ELECTRONIC LETTER

Single nucleotide polymorphism (SNP) analysis of mouse quantitative trait loci for identification of candidate genes

Y Yan, M Wang, W J Lemon, M You

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Mouse models are widely used for studying polygenic traits underlying various diseases to identify the low penetrance disease susceptibility genes. Classical genetic studies using cross breeding of mouse strains with differing susceptibilities to different diseases have identified chromosomal regions associated with predisposition to lung tumours, lung injury, disease resistance, and seizure.[1 2] The process, called quantitative trait loci (QTL) mapping, involves the use of evenly spaced polymorphic DNA markers to create landmarks across each chromosome to find correlations between marker alleles and the phenotypic variation.[3] Inbred mouse strains vary in their susceptibility to lung tumours, lung injury, disease resistance, and seizures, with two extreme strains being A/J (susceptible) and C57BL/6J (B6, resistant).[1 2] As the mouse genome sequences for both the A/J and B6 strains of mice have been completed, identification of candidate genes responsible for disease related QTL, mapped using these two strains of mice as parental strains, becomes feasible by undertaking single nucleotide polymorphism (SNP) analysis.

One set of mouse QTL is associated with genetic predisposition to lung tumour. In humans, the wide variety of carcinogens and varying degrees of exposure make identifying the predisposing genes difficult, but in a mouse model, such confounding variables can be controlled. There is evidence that, although lung cancer incidence is largely associated with environmental factors such as smoking or occupational exposure, genetic components are involved in lung cancer development. Genetic linkage studies using various strains of inbred mice mapped pulmonary adenoma susceptibility (Pas), pulmonary adenoma resistance (Par), and susceptibility to lung cancer (Sluc) loci.[1] Recently, we have mapped three major QTL, named mouse lung tumour (MLT) 1–3, in 25 strains of mice with known susceptibility to lung cancer development, using a whole genome LD analysis with 5638 genetic markers.[4] MLT1 locates between D4Mit50 and D4Mit361, MLT2 between D6Mit15 and D6Mit304, and MLT3 from D8Mit127 to D8Mit69. SNP analysis for candidate genes at these three lung tumour related loci was an objective of the present study.

The second set of mouse QTL is associated with genetic susceptibility to acute lung injury. Acute lung injury is a syndrome characterised by increased alveolar–capillary permeability and hypoxaemia. The most severe cases of acute lung injury culminate in respiratory distress, a condition that remains a serious clinical challenge in critical care medicine because of its high incidence and substantial mortality.[5 6] Two extreme inbred mice strains—A/J (susceptible) and C57BL/6J (resistant)—vary in their susceptibility to acute lung injury.[7 8] Four QTL on mouse chromosome 11, 13, 17, and 6 were identified as acute lung injury susceptibility loci, and were designated as Ali1, Ali2, Ali3, and Ali4, respectively.[8 9] SNP analyses between A/J and C57BL/6J mice for candidate genes in these regions were conducted in this study.

Key points

- Mapping of quantitative trait loci (QTL) for susceptibility to diseases in animal models is a powerful tool for identifying genes that may be relevant to humans.
- With the availability of the complete mouse genome sequence, SNP analysis allows the identification of potential candidates of various QTL mapped using mouse models.
- In the present study, the genes located within QTL regions linked to susceptibility to lung tumour development, lung injury, disease resistance (trypanosomiasis), and seizures were systematically characterised by SNP analysis.
- The combination of QTL mapping and SNP analysis is an effective approach for identifying candidate disease genes in mouse models of human disease. The approach can narrow down the list of candidates in QTL as a filtering tool for further screening and study.

The third set of mouse QTL is associated with genetic resistance to trypanosomiasis. Trypanosomiasis, or sleeping sickness, is a re-emerging public health problem of epidemic proportions in many parts of rural Africa. It is caused by a subspecies of *Trypanosoma brucei* and is transmitted by tsetse flies.[10] Multigene control of variation in susceptibility to trypanosomiasis has been mapped in mice, where the C57BL/6J strain is relatively resistant and the A/J strain is susceptible.[11 12] Kemp *et al* reported three QTL of trypanosomiasis resistance located on mouse chromosomes 17, 5, and 1 using the intercross of resistant C57BL/6J mice and susceptible A/J or BALB/c mice.[13] The loci were named Tir1, Tir2, and Tir3, respectively. Among these three QTLs, Tir1 and Tir2 were mapped using the intercross of A/J strain and C57BL/6J strain, whereas Tir3 was mapped using the intercross of C57BL/6J strain and BALB/c strain. Later, the Tir3 locus located on mouse chromosomes 1 was fine mapped by using G6 generation of advanced intercross lines between A/J and C57BL/6J mice.[14] On mouse chromosomes 1, they found three QTL peaks which were named Tir3a, Tir3b, and Tir3c.[14] These regions are examined for potential candidates in the present study.

The fourth set of mouse QTL is associated with genetic susceptibility to seizures. A seizure is a brief change in behaviour; it is the uncontrolled hypersynchronous electrical

Abbreviations: Ali, acute lung injury locus; Bis, β-carboline induced seizure locus; $GABA_A$, γ-aminobutyric acid; LD, linkage disequilibrium; MLT, mouse lung tumour; NCBI, National Center for Biotechnology Information; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; Tir, trypanosomiasis resistance locus; β-CCM, methyl-β-carboline-3-carboxylate

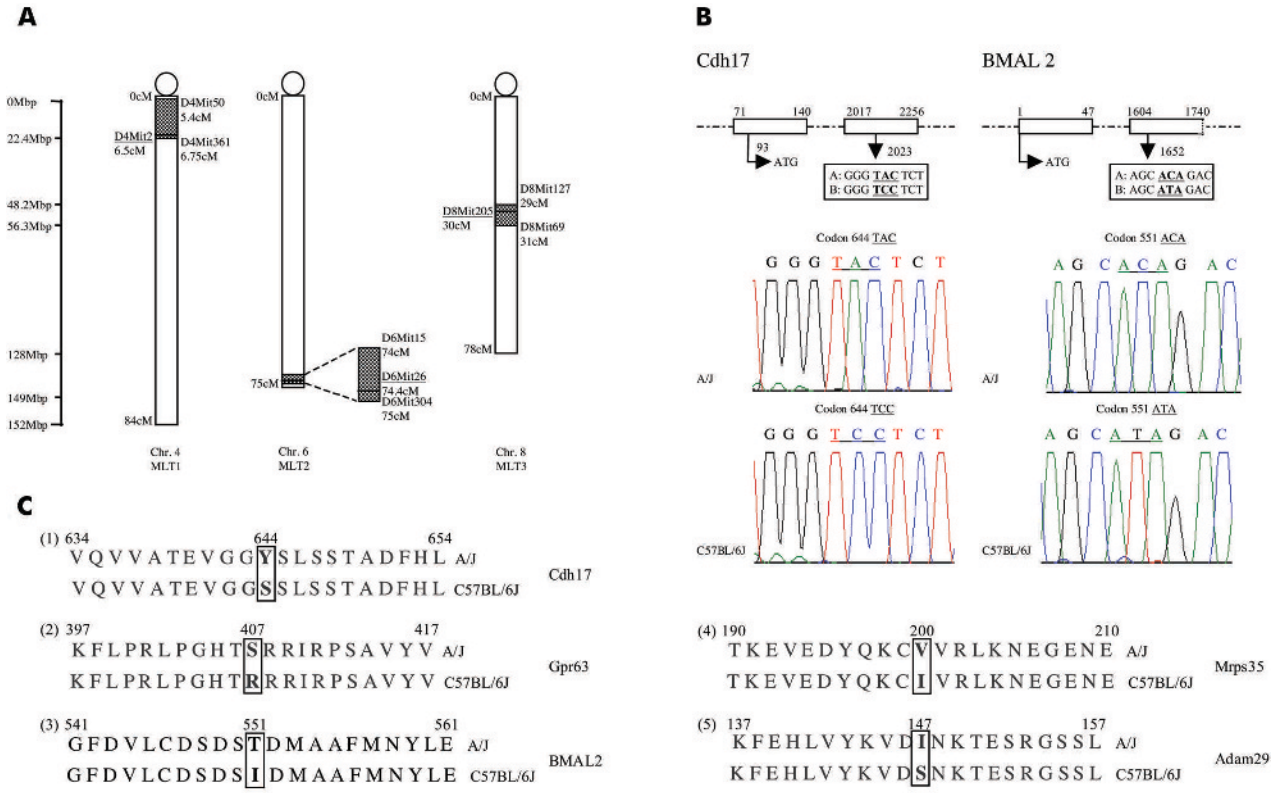

Figure 1 Physical position (MB) of MLTs and polymorphisms between A/J and C57BL/6J mice. (A) The Celera physical position (Mbp) single nucleotide polymorphism (SNP) analysis and the regions for MLT1, MLT2, and MLT3 quantitative trait loci (QTLs). Within the QTL rectangles in each chromosome, the parts of shadow denote the location of genes containing A-B SNPs. The horizontal lines within the shadow region denote the corresponding QTL peaks on each chromosome. (B) SNPs resulted in amino acid alterations in representative mouse lung tumour candidate genes. On the upper panels, only polymorphisms bearing exons are shown. Interrupted lines represent the intronic region and the omitted exon that did not show SNPs in this study. The lower panels show the chromatograms of amino acid alterations. (C) Functional polymorphisms in representative candidate genes for lung tumour susceptibility. In each group, the upper row shows the sequence of susceptible strain mouse of A/J, and the lower row the resistant strain of C57BL/6J. The boxed regions represent the polymorphisms between A/J and C57BL/6J mice. The numbers over the boxes are the codon numbers of each of the representative mouse lung tumour candidate genes. MLT, mouse lung tumour.

discharges of neurones in the brain that interfere with normal function.[15] Epilepsy is characterised by recurrent seizures and refers to a collection of disorders that affect 1–2% of the world's population.[16 17] Epilepsy genes fall into several quite distinct classes including those in which mutations cause abnormal brain development, progressive neurodegeneration, disturbed energy metabolism, or dysfunction of ion channels.[18] To define the genetic contributions affecting individual differences in seizure threshold, Gershenfeld *et al* mapped quantitative trait loci for seizures induced by the intraperitoneal injection of methyl-β-carboline-3-carboxylate (β-CCM), a $GABA_A$ (γ-aminobutyric acid) receptor inverse agonist and convulsant.[19] By using backcross and intercross populations between susceptible A/J strains and resistant C57BL/6J strain, they reported that the QTLs of seizure susceptibility loci were located on mouse chromosomes 10, 4, and 7, and were designated as Exq1, Bis1, and Bis4, respectively. In this study, these regions are further characterised for candidate genes using SNP analysis.

Genomic DNA contains a variety of polymorphisms, such as single nucleotide substitutions, insertion/deletions, and nucleotide repeat motifs. SNPs are changes in a single base at a specific position in the genome, in most cases with two alleles.[20] They are useful as marker in population genetics and evolutionary studies.[21 22] Depending on where an SNP occurs, it may have different consequences at the phenotypic level. SNPs in the regulatory regions of genes might influence the risk of common disease. SNPs in the 3′-UTR region may alter the stability of the mRNA by changing binding sites or secondary structure, thus making it more or less likely to be degraded. An SNP in the 5′ region may change binding sites and thereby modify the affinity for a transcription factor. Non-sense SNPs can introduce a premature stop-codon resulting in a truncated polypeptide, and often result in a loss of function. Missense mutations cause amino acid alterations that can be significant if the properties of the new amino acid (charge, polarity, and so on) are different from the one it replaced.

