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Searching for Genes that matter in Acute Kidney Injury: A Systematic Review

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Searching for Genes that matter in Acute Kidney Injury: A Systematic Review

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Abstract

Background and Objectives

Identifying patients who may develop acute kidney injury (AKI) remains challenging as clinical determinants explain only a portion of individual risk. Another factor that likely affects risk is intrinsic genetic variability. Therefore, we performed a systematic review of studies that related the development or prognosis of AKI to genetic variation.

Design

We searched MEDLINE, EMBASE, HuGEnet, SCOPUS and Web of Science for articles from 1950 to Dec 2007. Two independent researchers screened articles using predetermined criteria. Studies were assessed for methodological quality via an aggregate scoring system.

Results

The 16 included studies were of cohort or case-cohort design, and investigated 35 polymorphisms in 21 genes in association with AKI. Fifteen gene-gene interactions were also investigated in 4 separate studies. Study populations were primarily premature infants or adults who were critically ill or post-cardiac bypass. Among the studies, 5 different definitions of AKI were used. Only 1

polymorphism, *APO E e2/e3/e4*, had greater than one study showing a significant impact ($p<0.05$) on AKI incidence while of gene-gene interactions, this was true only with the *IL-6 -174G/C* and *TNF-α -308G/A* combination . The mean quality score of 5.8/10 (range 4-9), heterogeneity in the studies and the dearth of studies precluded additional meta-analysis of the results.

Conclusions

Current association studies are unable to provide definitive evidence linking genetic variation to AKI. Future success will require a narrow consensus definition of AKI, rigorous epidemiologic techniques and a shift from a priori hypothesis-driven to genome-wide association studies.

Keywords:

Acute Kidney Injury, Acute Renal Failure, Genetic Polymorphism, Single Nucleotide Polymorphism, Gene-gene Interaction, Genetic Susceptibility to Disease, Genetic Association Study, Genome-Wide Association Study

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Introduction

Acute kidney injury (AKI) is a complex disorder manifested by a rapid loss of renal function that results in retention of metabolic waste products.(1) A disease that primarily afflicts hospitalized patients, the incidence of AKI has steadily increased over the past decade, and while mortality has fallen with advances in renal replacement therapy (RRT), AKI still confers significant morbidity and mortality.(2-4) Several recent large studies of medical administrative databases have shown that in-hospital mortality for patients who develop AKI ranges from 20-28%; for those who require RRT, the mortality is higher, ranging from 28-33%.(3-5) Currently, there are no effective therapies to treat AKI. The present standard of care is to remove any potential instigating factors, optimize volume status and provide supportive care to allow renal function to recover.(1) As such, the ability to identify high-risk patients and potentially prevent AKI becomes crucial.

The current literature maintains that a patient's risk for AKI depends on a combination of acute insults and chronic co-morbidities. Acute risk factors include volume depletion, exposure to nephrotoxic agents or drugs such as aminoglycosides or radiocontrast dye, surgery, and the presence of the systemic

inflammatory response syndrome or sepsis.(6-9) Chronic risk factors include advanced age, chronic kidney disease (CKD), diabetes and congestive heart failure.(9, 10) However, models using these traditional risk factors remain inadequate.(6, 7, 11-14) Two patients with identical clinical risk factors often react differently to the same insult; one may suffer no harm while the other may require RRT. Furthermore, for those who develop RRT-requiring AKI, we as clinicians remain unable to predict which patients will progress to chronic dialysis and which will recover kidney function.

Consequently, there are likely to be clinically unobservable risk factors that contribute to one's susceptibility to AKI. Our understanding of epithelial, vascular and immune responses in kidney injury makes it likely that genetic variability in regulatory elements of these responses plays a major role in determining one's risk of AKI, given identical risk factors. This has been seen in other complex diseases, such as the discovery of a single nucleotide polymorphism (SNP) in complement factor H as a risk factor for age-related macular degeneration.(15) While there have not been any animal studies looking specifically at genetic polymorphisms and their impact on an organism's response to AKI, there has been strong evidence for differential gene expression in the murine model of AKI.(16) A genetic polymorphism that would alter that

pattern of gene expression would hence change the reaction of an organism or individual cell to an acute insult, thus making such polymorphisms a plausible risk factor for AKI.

In AKI, genetic variation in inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) have recently been proposed as risk factors.(13, 17-20) This is due to the role of inflammatory mediators in the pathophysiology of AKI, especially with ischemia and sepsis.(1, 21, 22) Genetic variations of vasomotor regulatory proteins, such as angiotensin converting enzyme (ACE) and endothelial nitric oxide synthase (eNOS), have also been investigated given the importance of vascular reactivity in the pathogenesis of AKI.(13, 21, 23-25)

However, it remains difficult to ascertain which polymorphisms are truly associated with AKI.(26) False positive reports are common in genetic association studies, and the plausibility of an association is highly dependent on the quality of the studies involved.(27) Therefore, we conducted this systematic review to evaluate the quality of published studies on genetic associations with AKI, and to ascertain if the current evidence demonstrates any polymorphism to be conclusively associated with AKI.

Hypothesis and Aims

The primary hypothesis of this study is as follows, that a given patient's risk of developing acute kidney injury after a known insult, such as ischemia, sepsis or nephrotoxic agents, is dependent on that patient's genetic makeup. That is, genetic polymorphisms that are commonly found in the population at large will render a patient either more or less susceptible to AKI. Our aim is to do a systematic review of the current existing literature on the topic and, where possible, perform a quality analysis of the studies found in order to determine the plausibility of the reported associations. Where appropriate, we plan to perform a meta-analysis to provide pooled odds ratios and relative risks.

Methods

Literature Selection

Studies were selected by searching MEDLINE, EMBASE, HuGEnet, SCOPUS and Web of Science for articles listed from 1950 up until December 2007. We used terms such as "acute kidney injury", "acute renal insufficiency" and "acute renal

failure" in combination with "genetic variation" and "polymorphism" as our search criteria. We included many terms in our search to achieve as high sensitivity as possible.

