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Mini review

Genomic screening and complications of hematopoietic stem cell transplantation: has the time come?

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Summary:

The occurrence of toxic complications following hematopoietic stem cell transplantation (HSCT) is highly variable and dependent on a multitude of host, donor, and treatment factors. The increasingly broad indications for HSCT and the need to provide this treatment option to older and/or more debilitated patients emphasizes the importance of refining our methods of predicting and ameliorating these toxicities. Late complications (occurring after day 100) also pose a threat to quality of life after HSCT. Genetic polymorphisms in key molecular pathways in the host are likely to contribute significantly to the observed variability in the development HSCT-associated complications. Hepatic veno-occlusive disease and acute lung injury, two of the most serious organ toxicities that occur, represent useful paradigms for the identification of genetic polymorphisms in enzyme systems that modulate local and systemic responses to oxidant stress during transplant conditioning therapy. Ongoing studies in this area are providing clues to the prevention of adverse clinical outcomes based on the genetic milieu. This review of studies in HSCT that explore genetic risk factors for transplant complications indicates that significant progress is being made in this rapidly evolving area. However, further large-scale clinical and translational studies are needed before genomic screening can be widely used to individualize treatment.
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Clinicians are continually challenged by the need to predict the responses of individual patients to potentially toxic treatments. The increasing promise of cure for patients who undergo hematopoietic stem cell transplantation (HSCT) for life-threatening disorders is overshadowed by the very real threat of short-term, often fatal, complications resulting from the transplant regimen and graft-versus-host disease (GVHD). However, significant variation is observed between similarly treated individuals in the development of complications. It is an appealing concept, therefore, and one to which many clinicians now subscribe, that a significant portion of this variability has a genetic basis. Identifying important genetic variables will allow for better prediction of HSCT-related outcomes, and in the process of identifying these susceptibilities, it may also be possible to gain sufficient knowledge of the underlying pathophysiology of these toxicities to develop targeted interventions.

This review will focus on genomic screening of the host for the prediction of specific complications, touching briefly on selected donor factors. Other rapidly evolving areas that are not covered include molecular genetic testing to predict graft failure and disease relapse after HSCT. The role of clinical and biochemical risk factors in the pre-transplant evaluation of HSCT candidates has also been reviewed recently in this journal.[1]

Importance of context-dependent genetic effects

The study of uncommon disease-associated mutations has been invaluable to our understanding of basic pathophysiology and to the development of rational treatment strategies for diseases such as chronic myelogenous leukemia, acute promyelocytic leukemia, sickle cell anemia, and metabolic disorders. These disorders result from genetic mutations that drastically alter or abolish the function of a key cellular protein. However, more common DNA variants, or polymorphisms (generally defined as $>1\%$ prevalence in a specific population), may have more subtle functional consequences. Single-nucleotide polymorphisms (SNPs) are by far the most prevalent type of variant in the human genome, occurring on average every several hundred bases, and they are amenable to high-throughput genotyping methods.[2,3] Most SNPs have no known effect on gene function, but a proportion of them may alter the expression and/or biological activity of encoded proteins, contributing to variation in disease susceptibility and treatment toxicity. Since millions of SNPs have already been identified in the human genome,

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this phenotypic variation is most likely the result of interactions between SNPs at many different loci (genetic epistasis) and from context-dependent effects rather than single-gene effects.[4]

While the knowledge that genetic factors and environmental factors (context) interact is not new, the study of such interactions is in its infancy. The concept *of environmentally determined genetic expression* (EDGE) provides a framework for considering the combinations of exposures, genetic and environmental, that define the thresholds for expression of specific phenotypes in an individual (Figure 1).[5] This concept is based on the following observations: (1) genetically encoded variations in expressed proteins have different effects in different environmental contexts; (2) a disease phenotype is determined by both the functional magnitude of the genetic change and the severity of the environmental change; (3) rare genetic disorders (eg inborn errors of metabolism) represent one extreme with little contribution from the environment, while massive environmental insults result in phenotypes independent of genetic variation; and (4) most diseases/phenotypes fall between these extremes. Exposure to high-dose chemotherapy is a significant insult that may unmask the effects of normally silent genetic polymorphisms, and HSCT is an ideal setting in which to study these effects.

Approaches to the identification of susceptibility genes in HSCT

Genetic modifiers in complex diseases have been identified using linkage analysis (combined with positional cloning), association studies, and recently, genome-wide and microarray-based studies in unrelated affected and unaffected individuals. Linkage analyses, historically favored by geneticists, are pedigree-based and therefore unhelpful in the study of phenotypes associated with sporadic exposures (eg HSCT). Association studies (case–control and cohort studies) are popular with investigators whose knowledge of the phenotype(s) under study suggests obvious hypotheses and candidate genes. The major criticism of association studies using this 'candidate-gene approach', however, has been their frequently inconsistent or nongeneralizable results due to poor selection of the study population, racial/ethnic and geographic factors that influence the genetic background on which modifier genes act, low statistical power, and linkage disequilibrium (the problem of distinguishing the effects of closely linked genes).[2,6,7] Genome-wide approaches suffer from some of the same drawbacks but are considerably more time-efficient, less prone to bias, and capable of identifying novel genes. Their chief disadvantage is that in the absence of *a priori* hypotheses, the very large number of statistical tests performed makes it difficult to distinguish true associations from random effects.

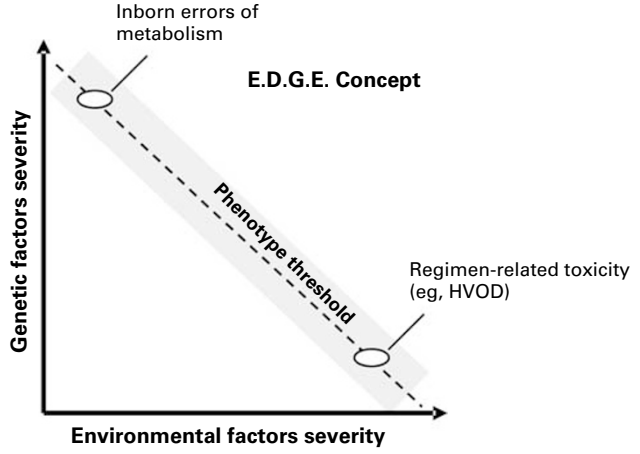

Figure 1 Concept of EDGE. An inborn error of metabolism, such as complete deficiency of a critical enzyme, has catastrophic consequences, whereas mildly diminished enzyme levels may be clinically silent except under stress conditions (eg high-dose chemoradiotherapy). HSCT = hematopoietic stem cell transplantation; HVOD = hepatic veno-occlusive disease (modified with permission from Summar *et al*[5]).

Genetic and molecular epidemiologic studies in HSCT

Complications occurring after HSCT may be divided into early, regimen-related organ toxicities such as hepatic veno-occlusive disease (HVOD), acute lung injury (ALI), and mucositis, and late complications that occur after post transplant day 100. Before considering the genes that have been associated with specific complications, it is instructive to review the role of oxidant stress in chemotherapy-related organ injury.

Oxidant stress is defined by the increased production of free radicals and other reactive oxygen/nitrogen species that damage cellular constituents.[8] The chemoradiotherapy conditioning regimens used in HSCT represent a tremendous oxidant stress on vital organs, and their intensity is the principal determinant of both HVOD and ALI.[9–14] Infection, accompanied by the release of inflammatory mediators, is a frequent contributor to oxidant stress in the post transplant period.[15,16] Nonmyeloablative regimens are now widely used in allogeneic HSCT with a significantly lower risk of acute organ toxicity, but some patients still develop complications, and myeloablative conditioning may be preferable in certain hematological malignancies.[17–19] Genetic polymorphisms in enzyme systems that normally constrain oxidant damage may partly explain the variation in the incidence and/or outcome of regimen-related complications.