Over the past few years, SNPs have been proposed as the next generation of markers for the identification of genes associated with complex diseases.[23–25] As A/J and C57BL/6J mice vary significantly in their susceptibility to lung tumour development, lung injury, disease resistance, and seizures, detailed SNP analysis between A/J and C57BL/6J mice is ideal for identifying potential candidate genes for relevant QTLs. This approach has been aided greatly by the fact that the genome sequences of both strains have been sequenced and assembled.

METHODS

Approach

Markers flanking each QTL were found in the Celera (Rockville, Maryland, USA) mouse genome database (www.celera.com). These markers were used to identify the reference DNA positions within the assembled genome for our database query. The Celera mouse SNP reference

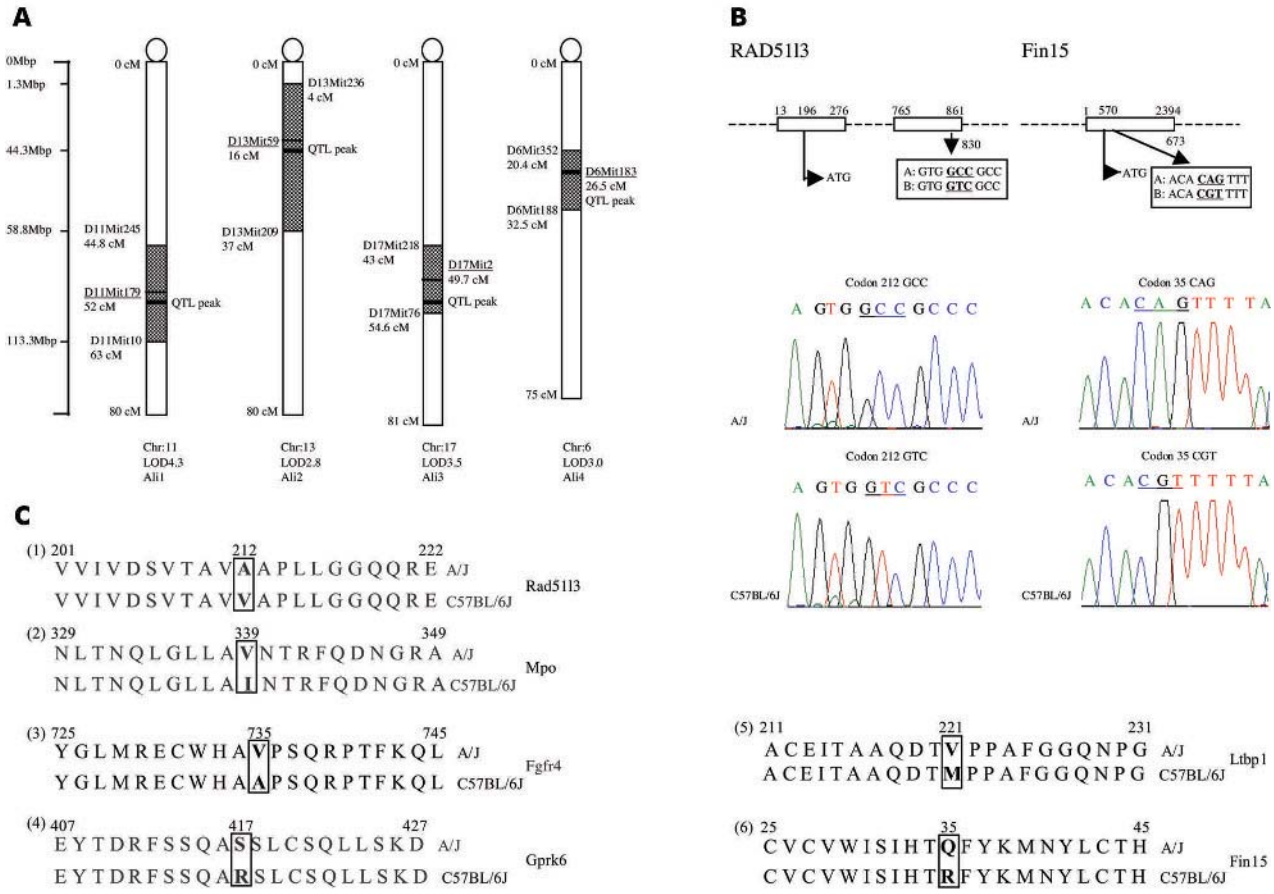

Figure 2 The physical position of acute lung injury (Ali) quantitative trait loci (QTLs); single nucleotide polymorphisms (SNPs) resulted in amino acid alterations and functional polymorphisms of acute lung injury between A/J and C57BL/6J mice. (A) The Celera physical position (Mbp) SNP analysis and the regions for Ali1, Ali2, Ali3, and Ali4 QTLs. Within the QTL rectangles in each chromosome, the parts of shadow denote the location of genes containing A-B SNPs. The horizontal lines within the shadow region denote the corresponding QTL peaks on each chromosome. (B) SNPs resulted in amino acid alterations in representative acute lung injury candidate genes. On the upper panels, only polymorphisms bearing exons are shown. Interrupted line represents the intronic regions and the omitted exon that did not show SNPs in this study. The lower panels show the chromatograms of amino acid alterations. (C) Functional polymorphisms in representative candidate genes for acute lung injury. In each group, the upper row shows the sequence of susceptible strain mouse of A/J, and the lower row the resistant strain of C57BL/6J. The boxed regions represent the polymorphisms between A/J and C57BL/6J mice. The numbers over the boxes are the codon numbers of each of the representative acute lung injury candidate genes.

database was interrogated for SNPs within each QTL and then filtered to keep only SNPs between A/J and C57BL/6J mice (A-B SNPs).

QTL associated with lung tumour development

Using linkage disequilibrium (LD) analysis of the whole genome, employing 5658 genetic markers in 26 strains of mice, we previously mapped three major loci: MLT1 near D4Mit2, MLT2 near D6Mit26, and MLT3 near D8Mit205, all showing highly significant LDs.[4] As a gradual decrease in LD surrounding each of the peak markers (D4Mit2, D6Mit26, and D8Mit205) was evident, we selected the following MLT candidate regions for SNP analysis: MLT1, located between D4Mit50 at 5.4 cMto D4Mit361 at 6.75 cM; MLT2, limited between D6Mit15 at 74 cM and D6Mit304 at 75 cM; and MLT3, the region from D8Mit127 at 29 cM to D8Mit69 at 31 cM (fig 1A).

QTL associated with acute lung injury

As shown in fig 2A, Ali1 is located from D11Mit245 at 44.8 cM to D11Mit10 at 63 cM; Ali2 is located from D13Mit236 at 4 cM to D13Mit209 at 37 cM; Ali3 is located from D17Mit218 at 43 cM to D17Mit76 at 54.6cM; and Ali4 is located from D6Mit352 at 20.4cM to D6Mit188 at 32.5 cM (fig 2A).[8 9]

QTL associated with resistance to trypanosomiasis

In Tir1 and Tir2 we selected DNA makers relative to LOD scores of 6.0 and 3.0, respectively,[13] with a focus on regions from 16.4 cM to 29.4 cM and from 41 cM to 57 cM. In Tir3, we selected those makers within about 5 cM around, respectively, Tir3a, Tir3b, and Tir3c when the LOD score was over 4.0.[14] The regions are from 58.7 cM to 74.3 cM (in Tir3a and Tir3b) and from 91.3 cM to 101 cM (in Tir3c) (fig 3A).

QTL associated with seizure

In Exq1, we combined the two QTLs on mouse chromosome 10 of the intercross F2 population and the backcross N2 population and selected those makers with a LOD score more than 3.0 for regions from D10Mit68 at 51.5 cM to D10Mit297 at 70 cM. In QTL Bis1 derived from backcross N2 population, we selected regions from D4Mit204 at 61.9 cM to D4Mit127 at 77.5 cM with a LOD score over 2.2. In QTL Bis4 derived from intercross F2 population, we selected those DNA makers

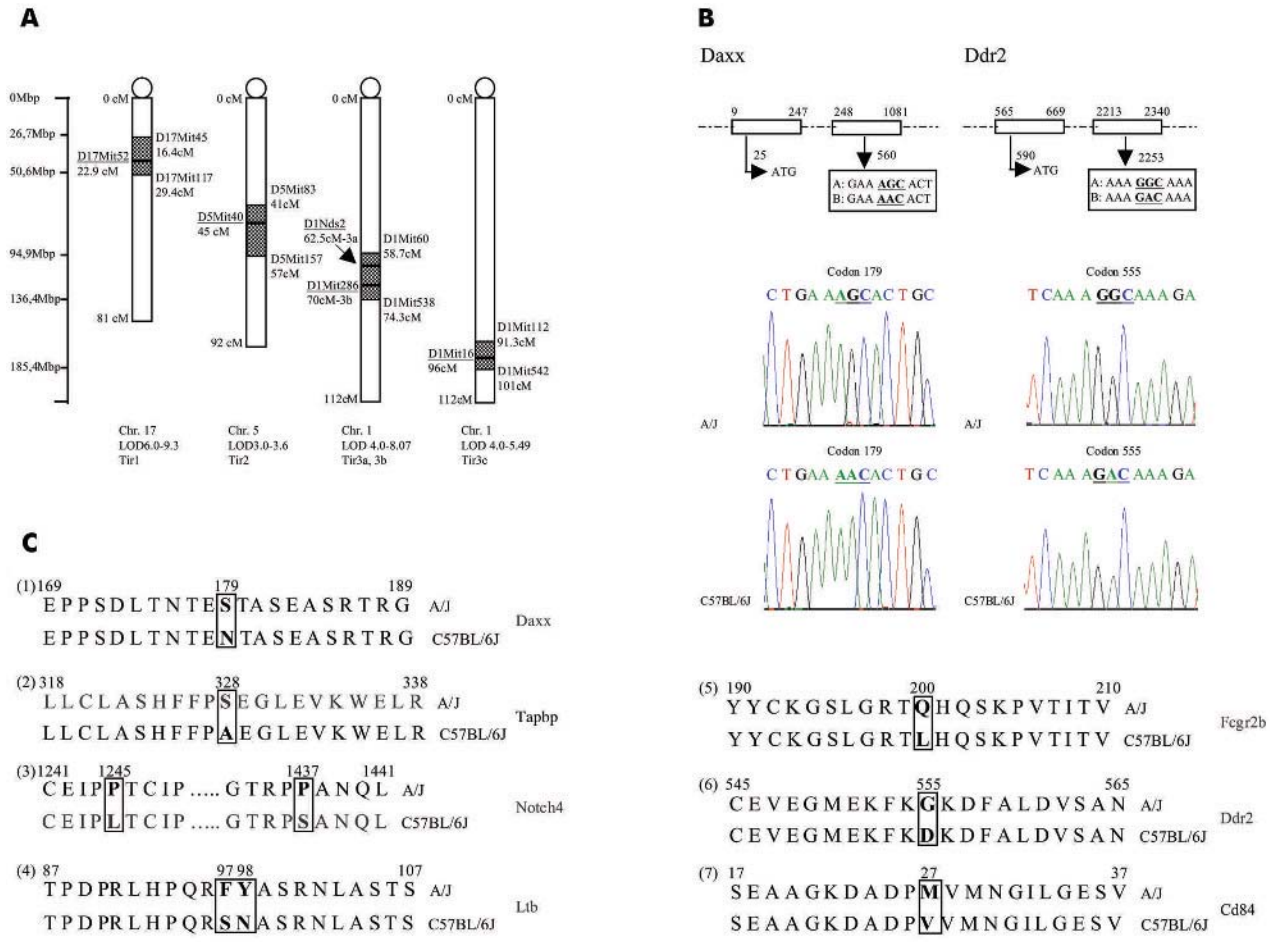

Figure 3 The physical position of trypanosomiasis resistant (Tir) quantitative trait loci (QTLs); single nucleotide polymorphisms (SNPs) resulted in amino acid alterations and functional polymorphisms of trypanosomiasis between A/J and C57BL/6J mice. (A) The Celera physical position (Mbp) SNP analysis and the regions for Tir 1–3 QTLs. Within the QTL rectangles in each chromosome, the parts of shadow denote the location of genes containing A-B SNPs. The horizontal lines within the shadow region denote the corresponding QTL peaks on each chromosome. (B) SNPs resulted in amino acid alterations in representative trypanosomiasis resistance candidate genes. On the upper panels, only polymorphisms bearing exons are shown. Interrupted lines represent the intronic region and the omitted exon that did not show SNPs in this study. The lower panels show the chromatograms of amino acid alterations. (C) Functional polymorphisms in representative candidate genes for trypanosomiasis resistance. In each group, the upper row shows the sequence of susceptible strain mouse of A/J, and the lower row the resistant strain of C57BL/6J. The boxed regions represent the polymorphisms between A/J and C57BL/6J mice. The numbers over the boxes are the codon numbers of each of the representative trypanosomiasis resistance candidate genes.

with a LOD score over 3.2 for the region from D7Mit117 at 11 cM to D7Mit145 at 26.4 cM (fig 4A).