Search terms were chosen by using keywords found in the Medical Subject Headings (MeSH) controlled language. This is the search language that is used by MEDLINE to index all articles found within that database. The MeSH language tree was searched for any and all keywords or categories associated with AKI or genetics. Where possible, we excluded more specific terms by moving up the MeSH language tree in order to use more inclusive search terms. For example, "genetic polymorphism" was included under "variation (genetics)", and "acute tubular necrosis" was included within "acute kidney failure." EMBASE search terms were found by matching MeSH terms to their equivalent EMTREE terms, a different controlled language that is used within the EMBASE database. Web of Science, SCOPUS and HuGEnet do not use controlled languages and therefore a larger number of relevant keywords were used. Our exact criteria are listed in Table 1 below.

Table 1 | Search criteria

MEDLINE
Anuria, acute kidney failure, acute renal insufficiency, uremia, oliguria, creatinine, acute nephropathy or acute kidney injury
AND
Gene frequency, genotype, phenotype, variation (genetics), gene expression, gene expression regulation, genes or nucleic acid regulatory sequences
EMBASE
Oliguria, creatinine blood level, creatinine, creatinine clearance, anuria, uremia, kidney tubule necrosis, acute kidney failure, acute kidney tubule necrosis, kidney dysfunction, acute nephropathy or acute kidney injury
AND
Allelism, genetic heterogeneity, genotype, human genetics, mutation, phenotype, population genetics, population genetic parameters, gene expression or gene
Web of Science, SCOPUS and HuGEnet
Acute renal insufficiency, acute nephropathy, acute kidney injury, acute tubular necrosis, acute kidney failure, kidney dysfunction or acute renal failure
AND
Gene frequency, genetic frequency, genotype, phenotype, gene variation, genetic variation, gene expression, genetic expression, polymorphism, nucleic acid regulatory sequences, allelism, genetic heterogeneity, mutation or population genetics

A strict set of selection criteria were then applied to the articles found by the search. We included any original study that pertained to associations between AKI and human gene polymorphisms or variability. Our exclusion criteria were the following: 1) Studies with less than 20 subjects; 2) Case reports and series; 3) Animal studies; 4) Outpatient studies; 5) Studies where the etiology of AKI was HUS/TTP, ischemia/reperfusion of a kidney allograft, IgA nephropathy or glomerulonephritis.

The reasons for each of the exclusion criteria are as follows. For criteria 1), the effect found in a study with fewer than 20 subjects was as likely to be from chance as an actual effect, despite achieving statistical significance. Therefore, all such small studies were excluded. Likewise, case reports and series would likely have small numbers and also would not be able to show an association, given that no comparison would have been made to a control group or an at risk population. Animal studies were excluded because we were interested primarily in how human genetic heterogeneity contributed to the risk of AKI. Outpatient studies were excluded as AKI is primarily a disease of hospitalized patients. Finally, the final criterion was included as we hoped to capture studies of AKI from ischemia or nephrotoxic injury, which are the commonest causes of AKI in hospitalized settings.

The titles and abstracts of the articles found by our search were screened using our selection criteria and articles of interest were selected for full article evaluation (figure 1); the bibliographies of relevant articles were also searched. Full articles were then reviewed by two researchers (JL and UP) independently and a final selection was made; disagreements were discussed and a consensus decision was reached as to whether or not to include an article. On one article pertaining to AKI in preeclampsia(28), our discussion was inadequate to achieve

consensus and a third researcher (CP) evaluated the study, which resulted in its exclusion.

Data Abstraction

The following data were extracted from each study: first author, journal, year of publication, number of cases/controls, ethnicity and the clinical setting in which AKI occurred. The gene polymorphisms or combinations thereof investigated by each of the studies were recorded, and the conclusions of the authors noted. Primary and secondary endpoints of each study were recorded as well. Study quality parameters were also collected. Studies were scored in 10 categories, which are described in table 2 below. Each study then received an aggregate quality score using a system adapted from Clark et al.(29) Cohort studies were automatically given one point in the control group category to facilitate comparison; this was deemed acceptable as cohort studies are better for studying high prevalence conditions such as AKI. A study was scored as 'good' if the score was 8-10, 'fair' if the score was 5-7 and 'poor' if the score was <4.

Table 2 | Scoring system for study quality used in this systematic review

Quality Criterion	Explanation	Scoring
Control Group	Was the control group equal or larger than the case group, and can it be replicated from the description given? Cohort studies were automatically given a point.	Yes=1 No=0
Hardy-Weinberg Equilibrium	Were the case and control groups assessed for Hardy-Weinberg Equilibrium?	Yes=1 No=0
Case Group	Can the case group be replicated from the description given, and was the disease state of interest adequately defined?	Yes=1 No=0
Primer	Was the primer sequence used for genotyping or a reference to one provided?	Yes=1 No=0
Reproducability	Can the genotyping method be reproduced from the description given, and was the method validated via a second technique?	Yes=1 No=0
Blinding	Were the researchers performing the genotyping blinded to the clinical status of the patient?	Blinded=1 Not blinded = 0
Power Calculation	Was a power calculation performed?	Yes=1 No=0
Statistics	Were the major findings presented with well described tests of tests of significance?	Yes=1 No=0
Corrected Statistics	If a study examined two or more polymorphisms, were the statistics corrected for the increased risk of a false-positive finding?	Corrected=1 No correction=0
Independent Replication	Was a second, confirmatory study performed?	Yes=1 No=0

Our initial research proposal also included a plan to pool odds ratios and relative risks for individual genetic polymorphisms in a meta-analysis. Pooling of results was not undertaken, however, primarily because of marked heterogeneity in study populations as well as the definition of AKI for studies of specific genetic polymorphisms.

Results

Our search returned 7273 unique articles, of which 43 were retrieved for full article review based upon our screening of titles and abstracts. Reasons for the exclusion of studies can be found in figure 1. Of those that required full article review, only 16 articles met our eligibility criteria and were included in the analysis; initial inter-reviewer agreement on article selection was excellent (40/43). Overall, these 16 articles investigated 21 candidate genes and 35 separate polymorphisms in association with AKI. Four studies investigated the role of gene-gene interactions (i.e. combinations of polymorphisms) describing 15 different combinations and their association with AKI. Of the 21 genes studied, 7 involved inflammatory pathways, 5 with oxidative stress or ischemia, 4 with vasomotor regulation, 2 with drug metabolism and 1 each involved in angiogenesis, coagulation regulation and cholesterol metabolism.