Hepatic veno-occlusive disease and acute lung injury

The pathophysiology of HVOD (also called sinusoidal obstruction syndrome) involves microvascular obliteration resulting from injury to hepatic sinusoidal and venular endothelium and zone 3 hepatocytes.[15] Nitric oxide (NO), a molecule with important roles as an antioxidant and in maintaining vascular patency, is produced in hepatocytes via the hepatic urea cycle exclusively (Figure 2). The hepatic urea cycle is the only nondietary source of arginine, the essential substrate for endothelial NO synthesis throughout the body. The enzyme carbamyl-phosphate synthetase I (CPSI) catalyzes the rate-limiting step of the

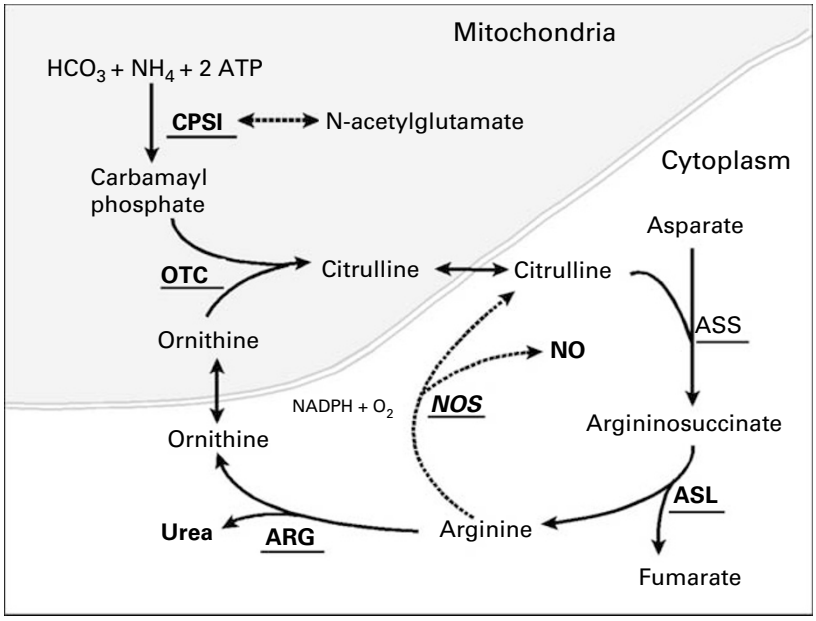

Figure 2 The hepatic urea cycle. Urea cycle enzymes are localized to either the mitochondrial or cytoplasmic spaces. Dysfunction of either CPSI or OTC results in a fall in citrulline, a rise in ornithine levels, and potentially reduced NO production. CPSI = carbamyl-phosphate synthetase 1; OTC = ornithine transcarbamylase; ASS = arginosuccinate synthetase; ASL = arginosuccinate lyase; ARG = arginase; NOS = nitric oxide synthetase (modified with permission from Summar *et al*).[21]

Table 1 The *CPSI* T1405N polymorphism and regimen-related organ toxicity in an HSCT cohort ($N = 195$)

CPSI SNP genotype	*HVOD N = 38 n(%)*	*ALI mortality N = 27 Deaths/no. with ALI (%)*	*Day 60 mortality N = 33 n(%)*
AA (n = 19)	0 (0)[a]	0/4 (0)[b]	1 (3)[c]
AC (n = 91)	22 (58)	11/15 (73)	14 (42)
CC (n = 85)	16 (42)	16/21 (76)	18 (55)

HVOD = hepatic veno-occlusive disease; ALI = acute lung injury; SNP = single-nucleotide polymorphism (T1405N).
[a]Fisher's exact P-value = 0.028 for comparison with other genotypes.
[b]Fisher's exact P-value = 0.008 for comparison of ALI mortality between *AA* and other genotypes.
[c]P-value not significant. Differences in risk of HVOD and ALI mortality remained significant ($P < 0.05$) in multivariable analysis.[24,32,33]

urea cycle, thereby controlling the availability of NO precursors.[20–22] A common SNP in the *CPSI* gene causes the substitution of asparagine (*Asn*) for threonine (*Thr*) at position 1405 (T1405N) in the critical cofactor-binding domain of the enzyme.[23] Summar *et al* studied urea cycle function and the prevalence of this SNP in relation to HVOD, ALI, and oxidant stress in 200 patients undergoing myeloablative HSCT at Vanderbilt Medical Center. Both toxicities were strictly defined and determined prospectively, before genetic testing.[15,24] In this cohort, citrulline levels fell by 57% ($P < 0.05$) during conditioning chemotherapy, and ornithine/citrulline (O/C) ratios rose nearly four-fold ($P < 0.05$), indicating impaired urea cycle function (Figure 2). Individuals with the *Asn1405* variant of CPSI (encoded by the *AA* genotype) had significantly higher plasma levels of citrulline and NO metabolites at baseline (reflecting superior urea cycle function) than those with the *Thr1405* variant (*CC* genotype). These data are consistent with *in vitro* studies of recombinant CPSI enzyme kinetics showing that the *AA*-encoded *Asn1405* variant is 20–30% more efficient than the *CC*-encoded variant.[5] The *CPSI AA* genotype was protective against HVOD and fatal lung injury after HSCT in the 195 patients analyzed (Table 1). These effects persisted after adjustment for other important risk factors (manuscript submitted). The antioxidant effects of NO may explain these findings, because plasma levels of 8-iso PGF_2-isoprostanes, the most sensitive and specific measures of oxidant stress *in vivo*, were significantly higher in patients who developed HVOD and/or ALI and in those who died by post transplant day 60 than in patients who did not suffer from these complications (Figure 3).[25] Consistent with these findings, the *CPSI* T1405N SNP appears to play a similar role in other phenotypes characterized by oxidant stress and vascular injury.[21,26,27]

Nitric oxide is also a regulator of intracellular iron metabolism during inflammation.[28] Iron augments oxidant liver injury.[29] Homozygotes and heterozygotes for the common hemochromatosis (*HFE*) mutation C282Y have increased mean liver iron content and circulating levels of reactive iron.[30,31] Another study in 166 patients from the same HSCT cohort found that *HFE* C282Y potentiates HVOD and that the *CPSI* SNP counterbalances this effect[32,33] (manuscript submitted). Multivariable analysis adjusting for other risk factors showed a significantly

increased risk of HVOD in the presence of one or two C282Y alleles (relative risk 3.7, 95% confidence interval 1.2–12.1). The risk of HVOD also increased progressively with the number of C282Y alleles present. A nonsignificant increase in day 60 mortality was noted in carriers of at least one *HFE* C282Y allele. A stratified analysis by *CPSI* genotype also showed that the *CPSI A* allele may reduce the impact of *HFE* C282Y on risk of HVOD in accord with its previously observed beneficial effect (Table 2). This study was limited primarily by the lack of quantitative data regarding iron stores in most patients, leaving unanswered the question of the mechanism of increased HVOD in carriers of *HFE* C282Y. However, carriage of one or two C282Y alleles was strongly associated with the need for vancomycin therapy during the conditioning phase ($P<0.001$), and pre transplant vancomycin therapy is a recognized HVOD risk factor.[15] Controlling for vancomycin use abolished the effect of *HFE C282Y* on HVOD, suggesting that infection may in fact be on the causal pathway. Since the *HFE* gene is on chromosome 6, another HLA class I-linked gene could conceivably be responsible for the observed effects. The *HFE* C282Y allele had no detectable effect on the incidence of ALI in this cohort.

