4 896A/G and TLR9 T−1486T/C increased the risk of low birthweight in term infants and TLR4 A896A/G also increased the risk of maternal anaemia. HSV2=herpes simplex virus 2. IRAK4=interleukin-1 receptor-associated kinase 4. LBP=lipopolysaccharide-binding protein. MBL=mannose-binding lectin. NOD=nucleotide-binding oligomerisation domain. RSV=respiratory syncytial virus. TLR=Toll-like receptor.

Table 2: **Association between innate immune gene polymorphisms and susceptibility to infectious diseases: complex inheritance**

	Polymorphisms	Type of infection	Effect of polymorphism on susceptibility in individuals with genetic variant
IRAK4 Medvedev et al,[33] Picard et al[34]	620–1AC/del (218stop); 821T/del (287stop); 877C/T (293stop)	Pyogenic bacterial infections	Increased
IκBα Courtois et al[35]	94G/T (S32I)	Bacterial infections	Increased
IKKγ Zonana et al,[36] Jain et al,[37] Jain et al,[38] Doffinger et al[39]	1217A/T (D406V); 1249C/T (C417R); 1259A/G (X420W); other mutations	Bacterial and mycobacterial infections	Increased

IκBα=inhibitor of nuclear factor κB (NFκB) kinase α. IKKγ=inhibitor of NFκB kinase γ. IRAK4=interleukin-1 receptor-associated kinase 4.

Table 3: **Association between innate immune gene polymorphisms and susceptibility to infectious diseases: monogenic inheritance**

	Comments
Internal validity	
Confounding	
Population stratification	Limited information on ethnicity
Failure to account for known confounders	Cohort not established in view of genetic study; insufficient clinical data; failure to adjust for multiple confounders in the analyses
Selection biases	No or insufficient attempt to ensure that cases and controls come from the same source population
Information biases	Information on exposure or study endpoints is gathered differently for cases and controls
Statistical analyses	
Limited power	No sample size calculation; insufficient sample size to detect a small effect; difficult and costly to collect large cohorts
Absence of a-priori specified hypotheses	Investigators rarely distinguish between hypothesis-testing and hypothesis-generating studies
No correction for multiple testing	Multiple endpoints and genetic markers are analysed, but only significant associations are reported
Causality	
Biological plausibility	
No functional data to support association	Biological systems not sensitive enough to illustrate functional association
Simplistic measure of genetic variability	Studies often limited to a few SNPs per gene
Failure to account for gene–gene and gene–environment interactions	Genetic and environmental background can be expected to influence most associations
Strength of the association	Any single genetic variant usually only has a small effect
Consistency of the association	Results are rarely replicated across studies
Dose-response effect	Alleles do not always display an additive mode of action

SNP=single nucleotide polymorphisms.

Table 4: **Common limitations in genetic association studies**

Blau syndrome,[133] early-onset sarcoidosis,[134] and graft-versus-host disease.[135] Genetic variations in NALP3 have been linked to three autosomal dominant diseases: Muckle-Wells syndrome,[136] familial cold auto-inflammatory syndrome, and chronic infantile neurological cutaneous and articular syndrome (also known as neonatal onset multisystemic inflammatory disease).[137] Loss-of-function mutations in the gene encoding another NLR-related protein, the class II transactivator, decrease expression of MHC II, resulting in type II bare lymphocyte syndrome.[138] So far, there are no data on mutations of NLR genes and susceptibility to, or outcome of, infectious diseases. However, in view of the part played by these molecules in inflammation, this area undoubtedly deserves further clinical investigation.

Limitations of genetic studies

Common limitations of genetic association studies are shown in table 4. Genetic studies done to date often fail on the following factors: (1) to properly account for confounding factors (such as lack of information on ethnicity), and selection and information biases (insufficient data on the source population of cases and controls or study endpoints); (2) to present appropriate statistical analyses (such as lack of sample size calculation and correction for multiple testing); and (3) to provide convincing information about biological plausibility. As an example, among five studies that assessed the effect of *TLR4* polymorphisms on susceptibility to, and outcome of, severe infections, only two included more than 100 patients,[105] two provided information about patient's ethnicity,[105,106] and only one limited the analysis to a specific ethnic group.[106]