Of the 16 included studies, 14 described genetic associations with AKI incidence (Table 3a) while 2 analyzed AKI outcomes (Table 3b). All studies had a cohort or case-cohort design; 11 were prospective studies. All study populations were of mixed gender; 12 studies investigated adults, while 4 studied neonates. Only 9 of 16 studies reported the ethnicity of their populations. The clinical setting where

Table 3a | Characteristics of Studies Examining Genetic Risk Factors for AKI Incidence

Article (author, year)	Type of study	Site	Gene Polymorphism(s) studied	# of patients	# of AKI cases	Study Population	Clinical Setting	Ethnicity	% Caucasian	Definition of AKI or clinical variable studied	Quality Score
Banyasz 2006	Retrospective case cohort	SC	VEGF -2578 C/A, +405 G/C, -460 T/C	128	41	VLBW infants	Critically ill	NR	…	Modi 1999†	5
Chew 2000	Prospective cohort	SC	APO E e2/e3/e4	564	...*	Adults	Post-CPB	NR	…	Delta Cr	7
Fekete 2003	Retrospective case cohort	SC	HSP72 +1267 A/G HSP73 +190 G/C	120	37	VLBW infants	Critically ill	Hungarian Ethnicity	100	Modi 1999	5
Gordon 2004	Prospective cohort	MC	TNF-a -238 G/A, -308 G/A LTA +365 C/G, +249G/A TNFRSF1A +1135 C/T, +36 A/G, -609 G/T TNFRSF1B +1663 A/G, +676 T/G	213	…	Adults	Critically ill with sepsis	Caucasian	100	SOFA renal score	9
Guadino 2002	Prospective cohort	SC	IL-6 -174 G/C	111	…	Adults	Post-CPB	NR	…	Delta Cr	6
Isbir 2007	Prospective case cohort	NR	ACE I/D APO E e2/e3/e4 AGTR1 +1166 A/C	248	54	Adults	Post-CPB	NR	…	Bellomo 2004 (RIFLE)‡	4
Luo 2004	Prospective cohort	SC	Haptoglobin phenotype (Hp2-2 vs (Hp2-1 and Hp1-1))	148	27	Adults	Post-CPB	Chinese, Malay Indian	0	Nash 2002§	4
MacKensen 2004	Prospective cohort	NR	APO E e2/e3/e4	130	…	Adults	Post-CPB	Mixed	NR	Delta Cr	7
Nobilis 2001	Retrospective case cohort	SC	ACE I/D AGTR1 +1166 A/C	110	42	VLBW infants	Critically ill	NR	…	Modi 1999	4

*Studies that looked at continuous outcome variables did not define cases

† Modi 1999 definition of AKI: serum Cr > 120 umol and/or serum urea > 9 mmol/L, and diuresis of 1.0 mL urine/kg/hr

‡ Bellomo 2004 (RIFLE) definition of AKI: increase in serum creatinine of 50% or greater

§ Nash 2002 definition of AKI: delta Cr > 0.5mg/dL from baseline of 1.9mg/dL or less, 1.0mg/dL from baseline of 2.0 to 4.9mg/dL and 1.5mg/dL for patients with baseline >5.0 mg/dL

Abbreviations: NR= not reported; SC= single centre; MC= multicentre; VLBW = very low birth weight; CPB = cardiopulmonary bypass; Delta Cr = change in serum creatinine in mg/dL

Table 3a continued | Characteristics of Studies Examining Genetic Risk Factors for AKI Incidence

Article (author, year)	Type of study	Site	Gene Polymorphism(s) studied	# of patients	# of AKI cases	Study Population	Clinical Setting	Ethnicity	% Caucasian	Definition of AKI or clinical variable studied	Quality Score
Stafford-Smith 2005	Prospective cohort	SC	ACE I/D Angiotensinogen +842 T/C AGTR 1 +1166 A/C eNOS +894 G/T IL-6 -174 G/C, -572 G/C, -597 G/A TNF-a +488 G/A, +376 G/A, -308 G/A APO E +448 T/C (APOE e4) APO E +586 C/T (APOE e2)	1671	…	Adults	Post-CPB	Caucasian, African-American	88	Delta Cr	8
Sirgo 2004	Prospective cohort	SC	PAI-1 4G/5G	150	11	Adults	Post-CPB	Caucasian	100	Doubling of Cr	4
Treszl 2002	Retrospective case cohort	SC	TNF-a -308 G/A IL-1b +3954 C/T IL-6 -174 G/C IL-10 -1082 G/A	92	36	VLBW infants	Critically ill with sepsis	NR	…	Modi 1999	7
Wattanathum 2005	Prospective cohort	SC	IL-10 haplotype (-592 C/A, -1082 A/G,+3367 G/A)	158	...*	Adults	Critically ill with sepsis	Caucasian	100	# of days free of renal dysfunction	5
Woodahl 2007	Retrospective cohort	SC	ABCB1 +1236C/T, +2677 G/T/A +3435 C/T,+1199 G/A	121	48	Adults	Post-HCT	NR	…	Doubling of Cr	6

*Studies that looked at continuous outcome variables did not define cases

Abbreviations: NR= not reported; SC= single centre; MC= multicentre; VLBW = very low birth weight; HCT = hematopoietic cell transplantation; Cr = creatinine

Table 3b | Characteristics of Studies Examining Genetic Risk Factors for AKI Outcome

Article (author, year)	Type of study	Site	Gene Polymorphism(s) studied	# of patients	# of AKI cases	Study Population	Clinical Setting	Ethnicity	% Caucasian	Definition of AKI or clinical variable studied	Quality Score
Perianayagam 2007	Prospective cohort	MC	NADPH Oxidase p22phox +242C/T Catalase -262 C/T	200	200	Adults	Hospitalized	Mixed	90	Dialysis or mortality	7
Jaber 2004	Prospective cohort	MC	TNF-a -308 G/A IL-10 -1082 A/G	61	61	Adults	Hospitalized	Mixed	93	Mortality and recovery of renal function	5

Abbreviations: MC = multicentre

AKI was studied fell primarily into two groups, post-cardiopulmonary bypass (7 studies) and critically ill with or without sepsis (6 studies). Of the remaining studies, two investigated all hospitalized patients with AKI, while one study investigated patients after hematopoietic cell transplant. AKI in all studies was caused by ischemia/reperfusion, sepsis or nephrotoxic agents.