Data filtering

Additional filtering of the A-B SNPs was done to increase confidence in the positive results. With screening, we sought to reduce the false negative rate and thereby to be inclusive, but owing to the tremendous number of SNPs (table 1) we needed also to minimise the false positive rate. To accomplish this, A-B SNPs that met the following criteria were selected for further analysis: (1) A/J and C57BL/6J that were polymorphic for the SNP; (2) SNPs appearing in the coding region, the 5′-regulatory region, or the 3′-untranslated region; (3) SNPs appearing in known genes; (4) exclusion of intronic, intergenic, and silent SNPs. We focused on those SNPs within the coding region, although there is a downside of possibly filtering out some genes responsible for the QTLs. This was done for two reasons: first, the function of the intronic and intergenic sequence is a matter of research beyond the scope of this study; second, the number of these SNPs made their analysis currently untenable. For the resulting A-B SNPs in genes, we chose those genes that might be a plausible link to the aetiology or pathophysiology of mouse lung tumour development, acute lung injury, trypanosomiasis, and seizures, respectively, for further analysis by undertaking sequence verification.

SNP annotation

SNP IDs were used to link SNPs with genes in which they occur and Celera's mechanism to link its genes with public sequences was used for reporting public accession numbers and description from the Celera database and/or GenBank.

Sequence verification of SNPs

A-B SNPs were sequence verified. Briefly, the sequence for the candidate genes was downloaded from the Celera database and polymerase chain reaction (PCR) primers flanking the SNPs designed such that approximately 200 base pair (bp) fragments were produced. PCR was done to amplify DNA harvested from normal lungs of A/J and C57BL/6J mice. PCR products were resolved on 1.2% ethidium bromide stained agarose gels and purified using QIAquick gel

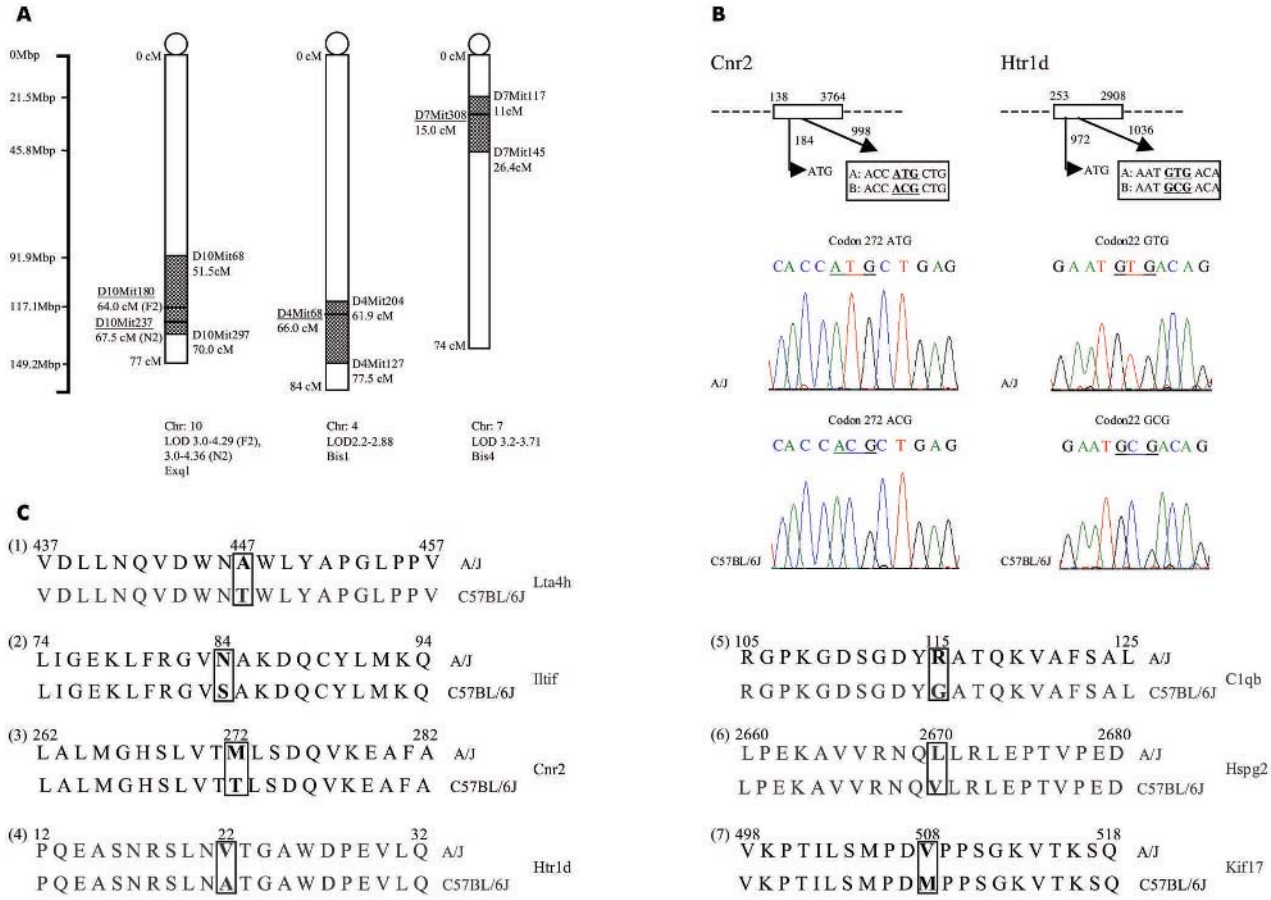

Figure 4 The physical position of seizure quantitative trait loci (QTLs); single nucleotide polymorphisms (SNPs) resulted in amino acid alterations and functional polymorphisms of seizure between A/J and C57BL/6J mice. (A) The Celera physical position (Mbp) SNP analysis and the regions for Exq1, Bis1, and Bis4 QTLs. Within the QTL rectangles in each chromosome, the parts of shadow denote the location of genes containing A-B SNPs. The horizontal lines within the shadow region denote the corresponding QTL peaks on each chromosome. (B) SNPs resulted in amino acid alterations in representative seizure candidate genes. On the upper panels, only polymorphisms bearing exons are shown. Interrupted lines represent the intronic region and the omitted exon that did not show SNPs in this study. The lower panels show the chromatograms of amino acid alterations. (C) Functional polymorphisms in representative candidate genes for seizure susceptibility. In each group, the upper row shows the sequence of susceptible strain mouse of A/J, and the lower row the resistant strain of C57BL/6J. The boxed regions represent the polymorphisms between A/J and C57BL/6J mice. The numbers over the boxes are the codon numbers of each of the representative mouse seizure candidate genes.

extraction kits (Qiagen, Hilden, Germany). Automated sequencing was undertaken using dideoxy terminator cycle sequencing kits (Applied Biosystems, Foster City, California, USA) and Applied Biosystems model 377 DNA sequencers (Perkin-Elmer, Foster City).

Confirmation of candidate genes

After sequencing analysis, we confirmed the SNPs from A/J and C57BL/6J mice by using software of SEQUENCHER (version 4.0.5, Gene Codes Corporation, Ann Arbor, Michigan, USA). The confirmed SNPs were compared with those public sequences of known genes in GenBank, correspondingly using online software of ''BLAST 2 SEQUENCES'' (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html). The reading frame was established and suitable candidates were those where the SNP could result in an amino acid alteration and where there was a plausible link to the aetiology or pathology of each disease.

Confirmation of potential functional domains

After the confirmation of amino acid alteration in candidates, the codon positions were checked with NCBI Conserved Domain Database by using the amino acid sequence of candidates (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). When the region of amino acid changed codon matched a known functional conserved domain, the domain was reported. If the region matched several known conserved domains, the highest score value domain was reported.

RESULTS

Candidate genes for lung tumour QTL

In this study, all nine confirmed SNPs in nine different genes within the MLT1 locus were of missense type. The human cytogenetic positions of these SNPs in MLT1 were located at 8q and 6q, respectively (table 2). Two of the candidates for MLT1 are Cdh17 and GPR63. Cdh17, locating at 8q22.1—also known as liver-intestine (LI) cadherin—represents a novel member of the cadherin superfamily. The amino acid at codon 644 was changed from TAC (tyrosine) in A/J mice to TCC (serine) in C57BL/6J mice for the Cdh17 gene (fig 1, panels B and C; table 2). GPR63 (G protein coupled receptor 63, located at human cytogenetic 6q16.1–q16.3)—also known as PSP24B—shares 57% identity with Xenopus PSP24.[26] At codon 407 of the GPR63 gene, the amino acid of AGT (serine) in A/J mice was substituted by AGG (arginine) in C57BL/6J mice (fig 1C; table 2). SNPs confirmed in MLT2 were located on human cytogenetic 12p11–p12 (table 2). LOC272322 is annotated as BMAL2 gene (brain-muscle ARNT-like

Table 1 Summary of single nucleotide polymorphisms found in the Celera mouse SNP reference database

Disease loci	QTLs	Total SNP	Filtered SNP	Genes (F)	Confirmed SNP	Genes
Lung cancer	MLT1	25 692	26	17	9	9
	MLT2	2477	10	7	3	3
	MLT3	10 245	4	4	1	1
	Total	38 414	40	28	13	13
Acute lung injury	Ali1	36 092	95	45	15	13
	Ali2	45 745	57	29	4	4
	Ali3	22 812	13	8	3	3
	Ali4	22 102	13	6	2	2
	Total	126 751	178	88	24	22
Trypanosomiasis resistance loci	Tir1	29 902	38	24	21	16
	Tir2	32 479	4	3	3	2
	Tir3	82 246	41	22	8	8
	Total	144 627	83	49	32	26
Seizures	Exq1	25 927	6	4	4	4
	Bis1	30 153	19	11	15	10
	Bis4	34 502	8	8	2	2
	Total	90 582	33	23	21	16

Criteria for filtered SNPs: (1) A/J and C57BL/6J were polymorphic for the SNP; (2) SNPs appeared in the coding region, the 5′ regulatory region, or the 3′ untranslated region; (3) SNPs appeared in known genes; (4) intronic, intergenic, and silent SNPs were excluded.
Total SNP = all SNPs found in the region; filtered SNP = SNP number meeting the criteria described in the note; Genes (F) = the number of gene holding filtered SNP; Confirmed SNP = the number of SNP that have been confirmed; Genes = the number of genes holding the confirmed SNP.
Ali, acute lung injury locus; Bis, -carboline induced seizure locus; Exq, exploratory and excitability locus; MLT, mouse lung tumour; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; Tir, trypanosomiasis resistance locus.

protein 2). In BMAL2, the missense polymorphisms gave rise to amino acid change at codon 551 (ACA/ATA) (table 2). At codon 551, threonine in A/J mice was changed to isoleucine in C57BL/6J mice (fig 1, panels B and C). Another gene in MLT2 is Mrps35 (mitochondrial ribosomal protein S35). In MLT3, we found only one SNP in ADAM29 (a disintegrin and metalloproteinase domain 29). Overall, we identified 13 candidate genes for mouse MLT QTLs.