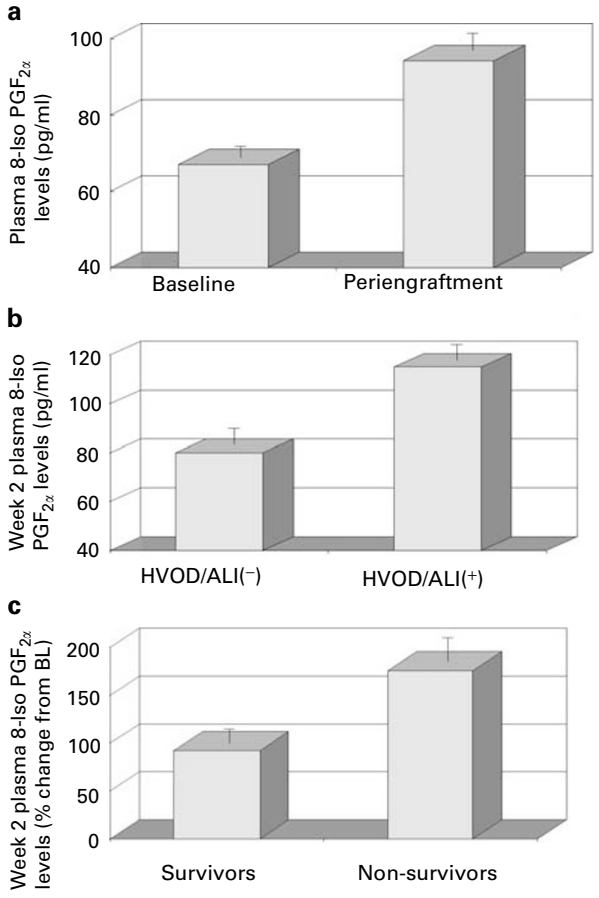

Figure 3 Oxidant stress in HVOD, ALI, and early mortality after HSCT. After conditioning therapy, plasma 8-iso $PGF_{2\alpha}$ (isoprostane) levels reach maximal levels during engraftment (measured at day 14). (**a**) Plasma isoprostane levels are significantly higher in patients who develop HVOD or ALI (**b**) and in nonsurvivors to day 60 (**c**) than in patients who do not have early complications (all *P*-values <0.05). BL = baseline.[24]

Glutathione (GSH) is perhaps the single most important cellular antioxidant in the liver and lungs, and considerable variation has been described in glutathione-metabolizing enzymes. A polymorphism in the *GST* gene that encodes glutathione *S*-transferase, which catalyzes the conjugation of busulfan and the active metabolite of cyclophosphamide to glutathione, has been reported to result in a significantly increased incidence of HVOD in thalassemic patients undergoing HSCT.[34] Pharmacokinetic studies suggest that this effect is due either to depletion of reduced glutathione or toxicity of GSH-conjugated busulfan metabolites.

Lung injury frequently follows HVOD, and shares with it many important features (Figure 4).[35–37] The final common pathway in ALI involves inflammatory cell activation, proteolytic enzyme and cytokine release, and damage to lung parenchyma, vascular endothelium, and airways. This sequence of events, combined with exuberant activation of the coagulation cascade and surfactant dysfunction, leads to reduced gas exchange. In mouse models, ALI is associated with enhanced expression of genes involved in oxidant stress and antioxidant responses, for example,

Table 2 Effects of *HFE* C282Y (upper panel) and stratification by *CPSI* T1405N genotype (lower panel) on HVOD and day 60 mortality[32,33]

No. of HFE C282Y alleles	*HVOD (95% CI)*[a] *(N = 30)*		*Day 60 mortality (95% CI)*[b] *(N = 24)*	
0 ($n=132$)	1.0		1.0	
1 or 2 ($n=34$)	3.7 (1.2, 12.1)		1.7 (0.4, 7.9)	
2 ($n=10$)	8.6 (1.5, 48.9)		0.4 (0.03, 6.3)	
CPSI variant	*HVOD cases by no. of HFE C282Y alleles*			*P-value (trend)*
	0 (wt) (%)	*1 (heterozygote) (%)*	*2 (homozygote) (%)*	
AA ($N=18$)	0	0	0	—
AC ($N=79$)	20	38	50	0.10
CC ($N=69$)	11	25	43	0.02

SNP = single-nucleotide polymorphism; HSCT = stem cell transplantation; HVOD = hepatic veno-occlusive disease, defined by strict Baltimore criteria.[15]
CPSI = carbamyl-phosphate synthetase I; 95% CI = 95% confidence interval.
Variants of CPSI enzyme: *AA*, *Asn1405*; *AC*, *Asn/Thr1405*; *CC*, *Thr1405*.
[a]Relative risk of HVOD, adjusted for previous radiation, donor type (related/unrelated), and pulmonary diffusing capacity for carbon monoxide at baseline (normal or $<70\%$ of normal).
[b]Relative risk of death on or before day 60 post transplant, adjusted for donor type and acute lung injury. (Patients with and without *HFE* mutations did not differ at baseline with respect to demographic variables, liver function, *CPSI* genotype, transplant type (allogeneic *vs* autologous), or conditioning regimen.)

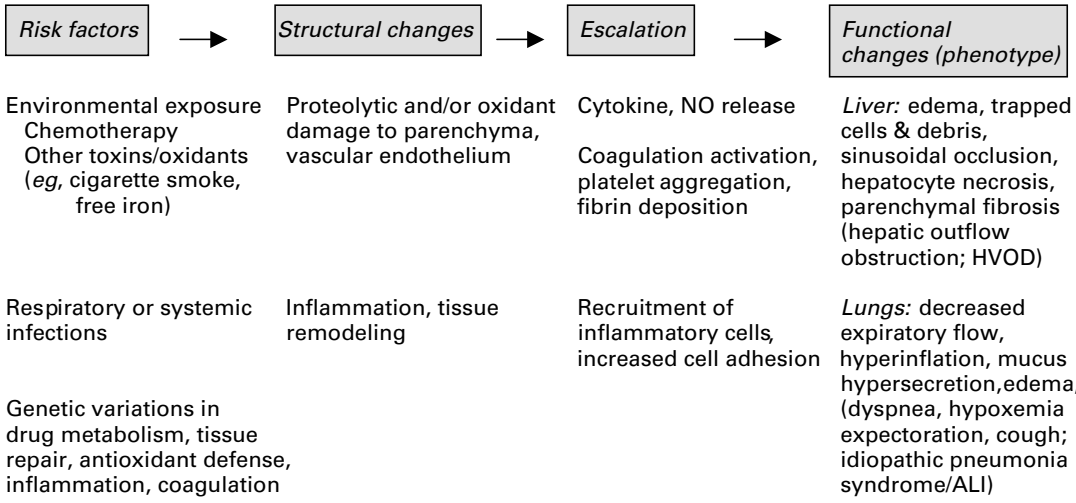

Figure 4 Common features in the pathophysiology of liver and lung injury. NO = nitric oxide; HVOD = hepatic veno-occlusive disease; ALI = acute lung injury.