The definition of AKI varied greatly in the included studies, as no fewer than 5 different definitions of AKI were used.(10, 30, 31) In addition, 4 studies opted to report only the change in serum creatinine; only the study by Stafford-Smith et al. stated a reason for doing so, that being the lack of a consensus definition for AKI at the time of the study. Two studies used hard endpoints of dialysis or mortality. The quality of studies was generally mediocre; mean quality score was 5.8/10 (range 4-9). Four studies were scored as 'poor', 10 were 'mediocre' while only 2 were of 'good' quality. Primarily, studies were lacking in the areas of reproducibility of genotyping methods, blinding, power calculations, corrected statistics for multiple comparisons and independent replication of results (Figure 2).(29)

Table 4 describes the specific polymorphisms studied and the authors' conclusions. In summary, nine polymorphisms (*NADPH Oxidase p22phox*

Table 4 | Gene characteristics

Gene	Polymorphism	Chromosome	Functional significance	Type of polymorphism	Study	Author's conclusion on association with AKI	Comparison	Association Variable	Magnitude of association	Significance of association
APO E	e2/e3/e4	19q13	Cholesterol metabolism	SNP	Isbir 2007	Yes	e4 vs. non-e4	AKI	OR=0.18†	p=0.002
	e2/e3/e4				MacKensen 2004	Yes	e4 vs. non-e4	Delta Cr	…	p=0.82
	e2/e3/e4				Chew 2000	Yes	e4 vs. e2, e3	Delta Cr	…	p=0.038, p=0.015
	e2/e3/e4				Stafford-Smith 2005	No	e4 vs. non-e4	Delta Cr	…	*p=0.009*‡
	e2/e3/e4				Stafford-Smith 2005	No	e2 vs. non-e2	Delta Cr	…	*p=0.32*§
NADPH Oxidase p22phox	+242C/T	16q24	Oxidative stress	SNP	Perianayagam 2007	Yes	CT/TT vs. CC	Dialysis or mortality	OR=2.11	p=0.01
Catalase	-262 C/T	11p13	Oxidative stress	SNP	Perianayagam 2007	No	CT/TT vs. CC	Dialysis or mortality	OR=1.05	p=0.86
ACE	I/D	17q23	Vasomotor regulation	Intron deletion	Isbir 2007	Yes	ID/DD vs. II	AKI	OR=2.37†	p=0.021
	I/D				Stafford-Smith 2005	No	II/ID vs. DD	Delta Cr	…	*p=0.004**
	I/D				Nobilis 2001	No	I vs. D allele	AKI	…	NS
AGT	+842 T/C	1q42	Vasomotor regulation	SNP	Stafford-Smith 2005	No	CC/CT vs. TT	Delta Cr	…	*p<0.0001*; 0.99*||
AGTR1	+1166 A/C	3q21-25	Vasomotor regulation	SNP	Isbir 2007	No	AA vs. AC/CC	AKI	OR=1.09†	p>0.05
	+1166 A/C				Stafford-Smith 2005	No	CC/CA vs. AA	Delta Cr	…	*p=0.84$*
	+1166 A/C				Nobilis 2001	No	C vs. A allele	AKI	…	NS
	+1166 A/C				Li 2007	No	AA vs. AC/CC	AKI	OR=0.34†	p=0.116
eNOS	+894 G/T	7q36	Vasomotor regulation	SNP	Stafford-Smith 2005	No	TT/TG vs. GG	Delta Cr	…	*p=0.17; 0.04**||
ABCB1	+1236 C/T	7q21	Drug metabolism	SNP	Woodahl 2007	No	TT vs. CC/CT	AKI	OR=1.9	p=0.21
	+2677 G/T/A				Woodahl 2007	No	TT vs. GG/GT	AKI	OR=1.6	p=0.34
	+2677 G/T/A				Woodahl 2007	No	TT vs. TA/GA	AKI	OR=2.5	p=0.33
	+3435 C/T				Woodahl 2007	No	TT vs. CT/CC	AKI	OR=1.1	p=0.82
	+1199 G/A				Woodahl 2007	No	GG vs. GA/AA	AKI	OR=3.2	p=0.14
CYP3A5	*1/*3	7q21	Drug metabolism	Splice variant	Woodahl 2007	No	*3/*3 vs. *1/*3 + *1/*1	AKI	OR=0.8	p=0.68
HSP72	+1267 A/G	6p21	Ischemia tolerance	SNP	Fekete 2003	Yes	GG vs. GA/AA	AKI	OR=3.17	p<0.01
HSP73	+190 G/C	11q24	Ischemia tolerance	SNP	Fekete 2003	No	G vs. C allele	AKI	…	NS
Haptoglobin	Hp2-2, Hp2-1, or Hp1-1	16q22	Fe metabolism, Anti-oxidant	Phenotype	Luo 2004	Yes	Hp2-2 vs. Hp1-1/2-1	AKI	*OR=5.4*	*p=0.03*

*Not significant after adjustment for multiple comparisons; †calculated from published data; ‡for caucasian subgroup; §for african american subgroup;