Candidate genes for acute lung injury QTL

As shown in table1, a total of 178 SNPs in 88 genes was found and those genes with potential functional association with acute lung injury were chosen for sequence verification. However, only 24 SNPs in 22 genes were confirmed by re-sequencing (table 3).

In the Ali1 locus, 15 SNPs in 13 genes have been sequence verified. Of the 13 genes, five—RAD51l3 (RAD51-like 3) (fig 2, panels B and C), Slfn2 (schlafen 2), Mpo (myeloperoxidase), Akap (A kinase anchor protein), and Map3k14 (mitogen activated protein kinase kinase kinase 14)—were considered candidates because of their likely link to acute lung injury (table 3).

In Ali2, four SNPs in four genes were identified. After comparing with the public sequences of known genes and excluding those without a plausible link to acute lung injury, two genes—Fgfr4 (fibroblast growth factor receptor 4) and Gprk6 (G protein coupled receptor kinase 6)—were considered candidates (table 3; fig 2C).

In Ali3, four SNPs were found in three genes. Among these, SNP mCV23088765 and mCV23090856 were found in Ltbp1 (latent transforming growth factor binding protein 1). SNP mCV23088765 could not be confirmed because of the extremely high GC content of the flanking sequence, but SNP mCV23090856 was confirmed. The SNP results in a valine alteration in A/J mice and a methionine alteration in C57BL/6J mice (fig 2C). The other candidate gene in this Ali3 locus is Xdh (xanthine dehydrogenase) (table 3).

In the Ali4 locus, only two SNPs were identified in two genes—Imap38 (immunity associated protein, 38 kDa) and Fin15 (fibroblast growth factor inducible 15) (fig 2, panels B and C); these were confirmed and are regarded as the candidate genes for acute lung injury (table 3).

Candidate genes for trypanosomiasis QTL

Among the 83 SNPs in 49 genes filtered, 32 SNPs in 26 genes were sequence verified. The 32 A-B SNPs are enumerated in table 4. In QTLs of Tir1 and Tir3, SNPs in the coding region between A/J and C57BL/6J mice were of missense type. There were no SNPs found in the coding regions in known genes except three silent mutations in Tir2 (table 4). Representative amino acid alterations are presented in fig 3, panels B and C. In the Tir1 locus, 21 SNPs in 16 genes were sequence verified. After sequence verification and examination of the open reading frames, 10 genes—Daxx, Tapbp, Abcb2, Abcb3, Psmb8, H2-Eb1, Bat2, Ltb, Rnf23, and Notch4—were considered as the candidate genes (table 4). On mouse chromosome 1, three QTLs (Tir3a, b, c) are responsible for mouse resistance to trypanosomiasis.[14] We categorised these as two continuous regions on chromosome 1 as shown in fig 3A. In this locus, we verified a total of eight SNPs in eight genes, and five genes—Ddr2, Fcgr2b, Ly9, CD84, and Fcer1a—were identified as the candidates (table 4).

Candidate genes for seizure QTL

After filtering the SNPs, 33 SNPs in 23 genes were identified. Twenty one SNPs in 16 genes were confirmed. Among these 16 genes, those related to seizure were considered as the candidates. The A-B SNPs listed in table 5 are filtered from the first query in the Celera database with the criteria described above. In Exq1, Bis1, and Bis4, SNPs in the coding regions between A/J and C57BL/6J mice were of mis-sense type as shown in table 5. In the Exq1 locus, four SNPs in four genes were sequence verified. After examination of the open reading frames, two genes (Lta4h and Iltif) were considered as candidates because of their potential link to seizure (table 5; fig 4C). In the Bis1 locus, 15 SNPs in 10 genes were sequence verified. Five genes (Cnr2, Htr1d, C1qb, Hspg2, and Kif17) were considered as the candidates (table 5). In the Bis4 locus, two SNPs in two genes, Ccne1 and Otog, were confirmed (table 5). However, the relation between these two genes and seizures is not clear at present.

After confirmation of the amino acid alteration and the position of the changed codon in each candidate gene, the changed codon regions were checked to see if they were within potential functional domains by using the NCBI

Table 2 Summary of confirmed SNPs with the confirmed amino acid alterations: mouse lung tumour

Chr	QTL	SNP ID	GenBank	Gene name	SNP type	Chr position	Cytogenetic	Conf amino acid	Description
4	MLT1	mCV23761092	NM_019753	Cdh17	Mis-sense	8620642	8q22.1	TAC(644)TCC	Cadherin 17
		mCV23760053	NM_177861	5330408M12Rik	Mis-sense	8866326	8q22.1	TAC(633)TTC	RIKEN cDNA 5330408M12 gene
		mCV23138760	XM_149412	C130086A10	Mis-sense	9785538		GGT(25)AGT	C130086A10
		mCV25009903	NM_145947	Slc26a7	Mis-sense	11352177	8q23	TTA(233)TTT	Solute carrier family 26, member 7
		mCV27558838	NM_026172	Decr1	Mis-sense	12727594	8q21.3	GTC(198)ATC	2,4-Dienoyl CoA reductase 1, mitochondrial
		mCV22925842	BC025158	2410005O16Rik	Mis-sense	16337274	8q21.2	AGC(24)GGC	RIKEN cDNA 2410005O16 gene
		mCV23438713	BC009809	Ggh	Mis-sense	16822496	8q12.2	CCA(248)GCA	-Glutamyl hydrolase
		mCV23250954	NM_030733	Gpr63	Mis-sense	21743685	6q16.1–q16.3	AGT(407)AGG	G protein coupled receptor 63
		mCV25107586	NM_026194	1810074P20Rik	Mis-sense	22013046	6q16.3	CGG(122)CAG	RIKEN cDNA 1810074P20 gene
6	MLT2	mCV23322871	AY005163	LOC272322	Mis-sense	146869761	12p12.2–p11.2	ACA(551)ATA	Brain-musde ARNT-like protein 2
		mCV23325259	NM_025620	2210417D09Rik	Mis-sense	147068643		GAA(103)CAA	RIKEN cDNA 2210417D09 gene
		mCV23327408	NM_145573	Mrps35	Mis-sense	147106699	12p11	GTC(200)ATC	Mitochondrial ribosomal protein S35
8	MLT3	mCV22993688	XM_146349	ADAM29	Mis-sense	55529448	4q34	ATT(147)AGT	A disintegrin and metalloproteinase domain 29

Chr = mouse chromosome; QTL = quantitative trait loci, MLT regions; SNP ID = specific identification number for the SNP from Celera discovery system; GenBank = transcript sequence associated with the gene containing the SNP; Gene name = name derived from Celera's annotation; Chr position = the base pair position of the SNP on the chromosome according to the Celera database; Conf amino acid = confirmed amino acid alteration with the order of A/J mice/C57BL/6J mice; Description = description derived from either Celara annotation or GenBank annotation.
MLT1/MLT2/MLT3, mouse lung tumour quantitative trait loci; SNP, single nucleotide polymorphism.

conserved domain database. Most of the candidates for the four diseases described above were within potential functional domains (table 6).

DISCUSSION

In this study, we systematically analysed single nucleotide polymorphisms in QTLs for four different diseases: lung tumours, lung injury, trypanosomiasis, and seizures. These QTLs were mapped by using A/J and C57BL/6J strains of mice as parental strains. Using this approach, we identified several candidates for each QTL (tables 2–5). The present study will facilitate the identification of modifier genes to lung tumour development, lung injury, trypanosomiasis, and seizures. The results from our study show the synergies between classical genetic studies and genomic tools such as the SNP database in dissecting the genetic basis of disease in mouse models.

Lung tumours

MLT QTL were mapped through LD analysis of the whole mouse genome using 5658 genetic markers in 25 strains of mice, resulting in three major QTLs: MLT1 (D4Mit2), MLT2 (D6Mit26), and MLT3 (D8Mit205) (fig 1A). We have identified 13 candidate genes for MLT1-3. Two candidates—Cdh17 and GPR63—were identified for the MLT1 locus. Cdh17, a liver-intestine (LI) cadherin, contains a tyrosine to serine change at codon 644. Expression of Cdh17 appears to correlate with tumour differentiation—for example, in ductal adenocarcinoma of the pancreas, LI-cadherin is highly expressed in well differentiated carcinoma but expression is reduced in less well differentiated areas and in poorly differentiated carcinomas.[27] Similarly, LI-cadherin is a marker for gastric intestinal metaplasia and well differentiated adenocarcinomas.[28] GPR63 (G protein coupled receptor 63) contains a valine to glycine change at codon 407. GPR63 (also known as PSP24B) shares 57% identity with Xenopus PSP24.[26] Xenopus PSP24 was originally identified as a functional lysophosphatidic acid (LPA) receptor. LPA may increases angiogenesis through vascular endothelial growth factor (VEGF), and also increases the level of cyclin D1 in ovarian cancer cells, increasing their proliferation.[29]

MLT2 (from 74 cM to 75cM on chromosome 6) was very close to the previously mapped pulmonary adenoma susceptibility 1 locus (Pas1, located at 72.2 cM on chromosome), a major locus affecting inherited predisposition to the development of lung tumours. SNPs that were confirmed in MLT2 include LOC272322 and Mrps35 (mitochondrial ribosomal protein S35). The LOC272322, containing a threonine to isoleucine change at codon 551, is considered to be the BMAL2 gene (brain-muscle ARNT-like protein 2). It belongs to the bHLH-PAS superfamily.[30] Members of the bHLH-PAS family are transcription factors that contain a basic helix-loop-helix (bHLH) DNA recognition motif located N-terminal to a PAS domain and composed of two imperfect direct repeats. Several lines of evidence indicate that proteins in the bHLH-PAS superfamily are involved in the regulation of cell growth and differentiation. Also, the BMAL2 gene is down-regulated in hepatocellular carcinoma, and overexpression of antisense BMAL2 RNA promotes cell proliferation.[30] Another confirmed known gene in MLT2 is Mrps35, which is reported to be overexpressed in testicular germ cell tumours.[31]

Only one SNP was verified for MLT3: ADAM29 (a disintegrin and metalloproteinase domain 29), containing an amino acid changing SNP in its coding sequence. Members of the ADAM family have been implicated in various important biological processes involving cell–cell and cell–matrix interactions, including fertilisation, muscle development, and neurogenesis. ADAM29 may function as a regulator of tumour metastasis.