Comparison of data is often impaired by the fact that apparently similar studies used markedly different controls groups and endpoints.[103–107] Proving causality is never trivial. Associations are likely to occur when non-causal markers are in linkage disequilibrium with the true disease locus. Although the replication of a finding in an independent sample decreases the risk of a false-positive result, the functional significance of the genetic variant should ultimately be shown in biological studies. However, proving biological plausibility may be difficult in view of the limitations of in-vitro studies used as proxy of complex in-vivo biological processes. For example, use of gene-silencing techniques often reduces the biological observation to that of an on/off system, which does not allow the detection of quantitative variations (ie, a dose-response effect) of gene expression or discrete functional alterations. With the increasing use of high-throughput genotyping techniques, the number of genetic associations that will be reported in the years to come will most probably exceed our capacity to do proper functional studies and hence to provide convincing evidence for biological plausibility.[139] Future

functional studies should therefore focus on genetic polymorphisms that exert a strong effect, have been replicated by independent investigators, and have potential diagnostic or therapeutic implications. To limit the importance of positive publication bias, it will be crucial for investigators and journal editors to become less reluctant to publish well-conducted negative studies.[139]

Future perspectives

In recent years, innate immunogenetic studies of inherited genetic disorders have provided researchers and clinical investigators with crucial information that has improved our understanding of the host defences against microbial pathogens. Table 5 shows examples of the effect of recent discoveries in the field of innate immunogenetics with foreseeable applications for the short, middle, and long term in areas such as vaccine development and predictive and preventive medicine. The persistence or emergence of potentially devastating infectious diseases, such as tuberculosis, malaria, HIV/AIDS, and, most recently, severe acute respiratory syndrome or avian influenza, underscore the need to develop new vaccines and therapeutic treatment strategies. A better understanding of microbial genomics and genetics and host innate immunogenetics is likely to provide important information for the development of new vaccines. Vaccine immunogenicity is determined not only by the chemical and physical nature of microbial antigens and adjuvants, but also by the genetic make-up of vaccine recipients. Analyses of polymorphisms of innate immune genes may also help understand why some individuals exhibit suboptimum responses to vaccination.[140] Immunosuppression as a result of myeloablative chemotherapy, solid organ or haematological stem-cell transplantation, or corticosteroid therapy for autoimmune diseases represent other clinical conditions for which immune gene polymorphisms may help to predict the risk of life-threatening infectious complications.

The recent discoveries of genes encoding TLRs, NLRs, and the related signal-transducing molecules has markedly improved our understanding of innate immunity. The availability of high-throughput genotyping techniques opens new perspectives to further improve our understanding of the pathogenesis of infectious diseases and for the development of new diagnostic, predictive, and preventive treatment strategies. Clinicians and researchers should be aware of the results and far-reaching implications of recent innate immunogenetic studies that have associated genetic polymorphisms with susceptibility to, or outcome of, infectious diseases. Collecting DNA should now be an integral part of epidemiological or clinical infectious disease studies. National and international consortia should be created to put together large cohort studies to promote and facilitate research in the field.

	Short term	Middle term	Long term
Basic research	Better understanding of gene function at the molecular level by the study of genetic polymorphisms	Detection of novel disease-specific genes by genome-wide scans; targeted drug discovery; gene therapy (chronic infections, inherited immune deficiencies)	
Vaccine development	Better understanding of individual responses to vaccines	Elaboration of vaccines with improved immunogenicity (use of innate immune adjuvants)	Genetic screening at birth, allowing customised vaccination programme
Preventive and predictive medicine	List of crucial polymorphisms associated with increased susceptibility to infection	Screening of individuals at high risk for infectious diseases and development of individualised prophylactic measures including antimicrobial prophylaxis; identification of new therapeutic targets	Genetic screening at birth, allowing customised prophylaxis in case of high-risk condition (immunosuppressive therapy, major surgery); development of new treatment modalities

***Table 5:* Examples of potential short, middle, and long-term applications and effects of innate immunogenetic studies for basic and translational research**

Search strategy and selection criteria

Relevant articles for this Review were identified by searching Medline (1966 to November, 2006) by use of the terms “genetics”, “single nucleotide polymorphisms”, “Toll-like receptors” or “TLRs”, “nucleotide-binding oligomerization domain receptors” or “NODS”, “immunology”, and “innate immunity”, and by extracting references from these articles. The Review was limited to articles published in the English language.

Conflicts of interest

We declare that we have no conflicts of interest.

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