||for caucasian and african american subgroups, respectively

Italics - results from multivariable analysis; NS = not significant

Table 4 continued | Gene characteristics

Gene	Polymorphism	Chromosome	Functional significance	Type of polymorphism	Study	Author's conclusion on association with AKI	Comparison	Association Variable	Magnitude of association	Significance of association
TNF-α	-308 G/A	6p21	Proinflammatory	SNP	Jaber 2004	Yes	AA/AG vs. GG	Mortality	*HR=2.47*	*p=0.04*
	-308 G/A				Stafford-Smith 2005	No	AA/AG vs. GG	Delta Cr	…	p=0.17†
	-308 G/A				Gordon 2004	No	A vs. G allele	Renal SOFA score	…	NS
	-308 G/A				Treszl 2002	No	A vs. G allele	AKI	…	NS
	-238 G/A				Gordon 2004	No	A vs. G allele	Renal SOFA score	…	NS
	+376 G/A				Stafford-Smith 2005	No	AA/AG vs. GG	Delta Cr	…	NS
	+488 G/A				Stafford-Smith 2005	No	AA/AG vs. GG	Delta Cr	…	NS
LTA	+365 C/G	6p21	Proinflammatory	SNP	Gordon 2004	No	C vs. G allele	Renal SOFA score	…	NS
	+249 G/A				Gordon 2004	No	G vs. A allele	Renal SOFA score	…	NS
IL-1b	+3954 C/T	2q14	Proinflammatory	SNP	Treszl 2002	No	T vs. C allele	AKI	…	NS
IL-6	-174 G/C	7p21	Inflammation modulator	SNP	Stafford-Smith 2005	No	GG vs. CC/CG	Delta Cr	…	NS
	-174 G/C				Guadino 2002	Yes	GG vs. CC/CG	Delta Cr	…	p<0.0001
	-174 G/C				Treszl 2002	No	C vs. G allele	AKI	…	NS
	-572 G/C				Stafford-Smith 2005	No	GG vs. CC/CG	Delta Cr	…	p<0.0001*†
	-597 G/A				Stafford-Smith 2005	No	AA/AG vs. GG	Delta Cr	…	NS
TNFRSF1A	+1135 C/T	12p13	Anti-inflammatory	SNP	Gordon 2004	No	C vs. T allele	Renal SOFA score	…	NS
	+36 A/G				Gordon 2004	No	A vs. G allele	Renal SOFA score	…	NS
	-609 G/T				Gordon 2004	No	G vs. T allele	Renal SOFA score	…	NS
TNFRSF1B	+1663 A/G	1p36	Anti-inflammatory	SNP	Gordon 2004	No	A vs. G allele	Renal SOFA score	…	NS
	+676 T/G				Gordon 2004	No	T vs. G allele	Renal SOFA score	…	NS
IL-10	-1082 G/A	1q31-32	Anti-inflammatory	SNP	Jaber 2004	Yes	GG/GA vs. AA	Mortality	*HR=0.36*	*p=0.03*
	-1082 G/A				Treszl 2002	No	G vs. A allele	AKI	…	NS
VEGF	-2578 C/A	6p12	Angiogenesis	SNP	Banyasz 2006	Yes	AA vs. AC/CC	AKI	*OR=0.2*	*p=0.021*
	-460 T/C				Banyasz 2006	No	CC vs. CT/TT	AKI	…	NS
	+405 G/C				Banyasz 2006	No	CC vs. GC/GG	AKI	…	NS
PAI-1	-675 4G/5G	7q21	Coagulation activation	SNP	Sirgo 2004	No	4G/4G vs. 4G/5G + 5G/5G	AKI	…	NS

*Not significant after adjustment for multiple comparisons; †For caucasian subgroup

Italics - results from multivariable analysis; NS = not significant

+242C/T; Haptoglobin Hp2-2, 2-1 or 1-1; Heat Shock Protein 72 (HSP72) +1267A/G; Apolipoprotein E (APO E) e2/e3/e4; Angiotensin Converting Enzyme (ACE) I/D; TNF-α -308G/A; IL-6 -174G/C; Interleukin-10 (IL-10) -1082G/A; Vascular Endothelial Growth Factor (VEGF) -2578C/A) were found to have significant associations in individual studies.(17, 19, 23, 32-36) Only one polymorphism, *APO E e2/e3/e4*, had an association with AKI demonstrated in multiple studies.

Cholesterol Metabolism Genes - Apolipoprotein E

Isbir et al.(23) found that in patients undergoing coronary artery bypass grafting (CABG), carriers of the *APO E e4* allele had a decreased risk of AKI compared with non-*APO E e4* patients (unadjusted odds ratio (OR)=0.18, p=0.002). Chew et al.(33) found the same result in a similar population, as those with the *e4* allele had a smaller postoperative change in creatinine compared to those with *e3* and *e2* alleles (p=0.015 vs. *e3*, p=0.038 vs. *e2*), even after adjustment for preoperative creatinine, age, bypass time, hypertension, diabetes and ejection fraction. Mackensen et al.(37), examining a similar group of 130 CABG patients, also found this allele to have a protective effect after adjustment for a given amount of ascending aortic atheroma burden. However, without adjustment no association was found. Finally, a large study of 1671 cardiac surgical patients by

Stafford-Smith et al.(13) found no significant association between *APO E* alleles and the degree of change of postoperative creatinine in Caucasian or African-American populations.

Oxidative Stress Genes

In a study of 200 patients with mixed-cause AKI, Perianayagam et al.(36) examined the association of polymorphisms in *NADPH Oxidase p22phox* and *Catalase*, two enzymes involved with the regulation of reactive oxygen species, with dialysis and mortality. Individuals with the *T* allele in the *NADPH Oxidase p22phox* gene possessed a greater risk of dialysis or mortality (unadjusted OR=2.11). This remained true after adjusting for race, gender, age, APACHE II score and CKD. No association was demonstrated between the *Catalase* gene and AKI. This study was unique in that it is one of two studies that examined associations with firm outcomes, i.e., dialysis or mortality.(19, 36)

Vasomotor Regulation Genes

For the *ACE I/D* polymorphism three studies were identified, of which only one found significant associations with AKI. Isbir et al. found that patients with the

ACE D allele exhibited increased risk of AKI following CABG (unadjusted OR=2.37, p=0.021). However, Stafford-Smith and colleagues also examined the *ACE I/D* polymorphism and found no association with AKI.(13) Another study of 110 very low birth weight (VLBW) infants also found no association.(24)

No significant associations were found with other genes involved with vasomotor regulation, including *Angiotensinogen* (AGT), *Angiotensin Receptor 1* (AGTR1) and *eNOS*.

Inflammatory and Anti-Inflammatory Genes

Six different studies examined a total of fifteen polymorphisms in seven genes involved in inflammatory and anti-inflammatory pathways. Jaber et al.(19), in a study of 61 patients with AKI requiring hemodialysis, found that high producers of TNF-α (*-308 A*-allele carriers) possessed an increased risk of death after adjustment for APACHE II score (adjusted hazard ratio (HR)=2.5, p=0.04). However, three other studies searched for an association between this polymorphism and AKI incidence, but found none.(13, 18, 20)

The same study by Jaber et al.(19) also found that IL-10 intermediate/high producers (*-1082 G*-allele carriers) had a decreased risk of death after adjustment for the multiple organ failure score (adjusted HR=0.36, p=0.36). Treszl et al. also investigated this polymorphism in VLBW infants but found no association with AKI incidence.(20)

The *IL-6 -174G/C* polymorphism was investigated in three studies, of which only Guadino et al.(17) found a significant association. This study found that in patients undergoing CABG, *IL-6 -174GG* carriers had significantly higher elevations in perioperative creatinine vs. non-*GG* carriers (p<0.0001). However, Stafford-Smith et al.(13) found no such association in a similar population, nor did Treszl et al.(20) in VLBW infants.