Table 3 Summary of confirmed single nucleotide polymorphisms with the confirmed amino acid alterations: acute lung injury

Chr	QTL	SNP ID	GenBank	Gene name	SNP type	Chr position	Conf amino acid	Description
11	Ali1	mCV24258169	AF034955	Rad51l3	Mis-sense	89500890	GCC(212)GTC	RAD51-like 3 (*S cerevisiae*)
		mCV24260314	AF099973	Slfn2	Mis-sense	89693576	ATG(207)CTG	Schlafen 2
		mCV24673293	AF081568	Tubd1	Mis-sense	93176928	TCA(322)ACA	Tubulin, delta 1
		mCV22737741	X15313	Mpo	Mis-sense	94393241	GTC(339)ATC	Myeloperoxidase
		mCV24690994	U84389	Akap	Mis-sense	95457054	ATC(486)ATG	A kinase anchor protein
		mCV24690995			Mis-sense	95457493	ATC(340)ACC	
		mCV24691005			Mis-sense	95457537	TTG(325)TTT	
		mCV24310400	AF208663	Coil	Mis-sense	95597299	TCG(129)TTG	Coilin
		mCV24318752	NM_021213	Pctp	Mis-sense	96598777	ACA(69)GCA	Phosphatidylcholine transfer protein
		mCV24803224	AF152924	Stxbp4	Mis-sense	97142831	CAT(494)CGT	Syntaxin binding protein 4
		mCV24760749	BC016219	0610040E02Rik	Mis-sense	103698905	CAA(141)CGA	RIKEN cDNA 0610040E02 gene
		mCV23634907	NM_011732	Nsep1	Mis-sense	107548610	GCC(5)GCA	Nuclease sensitive element binding protein 1
		mCV23222844	XM_122319	Itga2b	Mis-sense	111166183	CTG(944)CCG	Integrin α2b
		mCV22581801	AK017119	4933439F11Rik	Mis-sense	111597061	GAC(211)GCC	RIKEN cDNA 4933439F11 gene
		mCV23174311	NM_016896	Map3k14	Mis-sense	111945010	ACG(486)ATG	Mitogen activated protein kinase kinase kinase 14
13	Ali2	mCV24250326	BC013531	9430025F20Rik	Mis-sense	1545145	CGT(60)CAT	RIKEN cDNA 9430025F20 gene
		mCV23916243	AF291722	Skz1-pending	Mis-sense	18660515	ATG(192)GTG	SCAN-KRAB-zinc finger gene 1
		mCV23280804	X59927	Fgfr4	Mis-sense	53228374	GTC(735)GCC	Fibroblast growth factor receptor 4
		mCV22736530	AF040747	Gprk6	Mis-sense	53508536	AGC(417)CGC	G protein coupled receptor kinase 6
17	Ali3	mCV22724951	XM_123178	Xdh	Mis-sense	75214557	ATT(241)GTT	Xanthine dehydrogenase, exon 1
		mCV23088765	AF022889	Ltbp1	Mis-sense	76300864		Latent transforming growth factor binding protein 1
		mCV23090856				76360387	GTG(221)ATG	
		mCV23089854	U19891	Cebpa-rs1	Mis-sense	80240570	ACA(875)GCA	CCAAT/enhancer binding protein α (C/EBP), related sequence 1
6	Ali4	mCV25192983	AJ133125	Imap38	Mis-sense	46253620	CGT(151)TGT	Immunity associated protein, 38 kDa
		mCV24051845	U42384	Fin15	Coding Reg.	47711614	CAG(35)CGT	Fibroblast growth factor inducible 15

Chr = mouse chromosome; QTL = quantitative trait loci, acute lung injury locus regions; SNP ID = specific identification number for the SNP from Celera discovery system; GenBank = transcript sequence associated with the gene containing the SNP; Gene name = derived from Celera's annotation; Chr position = the base pair position of the SNP on the chromosome according to the Celera database; Conf amino acid = confirmed amino acid alteration with the order of A/J mice/C57BL/6J mice; Description = description derived from either Celara annotation or GenBank annotation.
Ali1/Ali2/Ali3/Ali4, acute lung injury quantitative trait loci; SNP, single nucleotide polymorphism.

Table 4 Summary of confirmed single nucleotide polymorphisms with the confirmed amino acid alterations: trypanosomiasis resistance

Chr	QTL	SNP ID	GenBank	Gene name	SNP type	Chr position	Conf amino acid	Description
17	Tir1	mCV24105117	D49544	Kifc1	Mis-sense	33637767	GGA(124)GGT	Kinesin family member C1
		mCV24105832	AF006040	Daxx	Mis-sense	33664471	AGC(179)AAC	Fas binding protein
		mCV24106508	BC015074	Tapbp	Mis-sense	33679020	TCG(328)GCG	TAP binding protein
		mCV23315924	BC013802	Abcb2	Mis-sense	33943094	CAT(88)CGT	ATP binding cassette, subfamily B (MDR/TAP), member 2
		mCV23315948			Mis-sense	33944221	GAC(130)GGC	
		mCV23317283	BC013785	Psmb8	Mis-sense	33954831	CGA(272)GGA	Proteosome (prosome, macropain) subunit, β type 8 (large multifunctional protease 7)
		mCV23318016	BC005578	Abcb3	Mis-sense	33966976	AGG(449)AAG	ATP binding cassette, sub-family B (MDR/TAP), member 3
		mCV23950081	U28489	H2-Eb1	Mis-sense	34071900	CCG(119)CGG	Histocompatibility 2, class II antigen E β
		mCV24297661	U43691	Notch4	Mis-sense	34678075	CCA(1245)CTA	Notch gene homolog 4 (Drosophila)
		mCV24297624			Mis-sense	34679577	CCT(1437)TCT	
		mCV24296571	NM_019873	Fkbpl	Mis-sense	34740447	ACC(6)ATC	FK506 binding protein-like
		mCV24295566	NM_031176	Tnxb	Mis-sense	34777277	CGT(965)AGT	Tenascin XB
		mCV22968016			Mis-sense	34792079	CCC(2056)TCC	
		mCV22968004			Mis-sense	34799613	TCT(2386)CCT	
		mCV22965186	NM_020027	Bat2	Mis-sense	35255946	ATT(564)GTT	HLA-B associated transcript 2
		mCV22964932	U16985	Ltb	Mis-sense	35291886	TTC(97)TCC	Lymphotoxin-β
		mCV22964931			Mis-sense	35291888	TAT(98)AAT	
		mCV24434154	AB046382	Rnf23	Mis-sense	36741549	GCC(11)ACC	Ring finger protein 23
		mCV23240622	AF220121	Trim10	Mis-sense	37296242	GTG(368)ATG	Tripartite motif protein 10
		mCV22570535	BC026834	Guca1a	Mis-sense	47835206	GTC(4)ATC	Guanylate cyclase activator 1a (retina)
		mCV23317880	BC006018	Mdfi	Mis-sense	48261627	GGG(67)CGG	MyoD family inhibitor
5	Tir2	mCV24595131	NM_010727	Lnx1	Silent	68872796	GAA(417)GAG	Ligand of numb-protein X 1
		mCV23061782			Silent	68885173	CTT(255)CTG	
		mCV23860455	M30643	Fgf5	Silent	92599216	CGA(203)CGG	Fibroblast growth factor 5
1	Tir3	mCV24438398	NM_022563	Ddr2	Mis-sense	169428973	GGC(555)GAC	Discoidin domain receptor family, member 2
		mCV24426932	NM_010187	Fcgr2b	Mis-sense	170416198	CAG(200)CTG	Fc receptor, IgG, low affinity IIb
		mCV24422422	AK002459	0610010E03Ri	Mis-sense	170598815	CTC(4)TTC	RIKEN cDNA 0610010E03 gene
		mCV24411266	NM_008534	Ly9	Mis-sense	171451576	GAG(571)GGG	Lymphocyte antigen 9
		mCV24401565	NM_013489	Cd84	Mis-sense	171707823	ATG(27)GTG	CD84 antigen
		mCV24580691	NM_010184	Fcer1a	Mis-sense	173074477	GAA(182)AAA	Fc receptor, IgE, high affinity I, α polypeptide
		mCV23713265	NM_023173	Dusp12	Mis-sense	176989475	ACG(42)GTG	Dual specificity phosphatase 12
		mCV24023687	NM_010094	Ebaf	Mis-sense	184999571	TCA(2)CCA	Endometrial bleeding associated factor

Chr = mouse chromosome; QTL = quantitative trait loci, trypanosomiasis resistance locus regions; SNP ID = specific identification number for the SNP from Celera discovery system; GenBank = transcript sequence associated with the gene containing the SNP; Gene name = derived from Celera's annotation; Chr position = the base pair position of the SNP on the chromosome according to the Celera database; Conf amino acid = confirmed amino acid alteration with the order of A/J mice/C57BL/6J mice; Description = derived from either Celara annotation or GenBank annotation.
Tir1/Tir2/Tir3, trypanosomiasis resistance quantitative trait loci; SNP, single nucleotide polymorphism.

Table 5 Summary of confirmed SNPs with the confirmed amino acid alterations: seizure locus

Chr	QTL	SNP ID	GenBank	Gene name	SNP type	Chr positive	Conf amino acid	Description
10	Exq1	mCV23280479	M63848	Lta4h	Mis-sense	92458232	GCC(447)ACC	Leukotriene A4 hydrolase
		mCV22619310	NM_011629	Nr2c1	Mis-sense	93147494	TTC(327)GCC	Nuclear receptor subfamily 2, group C, member 1
		mCV25154635	AK014477	4432405K22Rik	Mis-sense	114131473	GTA(586)ATA	RIKEN cDNA 4432405K22 gene, similar to hypothetical protein
		mCV25027371	AJ249491	Iltif	Mis-sense	117159257	AAT(84)AGT	Interleukin 10 related, T cell derived inducible factor
4	Bis1	mCV24806112	BC019461	4930506L07Rik	Mis-sense	129294444	CAG(100)CGG	RIKEN cDNA 4930506L07 gene
		mCV22573664	NM_019578	Extl1	Mis-sense	129550916	ATA(190)TTA	Exostoses (multiple)-like 1
		mCV22573663			Mis-sense	129551389	TCG(32)TTG	
		mCV22570075	NM_016799	Srrm1	Mis-sense	130522801	CCG(814)CTG	Serine/arginine repetitive matrix 1
		mCV22566918	BC006598	0610011E17Rik	Mis-sense	131066845	CTT(41)CAT	RIKEN cDNA 0610011E17 gene
		mCV24214187	NM_009924	Cnr2	Mis-sense	131118228	ATG(272)ACG	Cannabinoid receptor 2 (macrophage)
		mCV22559909	NM_008309	Htr1d	Mis-sense	131636502	GTG(22)GCG	5-Hydroxytryptamine (serotonin) receptor 1D
		mCV25390842	NM_009777	C1qb	Mis-sense	132088168	AGG(115)GGG	Complement component 1, q subcomponent, beta polypeptide
		mCV25359925	NM_008305	Hspg2	Mis-sense	132762092	CTG(2670)GTG	Perlecan (heparan sulphate proteoglycan 2)
		mCV24058160	NM_010623	Kif17	Mis-sense	137068356	GTG(508)ATG	Kinesin 17
		mCV22902154	NM_019763	Mint-pending	Mis-sense	140291439	CCG(2317)CAG	Msx2 interacting nuclear target protein
		mCV22902161			Mis-sense	140291628	GCG(2254)GTG	
		mCV22902179			Mis-sense	140295089	CAG(1098)CAC	
		mCV22902180			Mis-sense	140295255	GCG(1043)GTG	
		mCV22902197			Mis-sense	140295958	GCC(809)ACC	
7	Bis4	mCV22777117	NM_007633	Ccne1	Mis-sense	30257794	CAG(179)CAT	Cyclin E1
		mCV22884690	NM_013624	Otog	Mis-sense	38762493	CAG(2020)AAG	Otogelin

Chr = mouse chromosome; QTL = quantitative trait loci, seizure locus regions; SNP ID = specific identification number for the SNP from Celera discovery system; GenBank = transcript sequence associated with the gene containing the SNP; Gene name = derived from Celera's annotation; Chr position = the base pair position of the SNP on the chromosome according to the Celera database; Conf amino acid = confirmed amino acid alteration with the order of A/J mice/C57BL/6J mice; Description = description derived from either Celara annotation or GenBank annotation.
Bis1/Bis 4, -carboline induced seizure quantitative trait loci; Exq1, exploratory and excitability quantitative trait locus; SNP, single nucleotide polymorphism.