Table 3 Candidate gene classes in oxidant toxicities after HSCT (eg lung, liver)

Functional gene class	*Examples of genes*	*Reference(s)*
Cellular antioxidant synthesis, metabolism	*GCLC, GST*	34, 39, 40, 79
Cytokines, chemokines, and their receptors	*IL-10, TNF-α, MCP-1 CCR-1, IL-6, IFN-γ*	15, 16, 41–43, 48, 77, 121, 122
Vascular tone, integrity, remodeling	*ACE, MTHFR*, serotonin transporter[a]	44, 49–54, 74
Growth factors	*TGF-β*	123
Proteases/antiproteases	*MMP*[a], *BMPR* (type II)[a]	2, 29
Genes involved in iron transport and metabolism	*HFE*	32, 33, 96, 124
Nitrogen/NO metabolism	*CPSI*	5, 21
Cytochrome P-450 metabolism	*CYP3A, CYP2E*[a]	15, 125, 126
Coagulation factors	Factor V Leiden, Prothrombin	15, 38, 41, 46, 47
Mitochondrial haplotypes	None	127

Genes: GCLC, glutamate L-cysteine ligase; *ACE*, angiotensin-converting enzyme 1; *MTHFR*, methylenetetrahydrofolate reductase; *MMP*, matrix metalloproteinase; *TGF-β*, transforming growth factor beta; *BMPR*, bone morphogenetic protein receptor; *IL*, interleukin; *TNF-α*, tumor necrosis factor alpha; *MCP-1*, monocyte chemoattractant protein 1; *CCR-1*, chemokine receptor-1; *IFN-γ*, gamma interferon; *HFE*, hemochromatosis; *CPSI*, carbamyl-phosphate synthetase 1; *CYP*, cytochrome P450 oxidase; *GST*, glutathione S-transferase; GSH = reduced glutathione; NO = nitric oxide.
[a]These genes were not studied in the setting of HSCT.

antiproteases and extracellular matrix repair proteins. The expression of proteins involved in surfactant production is markedly reduced, whereas expression of procoagulant and antifibrinolytic proteins is consistently increased.[38] Polymorphisms in the glutamate-L-cysteine ligase catalytic subunit gene (*GCLC*), which encodes the rate-limiting enzyme in glutathione synthesis, are potentially important in recovery from ALI, and studies to determine their functional importance are currently in progress at Vanderbilt[39,40] (manuscript submitted). Other than these studies of HSCT-related lung injury conducted at our institution, a few studies of candidate genes in ALI have been conducted in the setting of sepsis, trauma, or lung transplantation, and in mouse models of allogeneic HSCT.[41–43] These genes, and others that have been implicated in chronic obstructive pulmonary disease and pulmonary hypertension, deserve further study as potential modifiers of both acute and chronic pulmonary complications after HSCT.[2,44,45] Polymorphisms in many of these genes, such as those encoding coagulation factors, matrix metalloproteinases, and inflammatory mediators, may also be relevant to HVOD.[29,46–48] Functional classes of genes that may influence oxidant toxicities after HSCT are listed in Table 3.

Other early complications

Studies that have demonstrated associations between specific genetic variants and early complications of chemotherapy and HSCT are summarized in Table 4.

Mucositis: methylenetetrahydrofolate reductase polymorphisms and methotrexate toxicity

In HSCT, mucositis is largely due to the conditioning regimen, particularly regimens that incorporate total-body irradiation (TBI) and methotrexate (MTX) administration for acute GVHD (aGVHD) prophylaxis. The enzyme methylenetetrahydrofolate reductase (MTHFR), which catalyzes the reduction of 5,10-methyleneTHF to 5-methylTHF, provides the carbon donor for methionine synthesis and regulates folate metabolism. This enzyme is therefore critical for normal DNA synthesis, methylation,

Table 4 Genetic association studies of common complications after chemotherapy and/or HSCT

Author (ref.)	*No. of transplants*	*Genes*	*Comment*
Infection			
Mullighan *et al*[69]	90	*MBL2*	Multicenter review of allo-HSCT patients and donors analyzed *MBL2* coding or promoter-region mutations in relation to neutrophil recovery, febrile days, culture-confirmed infection; in multivariable analysis, *MBL2* (d) coding mutations and absence of high-producing *HYA* promoter haplotype (r) associated with major bacterial infection after neutrophil recovery; this effect was independent of d/r genetic matching at *MBL2* loci; *MBL2* genotypes were not associated with aGVHD. No adjustment for factors other than genotype
Rocha *et al*[68]	107	*$F_c\gamma$RIIa, IIIb* *MPO*	Retrospective, single-center study of HLA-identical HSCT patients studied several host-defense and inflammatory-gene polymorphisms and risk of all infections within 180 days of follow-up; multivariable analysis (adjusting for factors other than genotype that affect hematopoietic recovery) found associations between *$F_c\gamma$RII* a (r) genotype and risk of overall infections, *MPO* (d) genotype and severe bacterial infection, *$F_c\gamma$RIIIb* (d) genotype and time to neutrophil recovery
Mucositis			
Ulrich *et al*[50]	220	*MTHFR*	Single-center study of CML patients who underwent allo-HSCT (Cy/TBI or Bu/Cy) and MTX prophylaxis for aGVHD analyzed oral mucositis index (OMI) score, engraftment time for platelets and neutrophils, bilirubin, and MTHFR activity in relation to *MTHFR* genotype; lower MTHFR activity (*TT* genotype) associated with slower platelet recovery and higher mean mucositis score
Robien *et al*[51]	133		Single-center study having overlap with above study population[50]; excluded patients who required leucovorin rescue; multivariable analysis adjusting for conditioning regimen, pre-transplant vitamin use, age, BMI $\geqslant$25 showed higher mean mucositis scores associated with *MTHFR* genotype (*TT*)
Kalayoglu-Besisik *et al*[53]	53		(Letter) Single-center Turkish study in HLA-identical HSCT recipients who got Bu/Cy conditioning, CSA and short-course MTX GVHD prophylaxis. Follow-up to engraftment or day 28 showed no difference in incidence of mucositis grade IV or less or in seventy of mucositis by *MTHFR* genotype.
Toffoli *et al*[49]	43		Single-center study in refractory ovarian cancer patients treated with chronic low-dose, oral MTX$\pm$carboplatin analyzed plasma homocysteine and MTX levels and mucositis severity in relation to *MTHFR* polymorphisms; patients with grade III or IV mucositis had $\uparrow$ prevalence of the *MTHFR* low-producing genotype and hyperhomocysteinemia compared to baseline and patients with other *MTHFR* genotypes. MTX levels were similar across genotypes, but not evaluable in $>$50% of patients with toxicity.
Toffoli *et al*[128]	6		(Letter) Case series in breast cancer patients undergoing adjuvant CMF chemotherapy showed increased toxicity in patients with *MTHFR TT* genotype
Chiusolo *et al*[54]	78		Single-center review of *MTHFR* genotype in patients receiving maintenance chemotherapy including MTX 15–30 mg/m^2 weekly for $\sim$2 years for acute leukemia; 61 patients evaluable for MTX intolerance, defined as neutrophil count $<0.5\times10^6$/l, $>$2-fold $\uparrow$ in bilirubin and liver enzymes, and/or mucositis (assessed by OMI), nausea, vomiting, diarrhea, or fever requiring dose reduction or treatment delays; significantly more MTX intolerance seen in patients with *TT vs* other genotypes
HVOD			
Kallianpur *et al*[32,33] Summar *et al*[24]	166 200	*HFE, CPSI*	Single-center case-cohort studies including auto- and allo-HSCT recipients, in which HVOD (Baltimore criteria) was assessed prospectively. Genetic analyses were blinded to outcome. Multivariable analysis showed progressive $\uparrow$ in adjusted relative risk of HVOD with $\uparrow$ number of *HFE* C282Y alleles present (0, 1, or 2 *C282Y* alleles); stratification by *CPSI* genotype suggested effect modification by *CPSI AA* genotype. Iron stores were not measured.
Srivastava *et al*[34]	114	*GST*	Single-center, pharmacokinetic study of *GST* polymorphisms in children with β-thalassemia major who underwent HSCT and Bu/Cy$\pm$ATG conditioning; found $\uparrow$ incidence of HVOD (Baltimore criteria) and lower Bu concentration in patients with *GSTM1* null genotype
Ertem and Akar[46]	10	Factor V Leiden	(Letter) Pediatric case series: allo-HSCT and Bu/Cy or Cy/ATG conditioning; 3 of 4 HVOD cases had factor V Leiden; no prothrombin mutations