Other Genes

Luo et al.(35), in a population of 148 CABG patients, found that *Haptoglobin 2-2* phenotype was associated with an increased risk of AKI (OR=5.4, p=0.03). In the *HSP72* gene, Fekete et al.(34) found that VLBW infants homozygous for the *G* allele were at increased risk for AKI (OR=3.17, p<0.01). This same group also

found that in the *VEGF -2578 C/A* polymorphism, VLBW infants homozygous for the *A* allele were protected against AKI (OR=0.2, p=0.021).(32)

None of the remaining polymorphisms investigated were found to be significantly associated with AKI.(38, 39)

Gene-gene Interactions

There were four studies that investigated gene-gene interactions and their association with AKI. The combinations studied were mostly those that augmented inflammatory or down-regulated anti-inflammatory pathways (Table 5). Wattanathum et al.(40) found that the *CGG* haplotype involving three separate polymorphisms in the *IL-10* gene (*-592 C/A, +734 A/G and +3367 G/A*) was associated with a greater degree of AKI in patients with sepsis from pneumonia. They postulated that this genotype is associated with lower anti-inflammatory IL-10 production, thus causing increased renal dysfunction.

Jaber et al.(19) investigated whether the combination of pro-inflammatory alleles from the *TNF-α -308G/A and IL-10 -1082 G/A* polymorphisms, namely the *TNF-α -308 AA* and *IL-10 -1082 AA/AG* genotypes, were associated with an increased

risk for dialysis or death. They found that patients with these genotypes had an elevated risk for dialysis or death after adjustment for APACHE II score (adjusted HR=5.17, p=0.005).

Table 5 | Gene combinations

Study	Gene polymorphism combinations	Author's conclusion on association with AKI	Magnitude of association	Significance of association
Wattanathum 2005	*IL-10 -592 C/A, +734 A/G and +3367 G/A*	yes	…	p=0.024
Stafford-Smith 2005	*eNOS +894 G/T and AGTR1 +1166 A/C*	no	…	*p=0.006**‡
	IL-6 -572 G/C and TNF-α -308 G/A	yes	…	*p=0.05*‡
	AGT +842 T/C and IL-6 -572 G/C	yes	…	*p=<0.0001*‡
	APO E e4 and AGT +842 T/C	no	…	*p=0.03**‡
	AGTR1 +1166 A/C and APO E e4	no	…	*p=0.02**‡
	eNOS +894 G/T and ACE I/D	no	…	*p=0.006**§
	AGT +842 T/C and APO E e2	no	…	*p=0.03**§
Jaber 2004	*IL-10 -1082 G/A and TNF-α -308 G/A*	yes	*HR=5.72*	*p=0.004*
Treszl 2002	*TNF-α -308 G/A and IL-6 -174 G/C*	yes	OR=6.07†	p<0.01
	TNF-α -308 G/A and IL-1b +3954 C/T	no	…	NS
	TNF-α -308 G/A and IL-10 -1082 G/A	no	…	NS
	IL-1b +3954 C/T and IL-6 -174 G/C	no	…	NS
	IL-1b +3954 C/T and IL-10 -1082 G/A	no	…	NS
	IL-10 -1082 G/A and IL-6 -174 G/C	no	…	NS

*Not significant after adjustment for multiple comparison; †calculated from published data

‡for caucasian subgroup; §For african-american subgroup

Italics - results of multivariable analysis

Treszl et al.(20) also investigated the *TNF-α -308G/A* polymorphism, but in combination with the *IL-6 -174G/C* polymorphism. VLBW infants who had a combination of the *IL-6 -174C* allele and the *TNF-α -308A* allele were at increased risk for developing AKI (OR=6.07, p<0.01). This finding was supported by Stafford-Smith et al.(13) who investigated the interaction of the *IL-6 -572G/C* (in linkage disequilibrium with *IL-6 -174C*) and *TNF-α -308G/A* polymorphisms and

found that the combination of *IL-6 -572C* and *TNF-α -308A* alleles were weakly associated (p=0.05) with AKI in Caucasians. This study also investigated the interaction between *AGT+842T/C* and *IL-6 -572G/C* polymorphisms, and found the combination to be significantly associated with AKI (p<0.0001).

Discussion

While there have been several descriptive reviews on this topic,(41-43) to our knowledge this is the first systematic review of genetic determinants of AKI. In summary, we found that there is no single polymorphism that can be conclusively described as a risk factor in AKI. The general dearth of studies, the lack of confirmatory studies and their overall mediocre quality led us to this conclusion.

Of the 35 individual polymorphisms whose association with AKI has been studied, only *APO E ε2/ε3/ε4* had a significant association in more than one study.(23, 33) Apolipoprotein E is an important protein in lipid metabolism. It is a component of very low density lipoproteins (VLDL) and is the primary ligand for the LDL receptor in the liver. It has three isoforms, each of which is encoded by a different allele of the *APO E* gene, *ε2, ε3* or *ε4.* This gene has been found to

influence the development of several complex diseases, including Alzheimer's disease and atherosclerosis.(44, 45) Recent evidence points to a regulatory role of *APO E* in inflammatory responses and may thus be affecting a given patient's susceptibility to AKI.(21, 46) However, the positive association found in 3 studies(23, 33, 37) was contradicted by the results from the largest, highest quality study by Stafford-Smith et al.(13) While it may be the case that the power of this study to find an effect was diluted by the need to correct for multiple comparisons, nevertheless its findings cast strong doubt on the positive findings of the other studies. Furthermore, the study by Mackensen et al only found the *APO ε4* allele to be protective after adjusting for ascending aortic atheroma burden; the association claimed by the authors here is especially weak given that no relationship was found after adjustment for aortic arch and descending aorta atheroma burden. Therefore, while a future higher-powered study may find a role of *APO E* in AKI, current evidence is inadequate to make such a claim.

Of the gene-gene interactions studied, the combination of the *IL-6 -174G/C* and *TNF-α -308G/A* polymorphisms is the most promising, as evidence for an association has been reported in two separate studies.(13, 20) The association between the *TNF-α -308 AA* and *IL-10 -1082 AA/AG* genotypes with dialysis or mortality demonstrated by Jaber et al., also requires mentioning.(19) While this

association has yet to be replicated, the individual polymorphisms had small but significant associations with poor outcomes, and the combined gene-gene interaction was associated with a much higher risk than either polymorphism individually, an effect known as multiplicative interaction. This group also demonstrated phenotypic differences in ex-vivo production of TNF-α and IL-10 between genotypic groups, which lends biological plausibility to this linkage.