Table 6 Potential function domains found in candidates with amino acid alterations caused by single nucleotide polymorphisms: four kinds of diseases

Category	QTL	Chr	SNP ID	Gene name	Domain description
Lung tumour	MLT1	4	mCV23761092	Cdh17	KOG4289, cadherin EGF LAG seven-pass G-type receptor
			mCV23250954	Gpr63	KOG4220, muscarinic acetylcholine receptor
	MLT2	6	mCV23322871	LOC272322	KOG3561, aryl-hydrocarbon receptor nuclear translocator
			mCV23327408	Mrps35	KOG3933, mitochondrial ribosomal protein S28
	MLT3	8	mCV22993688	ADAM29	KOG3607, meltrins, fertilins, and related Zn dependent metalloproteinases of the ADAMs family
Acute lung injury	Ali1	11	mCV24258169	Rad51l3	KOG1433, DNA repair protein RAD51/RHP55
			mCV22737741	Mpo	KOG2408, peroxidase/oxygenase
			mCV24690994	Akap	KOG2279, kinase anchor protein AKAP149, contains KH and Tudor RNA binding domains
			mCV23174311	Map3k14	KOG0198, MEKK and related serine/threonine protein kinases
	Ali2	13	mCV23280804	Fgfr4	KOG0200, fibroblast/platelet derived growth factor receptor and related receptor tyrosine kinases
			mCV22736530	Gprk6	KOG0986, G protein coupled receptor kinase
	Ali3	17	mCV22724951	Xdh	Xanthine dehydrogenase, iron-sulphur cluster and FAD-binding subunit A
Trypanosomiasis	Tir1	17	mCV24105832	Daxx	Daxx family
			mCV24297661	Notch4	KOG4289, cadherin EGF LAG seven-pass G-type receptor
	Tir3	1	mCV24438398	Ddr2	KOG1094, discoidin domain receptor DDR1
			mCV24426932	Fcgr2b	Immunoglobulin
			mCV24401565	Cd84	T cell surface antigen CD2 protein
Seizure	Exq1	10	mCV23280479	Lta4h	KOG1047, bifunctional leukotriene A4 hydrolase/aminopeptidase LTA4H
			mCV25027371	Iltif	Interleukin 10
	Bis1	4	mCV24214187	Cnr2	7 Transmembrane receptor (rhodopsin family).
			mCV25390842	C1qb	Complement component C1q domain.
			mCV25359925	Hspg2	KOG3513, neural cell adhesion molecule L1
			mCV24058160	Kif17	KOG0242, kinesin-like protein

QTL = quantitative trait loci found in different category; Chr = mouse chromosome; SNP ID = specific identification number for the SNP from Celera discovery system; Gene name = name as in Celera's annotation; Domain description = description as in NCBI Conserved Domain Database.
Ali1/Ali2/Ali 3, acute lung injury quantitative trait loci; Bis1, -carboline induced seizure quantitative trait locus; Exq1, exploratory and excitability quantitative trait locus; MLT1/MLT2/MLT3, mouse lung tumour quantitative trait loci.

Acute lung injury

In this study we identified several candidates for Ali1–4: Rad51l3, Mpo, Gprk6, and Xdh are candidates for mediating effects of early stage acute lung injury, while Fgfr4, Ltbp1, and Fin15 may play an important role in tissue repair after acute lung injury. We identified two novel candidates: Fgfr4 for Ali2 and Fin15 for Ali4.

Ali1 is located at 2 cM distal to D11Mit179[8] (fig 2A). We found 95 SNPs in 45 genes in Ali1. Among those genes that are likely to associate with acute lung injury, 15 SNPs in 13 genes were confirmed (table 3). Rad51l3 proteins—which belong to the Rad51 paralogs—are required for homologous recombinational repair (HRR) in vertebrates.[32] Purified RAD51l3 protein possesses single stranded DNA binding activity and DNA stimulated ATPase activity.[33] Thus this gene may play an important role in the repair of DNA damage induced by chemical irritants in acute lung injury. Mpo (myeloperoxidase) is released from cytoplasmic granules of neutrophils and monocytes by a degranulation process, and reacts with the H_2O_2 formed by the respiratory burst to form a complex that can oxidise a large variety of substances.[34] It is expressed in neutrophils recruited to the lung after chemical or immunological insults. Akap was considered to be a ''transduceosome'' by acting as an autonomous multivalent scaffold that assembles and integrates signals derived from multiple pathways.[35–37] Map3k (mitogen activated protein kinase kinase kinase) activates both the SEK1-JNK and MKK3/6-p38 MAP kinase pathways and constitutes a pivotal signalling pathway in cytokine and stress induced apoptosis.[38] Slfn is a new family of growth regulatory genes that affect thymocyte development and guide both cell growth and T cell development.[39] As the genes of Rad51, Mpo, Akap, Map3k14, and Slfn2 have a plausible link with inflammation from multiple causes, they are considered candidates for acute lung injury.

Ali2 is located 2 cM distal to D13Mit59[8] (fig 2A), near the gene encoding the antioxidant enzyme glutathione peroxidase (Gpx), which catalyses the production of uric acid, a potent antioxidant found in airway secretions. Our results showed that the likely candidates are fibroblast growth factor receptor 4 (Fgfr4) and G protein coupled receptor kinase 6 (Gprk6). The fibroblast growth factors and the corresponding receptors are implicated in the regulation of epithelial cell proliferation and differentiation. Gprk6 is highly expressed in peripheral blood leucocytes and in some myeloid and lymphoid cell lines.[40–42] Gprk6 may be implicated in acute lung injury by regulating the inflammatory response.

Ali3 is located on chromosome 17[8] (fig 2A). Candidates for Ali3 include Xdh and Ltbp1. The expression of the Xdh gene is regulated in a cell specific manner and is markedly affected by inflammatory cytokines, steroids, and physiological events such as hypoxia.[43] Ltbp1 is latent transforming growth factor β binding protein 1. Transforming growth factor (TGF) β cytokines are a multifunctional family that exert a wide variety of effects on both normal and transformed mammalian cells, including cell invasion, tissue remodelling, and wound healing. Latency associated proteins and latent TGFβ binding proteins regulate the secretion and activation of this cytokine.[44] TGFβ has been most thoroughly studied during the late phases of tissue repair, where it plays a critical role in the development of pulmonary fibrosis.[45] However, in a recent study, expression levels of several TGFβ inducible genes were found to be dramatically increased as early as two days after the induction of injury.[46] It has been reported that pharmacological inhibition of TGFβ protected wild-type mice from pulmonary oedema induced by bleomycin or *E coli*

endotoxin.[47] Thus Ltbp1 may function as a critical mediator in pulmonary repair after acute lung injury.

Ali4 is located on chromosome 6[9] (fig 2A). Candidates for this region are Imap38 (immunity associated protein, 38 kDa) and Fin15 (fibroblast growth factor inducible 15). Imap38 expression is found almost exclusively in the spleen, and Imap-like genes in mice are associated with proliferative and apoptotic events, suggesting a role in the control of cell death/survival.[48] The fibroblast growth factors (FGFs) play important roles in multiple physiological functions, including angiogenesis, mitogenesis, pattern formation, cellular differentiation, metabolic regulation, tissue repair, and oncogenesis. Although the function of Fin15 is unknown, the FGF-15 gene is expressed in a regionally restricted pattern in the developing nervous system, and may play an important part in regulating cell division and patterning within specific regions of the embryonic brain, spinal cord, and sensory organs.[49] Thus Fin15 may play a major role in tissue repair after acute lung injury.

Trypanosomiasis

We identified several candidates for Tir loci, including Daxx, MHC super family genes, CD84, Ly-9, Fcgr2b, Notch4, and Ddr2. Daxx (death associated protein) is a Fas binding protein, mainly located in the nucleus, functioning as a transcriptional regulator. It has been reported to mediate the Fas/JNK dependent signals in the cytoplasm as an apoptosis enhancer[50] inducing T cell death.[51] The protozoan parasite *Trypanosoma cruzi* causes a persistent, lifelong infection. During acute *Trypanosoma cruzi* infection in mice, many leucocytes undergo apoptosis. Fas induced apoptosis had been implied in modulation of the immune response against *Trypanosoma cruzi* by interfering with cytokine and NO (nitric oxide) production during the acute phase of the infection.[52–55] Thus Daxx may play an important role in trypanosomiasis by its apoptosis inducing effect.

MHC (major histocompatibility complex) encodes various genes that are essential for immune function.[56] Notch4 is a member of the Notch family of transmembrane receptors that is expressed primarily on endothelial cells. The Notch signalling pathway has an essential role in regulating embryonic vascular morphogenesis and remodelling[57]; expression of an activated form of Notch4 in embryonic vasculature leads to abnormal vessel structure and patterning and involves the modulation of the endothelial cell phenotype associating with vessel patterning and remodelling in phases of vascular development.[58] We speculate that Notch4 expression could affect vessel penetrability in the blood–brain barrier, allowing trypanosomes to invade the central nervous system in the second stage of the disease.

CD84 is a member of the CD2 subset of the Ig superfamily of cell surface molecules. It functions as a homophilic adhesion molecule and enhances interferon (IFN) secretion.[59] IFN is known to help interleukin-12 in reducing parasitaemia and prolonging survival time after acute infection.[60] In trypanosoma infection, the CD84 molecule may play a protective role in the acute phase.

Immunoglobulins have an important function in the immune system. We identified two genes of Fcer1a and Fcgr2b related to immunoglobulins in Tir3 region. Fcer1a (Fc receptor, IgE, high affinity, α polypeptide) is one subunit of the IgE receptor. The IgE receptor plays a central role in allergic disease, coupling allergen and mast cell to initiate the inflammatory and immediate hypersensitivity responses. The initiation of IgE mediated allergic responses requires the binding of IgE antibody to its high affinity receptor, Fcer1a.[61] Fcgr2b (Fc receptor, IgG, low affinity 2b) molecules have been reported to serve as a negative feedback regulator for B cell Ag receptor elicited activation of B cells; any impaired Fcgr2b function is possibly related to aberrant B cell activation.[62] Ly-9 (lymphocyte antigen 9) is a novel member of a subgroup of the immunoglobulin superfamily. Though the function of Ly-9 was unknown, other subgroups of lymphocyte antigen members are involved in adhesion reactions between T lymphocytes and accessory cells.[63]

Two Fc receptors and Ly-9 appear to be associated with the responses in the acute phase of infection. Ddr2 (discoidin domain receptor 2) is a tyrosine kinase receptor expressed in mesenchymal tissues, the ligand of which is fibrillar collagen. Olaso *et al*[64] reported that proliferation of $Ddr2^{(-/-)}$ fibroblasts was significantly decreased compared with $Ddr2^{(+/-)}$ cells. Thus Ddr2 may be involved in the diffuse myocardial fibrosis of chronic Chagasic cardiomyopathy.