Table 4 Continued

Author (ref.)	*No. of transplants*	*Genes*	*Comment*
HVOD			
Duggan *et al*[47]	66	Prothrombin	(Letter) Single-center case–control study in allo-HSCT patients with many different regimens and methods of GVHD prophylaxis. Prevalence of prothrombin mutation was 13% in patients with strictly defined HVOD compared to patients without ($P = 0.05$) and much higher than in the general population (no adjustment for other risk factors). No HVOD cases had the factor V Leiden mutation.
ALI			
Summar *et al*[24]	200	*CPSI*	Single-center studies in the same HSCT cohort found *CPSI AA* (r) and a triplet repeat polymorphism of *GCLC* (r) to be protective against fatal ALI in HSCT recipients, adjusting for conditioning regimen and other risk factors
Kallianpur *et al*[32,40]	132	*GCLC*	
Hildebrandt *et al*[43]	22	*CCR-1, MCP-1*	Single-center study that correlated BAL findings in mouse models of ALI or chronic noninfections lung dysfunction after allo-HSCT with BAL findings in allo-HSCT patients with ALI and in healthy volunteers; found ↑ MCP-1 and ↑ CCR2 expression in mouse lung after allo-HSCT, reduced ALI severity associated with CCR2−/− donor or pre-treatment with anti-MCP-1. Newly diagnosed ALI in humans also associated with ↑ MCP-1 in BAL fluid
GVHD (Single-institution studies in allo-HSCT, except where otherwise noted)			
Lin *et al*[60]	993	*IL-10*	All HLA-identical sibling donors; CSA and MTX used for GVHD prophylaxis; Recipient *IL-10*$^{-592}$A allele and linked promoter haplotype associated with ↓ risk of grade III or IV aGVHD. This result was confirmed in a separate cohort of 423 patients.
Socié *et al*[61]	100	*IL-10, IL-6*	HLA-identical sibling HSCTs; GVHD prophylaxis with CSA + MTX in 90% of patients; independent of other clinical risk factors, aGVHD was associated with polymorphisms in *IL-10* (d/r); *IL-6* (r) polymorphisms correlated with cGVHD
Middleton *et al*[62]	49	*IL-10, TNFα*	*TNFd3/d3* (r) genotype and ↑ *IL-10*$^{-1064}$ (r) microsatellite repeat length associated with ↑ grade III/IV aGVHD in matched-sibling HSCT (CSA monotherapy for GVHD prophylaxis)
Cavet *et al*[129]	144	*IL-10, TNFα*	HLA-identical sibling HSCTs; CSA and MTX used for GVHD prophylaxis; *TNF d3/d3* (r) genotype and *IL-10*$^{-1064}$ (r) microsatellite repeat length associated with grade III/IV aGVHD
Cavet *et al*[73]	80	*IFNγ, TNFα, IL-6, IL-10*	HLA-identical sibling HSCTs; GVHD prophylaxis was CSA ± MTX or corticosteroids; *IFNγ* (r) genotype (r) associated with more severe grades of aGVHD; confirmed association between *TNFd* (r) and *IL-10*$^{-1064}$ (r) mutations and aGVHD; *IL-6*$^{-174}$ (r) genotype correlated with ↑ risk of cGVHD and with trend to ↑ aGVHD
Remberger *et al*[65]	30	*TNFα*	All matched-unrelated donor HSCTs (molecular typing); *TNFd* microsatellite genotype (r) associated with ↑ risk of aGVHD grades II–IV and higher TNFα levels during conditioning. GVHD prophylaxis was CSA + MTX
Rocha *et al*[68]	107	*IL-10, IL-1Ra*	*IL-10* (r) and *IL-1Ra* (r) genotypes associated with ↑ risk of cGVHD; *IL-1Ra* (d) genotype associated with ↑ aGVHD grades II–IV
Nordlander *et al*[66]	196	*TNFα, IL-10*	HLA-identical sibling HSCT in 85 patients, 111 matched-unrelated donors; GVHD prophylaxis mostly CSA + MTX; *TNFd* (r) and *IL-10*$^{-1064}$ (r) genotypes correlated with ↑ aGVHD grades II–IV
Takahashi *et al*[64]	62 (aGVHD) 54 (cGVHD)	*TNFα, IL-10*	Related (matched or mismatched) and unrelated-donor HSCT with CSA + MTX for GVHD prophylaxis in most patients; donor-derived *TNF*$^{-308}$ and *IL-10* alleles (*TNF2 A*, *IL-10*$^{-1082}$*G*) associated with ↑ severe aGVHD and cGVHD
GVHD			
Gagne *et al*[59]	75	*KIR*	This study in unrelated (HLA-identical or mismatched) and related (HLA-identical) donor HSCT found 0% aGVHD in sibling HSCT and 100% in unrelated-donor transplants if recipient *KIR* genotype was included in the donor genotype (ie donor had the same number or more activating KIRs).
Renal failure			
Juckett *et al*[74]	106	*ACE*	Single-center retrospective study in 1-year survivors of allo-HSCT whose renal function was followed for up to 3 years; rate of decline in creatinine clearance associated with *ACE* genotype on multivariable analysis despite no difference in survival across genotypes. Conditioning regimen was the same in all patients but not standard for most institutions; GVHD prophylaxis was T-depletion of graft and CSA; renal shielding changed twice during the course of the study; ACE inhibitor drug use was not analyzed

Table 4 Continued

Author (ref.)	*No. of transplants*	*Genes*	*Comment*
Overall mortality Lin *et al*,[60] Cavet *et al*,[129] Rocha *et al*,[68] Srivastava *et al*,[34] Summar *et al*,[24] Kallianpur *et al*[32,33,40]		*IL-10* (r), *TNFα* (r), *$F_c\gamma$R IIIb* (d), *MPO* (d), *GST* (r), *CPSI* (r), *GCLC* (r)	Different polymorphisms in these genes associated with ↑ or ↓ survival in single-institution studies (see above)
Cook *et al*[58]	220	*KIR, HLA-C*	First study to demonstrate the importance of HLA-C group of recipient in survival in the absence of HLA mismatch, restricted to patients with myeloid leukemias. Donor *KIR2DS2* further decreases survival in homozygous *HLA-C2* recipient despite no detectable effect on aGVHD. No effect of GVHD prophylactic regimen was noted in the analysis

Genes listed are only those found to be significantly associated with complications of interest, not necessarily the only genes analyzed. (d) = donor mutation; (r) = recipient mutation. Estimated prevalence of variant genotypes alleles in Northern Europe and the US: *MTHFR* (10–12%TT), *HFE* 282Y heterozygote (10–12%), *CPSI* 1405N homozygote (11%), *ACE DD* genotype (29%), *GSTM1* null genotype (38–50%), *GCLC* allele for 7 triplet repeats in 5′ untranslated region (62% in Caucasians; 45% in African-Americans), factor V Leiden (5%), prothrombin *20210A* heterozygote (<2%).

and repair.[49] MTX inhibits MTHFR activity and independently inhibits DNA methylation. A common thermolabile polymorphism in the *MTHFR* gene, designated *C677T*, results in heterozygous (*CT*) or homozygous (*TT*) enzyme variants with significantly reduced activities when compared to the *CC* variant (30% in *TT* homozygotes). The frequencies of *CT* and *TT* genotypes in the HSCT population are estimated to be 43 and 10–12%, respectively. This SNP has been associated with increased oral mucositis and delayed platelet recovery in patients undergoing allogeneic HSCT for chronic myelogenous leukemia who received cyclophosphamide/TBI or busulfan/cyclophosphamide conditioning and MTX prophylaxis for GVHD.[50] Controlling for other risk factors, patients with lower MTHFR activity and the *TT* genotype had significantly higher mean mucositis scores. These results were confirmed by Robien *et al*[51] after excluding patients who required leucovorin rescue and adjusting for pre-transplant vitamin use. Whether the effect of genotype on mucositis is independent of MTX administration is unclear, however, since only patients receiving MTX were included in these studies, and approximately 10% of patients receiving the same regimens without MTX experience severe mucositis.[52] Moreover, the relative risk for development of severe (grade III–IV) mucositis in patients with the *TT* genotype as compared to the *CC* genotype was not reported, and the effect of genotype was not stratified by vitamin use. Of two other smaller studies, one found no significant difference in mucositis severity or in engraftment times by *MTHFR* genotype, and the other study showed an association between the *TT* genotype and intolerance to MTX in leukemia patients.[53,54] These associations need to be validated in larger prospective studies that incorporate assessment of pre-transplant nutritional status and the incidence of aGVHD across *MTHFR* genotypes before MTX dose adjustment based on *MTHFR* genotype can be recommended.