These results however do not belie the major finding of this review, that thus far findings in this field have been inconsistent and contradictory. There is significant inter-study heterogeneity of results, as seen here with *APO E* and other polymorphisms, such as TNF-α *-308G/A* and *IL-6 -174G/C*. Causes for such heterogeneity include the large number of comparisons performed, which significantly increases the number of associations found by chance.(47) Population stratification from ethnic admixture, variable linkage disequilibrium and population specific gene-gene or gene-environment interactions are also potential sources of heterogeneity. Therefore, it is recommended for authors to independently verify their results in an independent sample group prior to publication.(48) This was an area that was found to be particularly lacking in the studies included within this systematic review (figure 1). This important mechanism of quality control is especially necessary given that initial studies

tend to show more impressive associations then subsequent research, a problem that is particularly endemic in genetic association research.(27, 49)

Indeed, the problem of false-positive reports has the potential to derail any potential research in this field before it has truly had a chance to begin. It is for this reason that evaluating the quality of a genetic association study is critical in determining whether the association claimed by the authors is indeed true. In addition to the independent replication of results, other important standards would include explicit demonstration of genotyping techniques and quality controls, adequate correction for multiple comparisons and evaluation of deviations from Hardy-Weinberg equilibrium. Recommendations on study design and quality control methods for genetic association studies have recently been published by the NCI-NHGRI Working Group on Replication in Association Studies.(27) Adherence to these standards will ensure that associations found in future studies are robust and durable.

Another problem that we have found is one that is somewhat unique to AKI, that is the lack of a gold standard outcome which has also contributed to study heterogeneity.(50) We found 5 different definitions of AKI in the studies found by our search; such variability increases the number of spurious associations and

makes inter-study comparison difficult. Use of well-defined criteria, such as the RIFLE (Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage kidney disease) or AKIN (Acute Kidney Injury Network) criteria will decrease heterogeneity and facilitate comparisons in future investigations.(30, 51) Even in this case however, caution is advised, in that both the RIFLE and AKIN criteria were formed in order to maximize sensitivity for AKI. In particular, use of the most sensitive categories of these classification systems would result in unacceptable dilution of case groups with false positive subjects. Therefore, we recommend using stage 2 of the AKIN criteria (a 200% to 300% increase in serum creatinine, or <0.5mL/kg urine output for 12 hours) as a cutoff, which would allow the creation of case groups that are both highly specific and have a well-defined phenotype. Studying outcomes such as dialysis or mortality would also alleviate this problem; however, due to the rarity of such events, an adequately powered study examining such outcomes may not be feasible.

Novel biomarkers of kidney injury that are currently under study are another potential tool to be used in creating a highly specific AKI phenotype. These biomarkers, unlike creatinine (which is a marker of the glomerular filtration rate, or GFR), are indicators of cell injury, particular that of tubular cells.(52) The best

studied of these biomarkers is Neutrophil Gelatinase-associated Lipocalin (NGAL), which animal studies have shown to be an early marker of ischemic and nephrotoxic kidney injury.(53) This biomarker has also been shown as a potential AKI marker in humans that is detectable in both serum and urine.(54, 55) Another marker, interleukin-18 (IL-18), is a mediator of ischemic AKI and has been studied in multiple clinical scenarios of AKI.(56-58) As evidence accumulates for the role of these and other novel biomarkers in AKI, they can be incorporated into existing AKI definitions and be used to further increase case group specificity.(52, 54)

Our study has limitations in several regards. This systematic review is vulnerable to several types of bias, the first being publication bias.(59) The literature has an inherent bias towards the publication of studies that find positive results.(60) Negative studies may take longer to reach print, if at all.(61) We made no attempt to retrieve unpublished data, as there is no way to know whether such efforts would have successfully corrected the bias; indeed, including such data may even worsen the problem.(59) Therefore, despite using systematic methods and a highly sensitive search strategy, this may be a non-representative sample of existing genetic association studies of AKI.

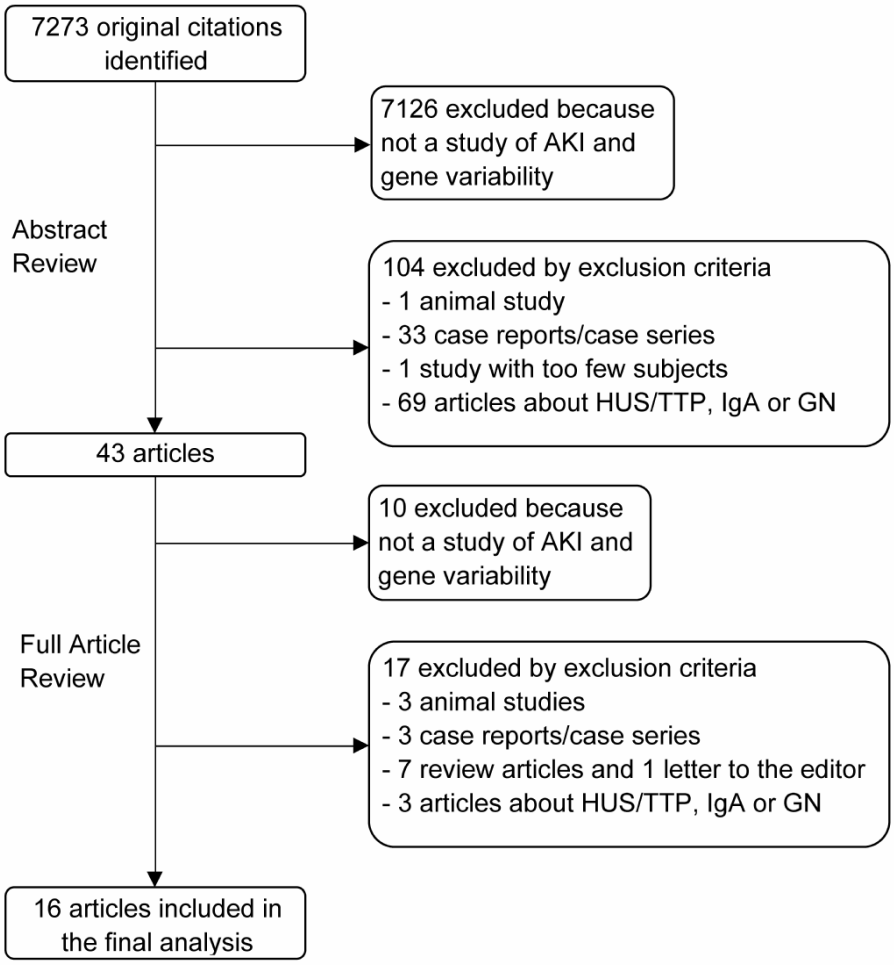
Another potential limitation is analysis reporting bias caused by researchers who report (or are forced to report due to space limitations) only a portion of their analyses.(62) This is especially salient in the reporting of gene-gene interactions, where many combinations of genetic polymorphisms may have been analysed for association but never reported.