Seizures

Epilepsy syndromes have been classified into more than 40 distinct types. During the past few years, several genes have been associated with epilepsy in human families and mouse models.[65] In this study we identified seven genes as the candidates. In Exq1 loci, two genes appear to be candidate genes (table 5). Lta4h (leukotriene A4 hydrolase) is a bifunctional zinc metalloenzyme that catalyses the final and rate limiting step in the biosynthesis of leukotriene B4, a classical chemoattractant and immune modulating lipid mediator.[66] Iltif is interleukin (IL)-10 related T cell derived inducible factor, also named interleukin-22. IL-22 is produced by T cells and induces the production of acute phase reactants in vitro and in vivo, suggesting a role in inflammation.[67] In the past few years, an autoimmune mechanism has emerged in a rare form of human epilepsy, Rasmussen's encephalitis.[16] Access of IgG to neuronal epitopes in the central nervous system may trigger complement mediated neuronal damage and contribute to the pathogenesis of epilepsy.[16] Perhaps the two genes Lta4h and Iltif can increase neuronal hyperexcitability by an autoimmune mechanism.

In Bis1 loci, five genes were found to be potential candidates. Cnr2 is G protein coupled peripheral cannabinoid receptor-2 (also referred to as Cb2). Cannabinoids have been shown to affect immune responses, acting on different populations of immune cells and also to inhibit T-type Ca^{2+} channels.[68] Cannabinoid acts to inhibit seizure spread in the CNS by an action on GABA,[69] and can be used as an antiepileptic.[70 71] The SNP identified in the present study may reduce the antiepileptic role of Cnr2 and increase the sensitivity of A/J strain mice to seizures.

Htr1d (5-hydroxytryptamine receptor 1D) was considered another candidate gene for its plausibility as a regulator of seizure threshold.[19] It is a known G protein coupled receptor and is expressed in the human cerebral cortex; it can couple to inhibition of adenylate cyclase activity.[72] Through this mechanism of inhibiting adenylate cyclase activity, the anticonvulsant galnon (a galanin receptor agonist) reduces the severity and increases the latency of pentylenetetrazole induced seizures in mice.[73]

C1qb (complement component C1q, B chain) mediates complement activation through the classical pathway and plays an important role in the development of antibody responses.[74 75] It may trigger complement mediated neuronal damage and contribute to the pathogenesis of seizures by allowing IgG access to neuronal epitopes in the CNS.[16]

Hspg2 (heparan sulphate proteoglycan of basement membrane) encodes perlecan (a ubiquitous heparan sulphate proteoglycan). Perlecan has an important role in neuromuscular function.[76] Mutation in Hspg2 can increase muscle hyperexcitability by modulation of ion channel expression or function through their interaction with perlecan.[77]

Kif17 (kinesin family member 17) is a neurone specific molecular motor in neuronal dendrites. It binds and

transports the NR2B subunit of N-methyl-D-aspartate receptors (NMDARs).[78] NMDARs play an important role in synaptic plasticity and neuronal morphogenesis.[78 79] Altering the expression of NMDARs in both the dentate gyrus and the subiculum can affect tissue excitability and seizure activity in seizure sensitive gerbils.[80] Moreover, presynaptic NMDARs can facilitate glutamate release onto principal neurones in the entorhinal cortex.[81] Excessive activation of glutamate receptors can destroy cortical neurones through an excito-toxic mechanism. The damaged cortex may trigger axonal sprouting and the formation of new circuits with increased excitability to seizure. Through this mechanism, Kif17 may play an important role in seizure sensitivity in mice.

Conclusions

We carried out SNP analysis based on mapped QTLs for susceptibility to diseases in animal models. Our results indicate that the combination of QTL mapping and SNP analysis is a powerful filtering tool to narrow down the gene list in QTL. Further mRNA expression screening of the candidates, for example by real time PCR, will be further improve the identification of candidate genes.

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Authors' affiliations

Y Yan, M Wang, W J Lemon, M You, Department of Surgery and Alvin J Siteman Cancer Center, Washington University in St Louis, St Louis, Missouri, USA

Correspondence to: Professor Ming You, Department of Surgery and Alvin J Siteman Cancer Center, Washington University in St Louis, School of Medicine, 660 S Euclid Avenue, Campus Box 8109, St Louis, MO 63110, USA; youm@msnotes.wustl.edu

REFERENCES

1 **Herzog CR**, Lubet RA, You M. Genetic alterations in mouse lung tumors: implications for cancer chemoprevention. *J Cell Biochem Suppl* 1997;**29**:49–63.
2 **Chen B**, Johanson L, Wiest JS, Anderson MW, You M. The second intron of the K-ras gene contains regulatory elements associated with mouse lung tumor susceptibility. *Proc Natl Acad Sci USA* 1994;**91**:1589–93.
3 **Schork NJ**, Nath SP, Lindpaintner K, Jacob HJ. Extensions to quantitative trait locus mapping in experimental organisms. *Hypertension* 1996;**28**:1104–11.
4 **Wang D**, Lemon WJ, You M. Linkage disequilibrium mapping of novel lung tumor susceptibility quantitative trait loci in mice. *Oncogene* 2002;**21**:6858–65.
5 **Fulkerson WJ**, MacIntyre N, Stamler J, Crapo JD. Pathogenesis and treatment of the adult respiratory distress syndrome. *Arch Intern Med* 1996;**156**:29–38.
6 **Luce JM**. Acute lung injury and the acute respiratory distress syndrome. *Crit Care Med* 1998;**26**:369–76.
7 **Wesselkamper SC**, Prows DR, Biswas P, Willeke K, Bingham E, Leikauf GD. Genetic susceptibility to irritant-induced acute lung injury in mice. *Am J Physiol Lung Cell Mol Physiol* 2000;**279**:L575–82.
8 **Prows DR**, Shertzer HG, Daly MJ, Sidman CL, Leikauf GD. Genetic analysis of ozone-induced acute lung injury in sensitive and resistant strains of mice. *Nat Genet* 1997;**17**:471–4.
9 **Prows DR**, Leikauf GD. Quantitative trait analysis of nickel-induced acute lung injury in mice. *Am J Respir Cell Mol Biol* 2001;**24**:740–6.
10 **Stich A**, Abel PM, Krishna S. Human African trypanosomiasis. *BMJ* 2002;**325**:203–6.
11 **Morrison WI**, Roelants GE, Mayor-Withey KS, Murray M. Susceptibility of inbred strains of mice to Trypanosoma congolense: correlation with changes in spleen lymphocyte populations. *Clin Exp Immunol* 1978;**32**:25–40.
12 **Morrison WI**, Murray M. Trypanosoma congolense: inheritance of susceptibility to infection in inbred strains of mice. *Exp Parasitol* 1979;**48**:364–74.
13 **Kemp SJ**, Iraqi F, Darvasi A, Soller M, Teale AJ. Localization of genes controlling resistance to trypanosomiasis in mice. *Nat Genet* 1997;**16**:194–6.
14 **Iraqi F**, Clapcott SJ, Kumari P, Haley CS, Kemp SJ, Teale AJ. Fine mapping of trypanosomiasis resistance loci in murine advanced intercross lines. *Mamm Genome* 2000;**11**:645–8.
15 **Ozuna J**. Seizure disorders and epilepsy. *Lippincotts Prim Care Pract* 2000;**4**:608–18.
16 **McNamara JO**. Emerging insights into the genesis of epilepsy. *Nature* 1999;**399**:A15–22.
17 **Kaneko S**, Okada M, Iwasa H, Yamakawa K, Hirose S. Genetics of epilepsy: current status and perspectives. *Neurosci Res* 2002;**44**:11–30.
18 **Robinson R**, Gardiner M. Genetics of childhood epilepsy. *Arch Dis Child* 2000;**82**:121–5.
19 **Gershenfeld HK**, Neumann PE, Li X, St Jean PL, Paul SM. Mapping quantitative trait loci for seizure response to a GABAA receptor inverse agonist in mice. *J Neurosci* 1999;**19**:3731–8.
20 **Gut IG**. Automation in genotyping of single nucleotide polymorphisms. *Hum Mutat* 2001;**17**:475–92.
21 **Jorde LB**, Watkins WS, Bamshad MJ, Dixon ME, Ricker CE, Seielstad MT, Batzer MA. The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *Am J Hum Genet* 2000;**66**:979–88.
22 **Hacia JG**, Fan JB, Ryder O, Jin L, Edgemon K, Ghandour G, Mayer RA, Sun B, Hsie L, Robbins CM, Brody LC, Wang D, Lander ES, Lipshutz R, Fodor SP, Collins FS. Determination of ancestral alleles for human single-nucleotide polymorphisms using high-density oligonucleotide arrays. *Nat Genet* 1999;**22**:164–7.
23 **Lander ES**, Schork NJ. Genetic dissection of complex traits. *Science* 1994;**265**:2037–48.
24 **Lander ES**. The new genomics: global views of biology. *Science* 1996;**274**:536–9.
25 **Kruglyak L**. The use of a genetic map of biallelic markers in linkage studies. *Nat Genet* 1997;**17**:21–4.
26 **Kawasawa Y**, Kume K, Nakade S, Haga H, Izumi T, Shimizu T. Brain-specific expression of novel G-protein-coupled receptors, with homologies to Xenopus PSP24 and human GPR45. *Biochem Biophys Res Commun* 2000;**276**:952–6.
27 **Takamura M**, Sakamoto M, Ino Y, Shimamura T, Ichida T, Asakura H, Hirohashi S. Expression of liver-intestine cadherin and its possible interaction with galectin-3 in ductal adenocarcinoma of the pancreas. *Cancer Sci* 2003;**94**:425–30.
28 **Grotzinger C**, Kneifel J, Patschan D, Schnoy N, Anagnostopoulos I, Faiss S, Tauber R, Wiedenmann B, Gessner R. LI-cadherin: a marker of gastric metaplasia and neoplasia. *Gut* 2001;**49**:73–81.
29 **Hu YL**, Albanese C, Pestell RG, Jaffe RB. Dual mechanisms for lysophosphatidic acid stimulation of human ovarian carcinoma cells. *J Natl Cancer Inst* 2003;**95**:733–40.
30 **Yeh CT**, Lu SC, Tseng IC, Lai HY, Tsao ML, Huang SF, Liaw YF. Antisense overexpression of BMAL2 enhances cell proliferation. *Oncogene* 2003;**22**:5306–14.
31 **Rodriguez S**, Jafer O, Goker H, Summersgill BM, Zafarana G, Gillis AJ, van Gurp RJ, Oosterhuis JW, Lu YJ, Huddart R, Cooper CS, Clark J, Looijenga LH, Shipley JM. Expression profile of genes from 12p in testicular germ cell tumors of adolescents and adults associated with i(12p) and amplification at 12p11.2–p12.1. *Oncogene* 2003;**22**:1880–91.
32 **Kurumizaka H**, Ikawa S, Nakada M, Enomoto R, Kagawa W, Kinebuchi T, Yamazoe M, Yokoyama S, Shibata T. Homologous pairing and ring and filament structure formation activities of the human Xrcc2*Rad51D complex. *J Biol Chem* 2002;**277**:14315–20.
33 **Braybrooke JP**, Spink KG, Thacker J, Hickson ID. The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2. *J Biol Chem* 2000;**275**:29100–6.
34 **Klebanoff SJ**. Myeloperoxidase. *Proc Assoc Am Physicians* 1999;**111**:383–9.
35 **Michel JJ**, Scott JD. AKAP mediated signal transduction. *Annu Rev Pharmacol Toxicol* 2002;**42**:235–57.
36 **Diviani D**, Scott JD. AKAP signaling complexes at the cytoskeleton. *J Cell Sci* 2001;**114**:1431–7.
37 **Jones SL**. Protein kinase A regulates beta2 integrin avidity in neutrophils. *J Leukoc Biol* 2002;**71**:1042–8.
38 **Matsuzawa A**, Ichijo H. Molecular mechanisms of the decision between life and death: regulation of apoptosis by apoptosis signal-regulating kinase 1. *J Biochem* 2001;**130**:1–8.
39 **Schwarz DA**, Katayama CD, Hedrick SM. Schlafen, a new family of growth regulatory genes that affect thymocyte development. *Immunity* 1998;**9**:657–68.
40 **Chuang TT**, Sallese M, Ambrosini G, Parruti G, De Blasi A. High expression of beta-adrenergic receptor kinase in human peripheral blood leukocytes. Isoproterenol and platelet activating factor can induce kinase translocation. *J Biol Chem* 1992;**267**:6886–92.
41 **De Blasi A**, Parruti G, Sallese M. Regulation of G protein-coupled receptor kinase subtypes in activated T lymphocytes. Selective increase of beta-adrenergic receptor kinase 1 and 2. *J Clin Invest* 1995;**95**:203–10.
42 **Haribabu B**, Snyderman R. Identification of additional members of human G-protein-coupled receptor kinase multigene family. *Proc Natl Acad Sci USA* 1993;**90**:9398–402.
43 **Hoidal JR**, Xu P, Hueckstead T, Sanders KA, Pfeffer K, Sturrock AB. Lung injury and oxidoreductases. *Environ Health Perspect* 1998;**5**:1235–9.
44 **Oklu R**, Hesketh R. The latent transforming growth factor beta binding protein (LTBP) family. *Biochem J* 2000;**3**:601–10.
45 **Giri SN**, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor beta on bleomycin induced accumulation of lung collagen in mice. *Thorax* 1993;**48**:959–66.
46 **Kaminski N**, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA. Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. *Proc Natl Acad Sci USA* 2000;**97**:1778–83.
47 **Pittet JF**, Griffiths MJ, Geiser T, Kaminski N, Dalton SL, Huang X, Brown LA, Gotwals PJ, Koteliansky VE, Matthay MA, Sheppard D. TGF-beta is a critical mediator of acute lung injury. *J Clin Invest* 2001;**107**:1537–44.
48 **Stamm O**, Krucken J, Schmitt-Wrede HP, Benten WP, Wunderlich F. Human ortholog to mouse gene imap38 encoding an ER-localizable G-protein