Acute graft-versus-host disease

Recent interest has focused on the role of killer immunoglobulin-like receptors (KIRs) present on NK cells and some T cells, in modulating lymphocyte responses and hence the risk of aGVHD after HSCT. Encoded by highly polymorphic genes in the leukocyte receptor cluster on chromosome 19, KIRs bind to class I HLA molecules that are expressed on most nucleated cells. Recipient cells that lack the appropriate HLA class I ligand fail to bind and inhibit KIR-expressing donor cells, becoming targets for donor NK and T-cell alloreactivity. Since NK cells also express activating KIRs, a delicate balance between activating and inhibitory stimuli on donor *vs* host NK and T cells seems to dictate whether graft rejection or GVHD predominates after HSCT. The natural ligands for activating KIRs are unknown. Binding of the HLA-C molecule to inhibitory KIRs is sensitive to a polymorphism in the *HLA-C alpha*-helix. Group 1 HLA-C molecules carry an asparagine residue at position 80 (encoded by the *C1* allele), whereas group 2 molecules possess a lysine at this position (encoded by the *C2* allele). Two studies reported a reduced incidence of aGVHD and improved overall survival in haploidentical and unrelated-donor HSCT when there was KIR incompatibility in the graft-versus-host direction (defined as the absence in the recipient of class I allele group(s) recognized by donor KIRs).[55,56] In haploidentical transplants for high-risk acute myeloid leukemia, lower rates of graft failure, relapse, and surprisingly, a reduced incidence of severe aGVHD may contribute to improved survival.[55] A third study showed conflicting results and no differences in survival or aGVHD between KIR-compatible and KIR-incompatible transplants.[57] These studies, however, did not directly assess KIR/HLA genotypes in donors and recipients and instead used a variety of methods to infer them.[58] A more recent genetic study by Gagne *et al*[59] did show that donor–recipient *KIR* genotype combinations are important determinants of aGVHD in both sibling and unrelated-donor HSCT. Cook *et al*[58] further demonstrated by donor/recipient *KIR* genotyping that the HLA-C group of the recipient is also a major factor in determining outcome in HLA-identical sibling-donor HSCT if the recipient has a myeloid leukemia. Patients homozygous for the group 2 *HLA-C* allele (*C2*) who by definition lack the *C1* allele (ligands for an inhibitory KIR) had reduced overall survival when

compared with recipients who possessed at least one *C1* allele (31.6 *vs* 56.1% at 4 years, respectively, $P<0.005$). This difference in survival was only significant when the donor also had the activating *KIR2DS2* genotype; in this case, unopposed donor alloreactivity might occur even in the absence of HLA mismatch. No significant differences in the rates of aGVHD higher than grade II or in causes of mortality were noted between recipient *C1*/donor *KIR* combinations, and method of GVHD prophylaxis had no effect on outcome. The preponderance of evidence therefore suggests that KIR ligand incompatibility is a favorable factor in HLA-mismatched, unrelated-donor HSCT and HLA-identical sibling HSCT for myeloid leukemias (Table 4). Further studies are needed to clarify these effects in different HSCT scenarios. Independent of HLA matching, *KIR* and *HLA-C* genotyping of donor–recipient pairs may prove to be helpful in prioritizing HSCT among treatment options, in selecting donors, and in predicting clinical outcome in sibling- and unrelated-donor HSCT.

Given the role of cytokines in inflammation and tissue damage, prominent features of the graft-versus-host response, it is not surprising that a growing body of research has uncovered cytokine gene polymorphisms that affect cytokine levels during stress and hence outcomes after allogeneic HSCT. Lin *et al*[60] analyzed SNPs in several cytokine genes (interleukin-1β, interleukin-1-receptor antagonist, interleukin-6, interleukin-10 and tumor necrosis factor-α) in allogeneic HSCT recipients and their HLA-identical sibling donors in relation to the risk of aGVHD. Homozygosity for an SNP in the *IL-10* promoter region (-592 *AA* genotype) in the recipient was protective against grade III–IV aGVHD (hazard ratio 0.4, 95% CI 0.2–0.9, $P=0.02$) and death in remission (hazard ratio 0.6, 95% CI 0.3–1.0) compared with the *CC* genotype. Intermediate risks of aGVHD and death in remission were observed in *AC* heterozygotes. All patients received the same GVHD prophylaxis, and the analysis of GVHD was controlled for age at HSCT, sex of the donor–recipient pair, use of TBI, year of transplant, and diagnosis. Probability of overall survival at 3 years, adjusted for age at HSCT, duration of disease, and diagnosis, similarly correlated with genotype (71, 56, and 57% for *AA*, *AC*, and *CC* genotypes, respectively). The incidence of chronic GVHD (cGVHD) was unaffected. Although IL-10 levels were not measured in this study, correlations between genetic variants in the promoter region, higher IL-10 levels, and reduced aGVHD have been demonstrated in other smaller studies.[61–65] Indeed, higher levels of IL-10 produced by antigen-presenting cells in the host may induce greater tolerance in donor T lymphocytes to alloantigens expressed on host cells. Variations in the other cytokine genes tested in either the donor or the recipient have not shown these associations with outcome. Since clusters of SNPs in the *IL-10* promoter region (haplotypes) are in linkage disequilibrium (ie there is a nonrandom distribution of individual SNPs within these clusters in the population), individual *IL-10* promoter haplotypes have been investigated. Since the beneficial effect on GVHD and survival was tied to the *T-C-A-T-A* haplotype defined by five SNPs, including the one at position -592, it is unclear whether the $-592A$ allele or the entire haplotype is responsible for this effect.[60] A higher frequency of the $-592A$ allele in some Asian populations (67% in Japanese) as compared to Caucasian populations (24%) may account for lower incidence and severity of aGVHD in the Japanese and for differences between other racial/ethnic groups. Knowledge of the recipient's *IL-10* promoter-region genotype would substantially inform the pre-transplant risk assessment, prioritization of HSCT among the available treatment options, and selection of conditioning regimens to minimize the risk of severe aGVHD in individual patients.