In conclusion, our present understanding of AKI suggests that genetic heterogeneity in pathways that regulate vascular and inflammatory responses to injury provide a plausible explanation for individual variability in susceptibility to AKI. Continued efforts in this field are important, as finding genetic risk factors will allow us to identify patients at risk and implement preventive therapies. Identification of culprit genes may also elucidate the true pathophysiology of AKI. Although some genes show promise, existing candidate gene studies have been unable to find conclusive evidence to confirm any association.

An alternative approach will be to use genome-wide association (GWA) studies. This technique has been harnessed to successfully investigate genetic vulnerabilities in complex diseases like macular degeneration and diabetes.(15, 63) GWA studies have the advantages of searching for candidate

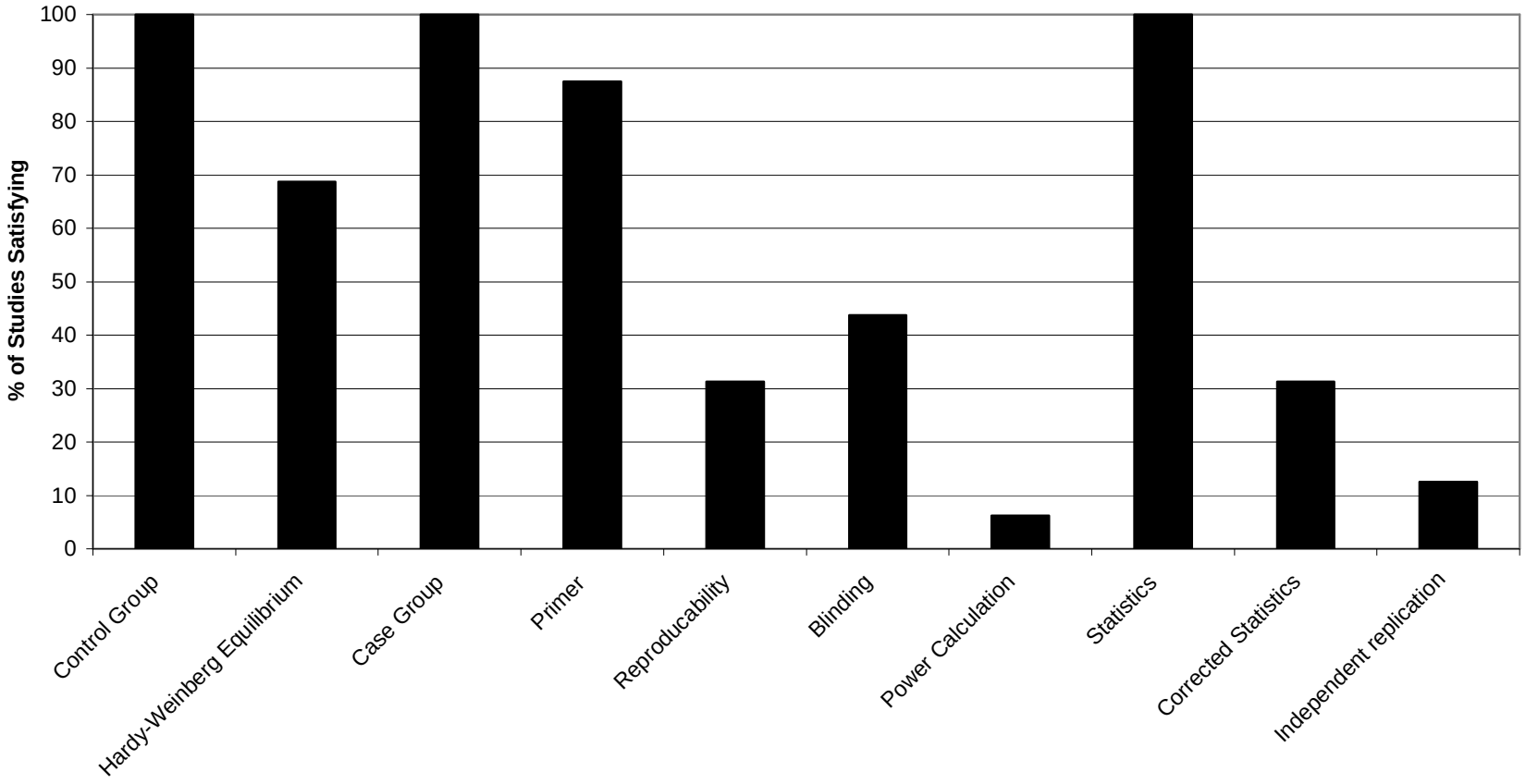
polymorphisms unhindered by previous hypotheses and greater power in demonstrating the effects of gene-gene interactions or high risk haplotypes.(64) This is especially important for complex disorders, where increased genetic risk may be from several different polymorphisms acting together. A pitfall is that GWA studies have unprecedented potential for false-positive results given the immense number of statistical tests performed; this problem can be somewhat alleviated by multistage designs and stringent requirements for statistical significance.(64) Nevertheless, the success of future work will likely depend on harnessing this revolutionary technique and the application of a true consensus definition of AKI.

Figure 1 | Selection of studies



Abbreviations: AKI - acute kidney injury; HUS/TTP - hemolytic uremic syndrome/thrombotic thrombocytopenic purpura; IgA - Immunoglobulin A nephropathy; GN - glomerulonephritis

Figure 2 | Percentage of all studies reviewed that achieved each of the ten quality criteria*



*Based on Clark MF, Baudouin SV: A systematic review of the quality of genetic association studies in human sepsis. Intensive Care Med 32: 1706-1712. 2006; please see table 2 for full descriptions of each criteria.

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