belongs to a gene family clustered on chromosome 7q32-36. *Gene* 2002;**282**:159–67.
49 **McWhirter JR**, Goulding M, Weiner JA, Chun J, Murre C. A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. *Development* 1997;**124**:3221–32.
50 **Yang X**, Khosravi-Far R, Chang HY, Baltimore D. Overexpression of Daxx enhances Fas-mediated apoptosis and activates the Jun N-terminal kinase (JNK) pathway. *Cell* 1997;**89**:1067–76.
51 **Ju ST**, Panka DJ, Cui H, Ettinger R, el-Khatib M, Sherr DH, Stanger BZ, Marshak-Rothstein A. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. *Nature* 1995;**373**:444–8.
52 **Martins GA**, Petkova SB, MacHado FS, Kitsis RN, Weiss LM, Wittner M, Tanowitz HB, Silva JS. Fas-FasL interaction modulates nitric oxide production in Trypanosoma cruzi-infected mice. *Immunology* 2001;**103**:122–9.
53 **Zuniga E**, Motran CC, Montes CL, Diaz FL, Bocco JL, Gruppi A. Trypanosoma cruzi-induced immunosuppression: B cells undergo spontaneous apoptosis and lipopolysaccharide (LPS) arrests their proliferation during acute infection. *Clin Exp Immunol* 2000;**119**:507–15.
54 **Nunes MP**, Andrade RM, Lopes MF, DosReis GA. Activation-induced T cell death exacerbates Trypanosoma cruzi replication in macrophages cocultured with CD4+ T lymphocytes from infected hosts. *J Immunol* 1998;**160**:1313–19.
55 **Lenardo MJ**. Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. *Nature* 1991;**353**:858–61.
56 **Banerji J**, Sands J, Strominger JL, Spies T. A gene pair from the human major histocompatibility complex encodes large proline-rich proteins with multiple repeated motifs and a single ubiquitin-like domain. *Proc Natl Acad Sci USA* 1990;**87**:2374–8.
57 **Krebs LT**, Xue Y, Norton CR, Shutter JR, Maguire M, Sundberg JP, Gallahan D, Closson V, Kitajewski J, Callahan R, Smith GH, Stark KL, Gridley T. Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev* 2000;**14**:1343–52.
58 **Uyttendaele H**, Ho J, Rossant J, Kitajewski J. Vascular patterning defects associated with expression of activated Notch4 in embryonic endothelium. *Proc Natl Acad Sci USA* 2001;**98**:5643–8.
59 **Martin M**, Romero X, de la Fuente MA, Tovar V, Zapater N, Esplugues E, Pizcueta P, Bosch J, Engel P. CD84 functions as a homophilic adhesion molecule and enhances IFN-gamma secretion: adhesion is mediated by Ig-like domain 1. *J Immunol* 2001;**167**:3668–76.
60 **Hunter CA**, Slifer T, Araujo F. Interleukin-12-mediated resistance to Trypanosoma cruzi is dependent on tumor necrosis factor alpha and gamma interferon. *Infect Immun* 1996;**64**:2381–6.
61 **Garman SC**, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS. Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. *Nature* 2000;**406**:259–66.
62 **Xiu Y**, Nakamura K, Abe M, Li N, Wen XS, Jiang Y, Zhang D, Tsurui H, Matsuoka S, Hamano Y, Fujii H, Ono M, Takai T, Shimokawa T, Ra C, Shirai T, Hirose S. Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. *J Immunol* 2002;**169**:4340–6.
63 **Sandrin MS**, Gumley TP, Henning MM, Vaughan HA, Gonez LJ, Trapani JA, McKenzie IF. Isolation and characterization of cDNA clones for mouse Ly-9. *J Immunol* 1992;**149**:1636–41.
64 **Olaso E**, Labrador JP, Wang L, Ikeda K, Eng FJ, Klein R, Lovett DH, Lin HC, Friedman SL. Discoidin domain receptor 2 regulates fibroblast proliferation and migration through the extracellular matrix in association with transcriptional activation of matrix metalloproteinase-2. *J Biol Chem* 2002;**277**:3606–13.
65 **Meisler MH**, Kearney J, Ottman R, Escayg A. Identification of epilepsy genes in human and mouse. *Annu Rev Genet* 2001;**35**:567–88.
66 **Rudberg PC**, Tholander F, Thunnissen MM, Samuelsson B, Haeggstrom JZ. Leukotriene A4 hydrolase: selective abrogation of leukotriene B4 formation by mutation of aspartic acid 375. *Proc Natl Acad Sci USA* 2002;**99**:4215–20.
67 **Xu W**, Presnell SR, Parrish-Novak J, Kindsvogel W, Jaspers S, Chen Z, Dillon SR, Gao Z, Gilbert T, Madden K, Schlutsmeyer S, Yao L, Whitmore TE, Chandrasekher Y, Grant FJ, Maurer M, Jelinek L, Storey H, Brender T, Hammond A, Topouzis S, Clegg CH, Foster DC. A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. *Proc Natl Acad Sci USA* 2001;**98**:9511–16.
68 **Chemin J**, Monteil A, Perez-Reyes E, Nargeot J, Lory P. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *Embo J* 2001;**20**:7033–40.
69 **Consroe P**, Benedito MA, Leite JR, Carlini EA, Mechoulam R. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. *Eur J Pharmacol* 1982;**83**:293–8.
70 **Karler R**, Turkanis SA. The cannabinoids as potential antiepileptics. *J Clin Pharmacol* 1981;**21**:437–48S.
71 **Munro S**, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;**365**:61–5.
72 **Weinshank RL**, Zgombick JM, Macchi MJ, Branchek TA, Hartig PR. Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1D alpha and 5-HT1D beta. *Proc Natl Acad Sci USA* 1992;**89**:3630–4.
73 **Saar K**, Mazarati AM, Mahlapuu R, Hallnemo G, Soomets U, Kilk K, Hellberg S, Pooga M, Tolf BR, Shi TS, Hokfelt T, Wasterlain C, Bartfai T, Langel U. Anticonvulsant activity of a nonpeptide galanin receptor agonist. *Proc Natl Acad Sci USA* 2002;**99**:7136–41.
74 **Armbrust T**, Nordmann B, Kreissig M, Ramadori G. C1Q synthesis by tissue mononuclear phagocytes from normal and from damaged rat liver: up-regulation by dexamethasone, down-regulation by interferon gamma, and lipopolysaccharide. *Hepatology* 1997;**26**:98–106.
75 **Rozovsky I**, Morgan TE, Willoughby DA, Dugichi-Djordjevich MM, Pasinetti GM, Johnson SA, Finch CE. Selective expression of clusterin (SGP-2) and complement C1qB and C4 during responses to neurotoxins in vivo and in vitro. *Neuroscience* 1994;**62**:741–58.
76 **Arikawa-Hirasawa E**, Le AH, Nishino I, Nonaka I, Ho NC, Francomano CA, Govindraj P, Hassell JR, Devaney JM, Spranger J, Stevenson RE, Iannaccone S, Dalakas MC, Yamada Y. Structural and functional mutations of the perlecan gene cause Schwartz-Jampel syndrome, with myotonic myopathy and chondrodysplasia. *Am J Hum Genet* 2002;**70**:1368–75.
77 **Nicole S**, Davoine CS, Topaloglu H, Cattolico L, Barral D, Beighton P, Hamida CB, Hammouda H, Cruaud C, White PS, Samson D, Urtizberea JA, Lehmann-Horn F, Weissenbach J, Hentati F, Fontaine B. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). *Nat Genet* 2000;**26**:480–3.
78 **Setou M**, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 2000;**288**:1796–802.
79 **Guillaud L**, Setou M, Hirokawa N. KIF17 dynamics and regulation of NR2B trafficking in hippocampal neurons. *J Neurosci* 2003;**23**:131–40.
80 **Suh JG**, Ryoo ZW, Ho Won MH, Oh YS, Kang TC. Differential alteration of NMDA receptor subunits in the gerbil dentate gyrus and subiculum following seizure. *Brain Res* 2001;**904**:104–11.
81 **Woodhall G**, Evans DI, Cunningham MO, Jones RS. NR2B-containing NMDA autoreceptors at synapses on entorhinal cortical neurons. *J Neurophysiol* 2001;**86**:1644–51.