Some studies suggest that recipient SNPs in the *TNF-*α gene (putative high-producer *TNF d3* and *d4* alleles) and *IFN-*γ gene may also predict moderate to severe aGVHD in HLA-matched sibling HSCT, but some data are inconsistent.[61,62,65–67] Weak associations with aGVHD are reported for other *IL-10* microsatellite promoter-region SNPs (-1082, -1064, -3575 genotypes), but these effects are difficult to distinguish from those of the -592 SNP or the entire *IL-10* haplotype, as previously mentioned. Since *TNF-*α and *HLA* haplotypes are tightly linked, the effects of *TNF-*α polymorphisms are also difficult to resolve from those of other immune response genes. Furthermore, these effects appear to be confined to HLA-identical sibling HSCTs. One study that was unable to show an effect of SNPs in the *IL-10* gene may have been underpowered due to a large proportion of unrelated-donor transplants.[66]

Studies of polymorphisms in genes encoding the adhesion molecules PECAM-1 and CD31 in relation to aGVHD have also been conflicting.[67]

Susceptibility to infection

Polymorphisms in several genes involved in host defense and the inflammatory response have been investigated in relation to the risk of infection in immunocompromised populations (Table 4).[68–70] These genes include *TNF-*α, *TNF-*β, *IL-1*β receptor antagonist, *IL-6*, *IL-10*, adhesion molecules, myeloperoxidase, F_c-γ receptors, mannose-binding lectin, and toll-like receptor genes. One study documented independent associations of both donor and recipient polymorphisms in the mannose-binding lectin gene *MBL2* (coding and promoter regions) and risk of major infection after allogeneic HSCT.[69] The time to neutrophil recovery, number of febrile days, incidence and severity of GVHD, and incidence of clinically significant, culture-confirmed infections were monitored in 97 HSCT recipients who received standard supportive care and GVHD prophylaxis. The MBL protein participates in the innate immune response by binding to carbohydrate moieties on microbial pathogens, leading to their opsonization and phagocytosis. Low, intermediate, and high MBL levels correlated with specific *MBL2* haplotypes defined by several combinations of coding and promoter-region SNPs. There were no associations with GVHD overall, neutrophil recovery, length of hospitalization, early death, or duration of neutropenic fever, but a nonsignificant trend toward more febrile days was observed in donor–recipient pairs with *MBL2* mutations. Replacement with purified MBL has been found to be safe and may be beneficial in MBL-deficient patients.[71,72]

Late complications

Chronic graft-versus-host disease

Although cGVHD is largely predicted by the occurrence of aGVHD, the results of genetic studies in this area have been discordant. Polymorphisms in *TNF-α* and *IL-10* genes that associate with aGVHD have not generally been reported to predict cGVHD, with rare exceptions.[68] However, the *IL-6*$^{-174}$ *G* allele, which correlates with lower *in vitro* and *in vivo* IL-6 production, leads to a dose-dependent increase in risk of cGVHD (highest risk with homozygosity for the *G* allele). After adjustment for other risk factors, homozygosity for the promoter-region *IL-6*$^{-174}$*GG* genotype does not predict increased aGVHD, but it is significantly associated with incident cGVHD.[73] These discrepancies may be due to different effects of proinflammatory cytokines at different stages in the evolution of aGVHD and cGVHD and warrant further study.

Chronic renal failure

Damage to the renal microvasculature and parenchyma due to the preparatory regimen, aggravated by the use of nephrotoxic medications and in some cases, by sepsis, is implicated in the pathophysiology of chronic renal failure occurring after HSCT. Angiotensin-converting enzyme (ACE) inhibitors have proven effective in preventing and treating this syndrome in animal models, and an association between decline of renal function in patients after HSCT and polymorphisms within the *ACE* gene has been reported.[74] The *ACE* gene encodes the enzyme responsible for cleaving angiotensin I to angiotensin II, which in turn has important effects on vascular tone, growth, and remodeling. A common polymorphism in the *ACE* gene, identified in 1990, is based on the insertion (*I* allele) or deletion (*D* allele) of a 287-base-pair intron. Individuals homozygous for the *D* allele have consistently ($\sim$60%) higher plasma ACE activity, and progressively decreasing amounts of immunoreactive ACE are associated with the other two genotypes, *DI* and *II*, respectively.[75] The *DD* genotype has also been associated with more rapid progression and increased severity of diabetic nephropathy, another form of renal disease in which oxidant injury is implicated. Juckett *et al* examined the effects of *ACE* genotype and other factors in a cohort of adult recipients of allogeneic HSCT between 1985 and 1996. Patients surviving at least 1 year and whose renal function was assessed for up to 3 years post transplant were studied. The strength of this study was that all patients received T-cell-depleted grafts, aGVHD prophylaxis with cyclosporine, and the same conditioning regimen. Independent of renal shielding and other clinical factors, the *DD* genotype predicted a slower decline in creatinine clearance during the year following transplant than the *II* genotype ($P = 0.040$ for a difference in slope of 1.13 ml/min/month). Importantly, *ACE* genotype did not affect survival over the course of the study, and genotype distribution was in Hardy–Weinberg equilibrium. The preservation of renal function in HSCT patients with the *DD* genotype contrasts with studies in patients with diabetic nephropathy, in whom the *II* genotype tends to be protective and correlates with improved response to ACE inhibition. It is speculated that the deleterious remodeling effects of angiotensin on the kidney in other chronic renal diseases may be offset in HSCT patients by other protective effects of ACE involving tissue repair, free-radical scavenging, responses to nephrotoxic medications, *etc*.[76] The use of ACE inhibitors is beneficial in animal models of BMT nephropathy and in transplant patients with nephropathy, however, and the reasons for this are unclear. Activation of the renin–angiotensin-activating system may initially preserve renal perfusion during hypotensive episodes but later contribute to progression of radiation-induced renal injury, which is amenable to ACE inhibition. Selection bias was a concern in this study, because only a fraction of the eligible patients survived to participate, had adequate follow-up data, and conditioning was not standard.[74] Doses of TBI were higher than those used than at most centers, and the development of renal failure in patients who receive 12 Gy TBI or less, with or without renal shielding, may be less dependent on *ACE* genotype. Furthermore, ACE inhibitors are widely used to treat hypertension after HSCT, and this study did not control for the use of ACE inhibitors. Therefore, the significance of polymorphisms in the *ACE* gene in chronic renal failure developing after HSCT remains unclear.

Future directions

Genetic predisposition to other organ toxicities in the HSCT setting remains unexplored, including hemorrhagic cystitis and the bronchiolitis obliterans syndrome (BOS). Numerous studies of chronic obstructive pulmonary disease in animal models and in humans have identified genes that modulate the inflammatory response in the lung to oxidant insults, promoting damage to the airways and lung parenchyma.[2] These include the matrix metalloproteinases (*MMP1*, *MMP9*, and *MMP12*), heme oxygenase-1, and α-1-antitrypsin genes. Similarly, polymorphisms in *IL-6* and *IFN-γ* genes that regulate the production of these cytokines have been shown to correlate with the development of BOS after lung transplantation, consistent with a proinflammatory role for IL-6 in the pathogenesis of this disease.[77] The role of these genes in both early and late pulmonary complications of HSCT setting should be evaluated. Polymorphisms that increase the risk of cGVHD might also be expected to increase the incidence of BOS.[78]

Common variations in the genes encoding drug-metabolizing enzymes, drug receptors, and drug transporters may give rise to individual variability in the toxicity of chemotherapeutic drugs.[79] The role of genetic polymorphisms in the cytochrome P-450 enzyme system in the liver in transplant toxicity has not been sufficiently explored.[80,81]

Larger cohort studies are needed to confirm the associations that have been observed between specific genetic variants and HSCT-related complications and to identify other genes that modify risk. The benefits of these studies to patients are obvious: the potential for improved risk stratification and development of more effective interventions that target high-risk individuals. The value of oral supplementation with citrulline, bypassing CPSI in

the generation of NO precursors, is currently being tested prospectively in a randomized, placebo-controlled trial in patients undergoing myeloablative HSCT at our institution. If supplementation is effective, it will constitute a significant addition to our armamentarium in supporting these critically ill patients.

Adverse effects of iron

There is growing evidence that elevated iron stores are detrimental to organ function in patients undergoing HSCT.[82–89] Iron present in excess amounts or in reactive forms is a potent pro-oxidant and probably a radiosensitizing agent, because it can rapidly generate highly toxic free radicals *in vivo* (Figure 5).[8,90–92] Iron overload is common in this population due to red cell transfusion and/or dyserythropoiesis.[93–95] Prevalent iron-loading genetic mutations such as *HFE* C282Y may also augment organ toxicity (eg HVOD) by promoting infection, increasing reactive iron levels, and/or impairing the re-uptake of iron that is released during myeloablation.[32,96–103] These mutations may also predispose HSCT survivors to secondary myelodysplasia and other malignancies.[104–109] The effects of iron in the HSCT setting deserve further investigation, since they contribute to long-term morbidity and are potentially modifiable by antioxidant therapy, phlebotomy, and chelation.[110–114]

Autoimmune disease and heritable malignancy

The risk of transmission of genetic disease from donor to host is neither well recognized nor routinely considered during screening of potential HSCT donors. The urgency of finding suitable HLA-matched donors for the treatment of life-threatening diseases has taken precedence over such concerns, but this may change as the donor pool grows. Case reports in allogeneic transplant recipients have suggested that the adoptive transfer of autoimmune diseases such as vitiligo, thyroid disease, psoriasis, and even fulminant inflammatory bowel disease, may occur.[115] Currently, however, there is no donor screening based on a positive personal or family history of autoimmune disease. Heritable cancer syndromes also constitute an important category of potentially transmissible genetic disease, and the absence of a positive family history in the donor is unhelpful. Genetic disorders associated with an increased risk of hematopoietic malignancy that are relatively common include Down's syndrome, Noonan's syndrome, and type 1 neurofibromatosis.[116] Furthermore, the prevalence of mutations underlying many autosomal recessive genetic diseases (eg ataxia-telangiectasia, Fanconi's anemia, hereditary hemochromatosis), if not the prevalence of the diseases themselves, is higher than was reported before genetic testing became available. Carriers of these mutations may be at increased risk of leukemia and other malignancies. For most such genetic disorders, however, the nature and magnitude of the risk to recipients remain poorly defined. Clinical factors that increase the risk of aGVHD have been associated with secondary malignancies after HSCT, but whether this correlation extends to genetic risk factors for aGVHD is unknown.

Use of DNA microarray technology

Microarray technology employs immobilized DNA probes to detect complementary gene sequences and may be used to analyze the expression patterns of thousands of genes simultaneously. Genome-wide analyses and functional genomic approaches that evaluate the expression of sets of related genes can provide insights into the genetic basis of HSCT-related toxicities. In this context, the SNP profiles obtained from microarrays can provide clues to the molecular pathways involved in the development of specific complications. Ultimately, they might be used for individual risk stratification and treatment planning. Microarrays may be prone to inconsistent results, however.[117] At present, both the statistical tools to handle large genetic studies and our understanding of proteomics lag significantly behind our ability to generate complex genetic data. At present, microarray technology may be most valuable as a tool to validate and refine (eg using multiplexed PCR assays) small, predictive panels of genes that are initially identified by other means.[117,118]

Translational studies

The EDGE model, while clearly an oversimplification of gene–environment interactions, illustrates for the *CPSI* gene example the value of moving beyond simple genetic association studies to relate qualitative and quantitative genetic changes to observed pathophysiology. This may involve a combination of *in vivo* and *in vitro* studies, as in the case of *CPSI*. Whereas common functional polymorphisms in *CPSI* that decrease urea cycle efficiency may be clinically silent under normal conditions, a shortage of NO substrates during the stress of HSCT may result in a deficiency of NO, thereby promoting severe oxidant damage in susceptible tissues (eg liver and lungs). The genetic axis for many disease states (eg the toxicities of HSCT) will consist of several genetic modifiers, such as polymorphisms in *CPSI*, *MTHFR*, *GST* and the major *HFE* locus. The different functional forms of each gene product will further subdivide this axis. Indeed, the complexity of this model for many phenotypes will require

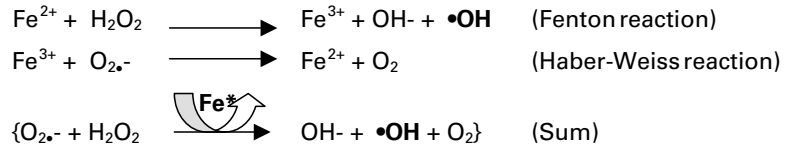

Figure 5 The iron-catalyzed Haber–Weiss reaction: mechanism of iron-mediated oxidant toxicity *in vivo*. Only trace amounts of redox-reactive iron (Fe) are required to generate large quantities of highly toxic free radicals (eg the hydroxyl radical, •OH).

not just larger study populations but also increasingly sophisticated probabilistic models. Conventional logistic regression and multiple linear regression models rapidly lose power in multilocus studies due to empty cells, and they may often miss significant gene–gene or gene–environment effects. Newer, evolving statistical tools, such as multifactor dimensionality reduction (MDR), may provide better modeling of these complex interactions.[119,120]

It is premature to recommend changes in treatment for patients based on the results of genetic tests that need further validation of their utility in the clinical arena. Given the weight of evidence favoring some genotype–phenotype associations, however, fine-tuning of care might begin with testing for common polymorphisms in patients who are already at high risk of complications based on other criteria. Genetic polymorphisms (eg *MTHFR*, *MBL2*, *CPSI*, *HFE*) for which potential interventions exist are listed in Table 5. Protein or enzyme levels correlate well in some cases with genotype, and direct measurement of these levels rather than genotyping may be more readily performed (eg *MBL2*). Since the benefits of supportive interventions during HSCT such as oral or parenteral glutamine, citrulline, and chelation to reduce the iron load are currently unclear, their use in the context of genetic testing should be studied on formal protocols.

Conclusion

There are relatively few published studies that investigate host genetic susceptibilities in the development of common organ toxicities after HSCT, probably due to the difficulty of studying toxicities for which the underlying pathophysiologic mechanisms are not well defined. We have also learned from the literature concerning clinical predictive factors in HSCT that differences in patient selection, treatment regimens, supportive care, and definitions of toxicity between transplant centers may make it hard to interpret and generalize the results of single-center studies.

The compensatory response to oxidant stress is a useful example of the candidate-gene approach to complications of HSCT that should be exploited further. As the studies reviewed here illustrate, mutations that cause uncommon diseases may, at lower 'allele dosages' (eg heterozygosity for the *CPSI* T1405N or the *HFE* C282Y mutation), display important phenotypes under stress conditions. Varying degrees of functional CPSI deficiency, for example, give rise to phenotypes ranging from overwhelming hyperammonemia in the newborn period (complete CPSI deficiency) to marginal urea cycle reserve that is unmasked in the context of high-dose chemoradiotherapy (diminished CPSI efficiency).

Finally, due to the likelihood that combinations of SNPs comprising individual haplotypes, rather than individual

Table 5 Potential uses of genetic testing in HSCT requiring further study

Risk factors	*Complication(s)*	*Genetic tests (reference lab/link)*	*Consideration(s)*
Pre-transplant liver dysfunction; prior abdominal XRT; iron loading states (transfusions, myelodysplasia); gemtuzumab ozogamicin exposure; fever during conditioning	HVOD or liver dysfunction; infection	*HFE* C282Y www.genetests.org or www.questdiagnostics.com	Chelation, glutamine ± vitamin E, apo-transferrin infusion, amifostine, oral citrulline, defibrotide; low threshold for empiric antifungals
		MBL2 www.ibtreflab.com[a]	Infusion of purified MBL if level <100 ng/ml
Pulmonary D_LCO $<70\%$ of normal at baseline; prior thoracic XRT; planned TBI	ALI	*CPSI* T1405N *Molecular Pathology Laboratory, Vanderbilt Medical Ctr* (www.genetests.org)[b]	Oral citrulline, parenteral glutamine
Fever/infection before or during conditioning phase; recurrent bacteremia; prolonged immunosuppression; poor engraftment	Bacterial sepsis	*MBL2* (see above)	Infusion of purified MBL
MTX prophylaxis and TBI	Mucositis	*MTHFR* (thermolabile, *C677T*) www.genetests.org	Topical oral or parenteral glutamine, vitamin E; amifostine; ?MTX dosing change
Risk of severe aGVHD	aGVHD	*IL-10*$^{-592}$, *KIR* and *HLA-C* typing www.ihwg.org/components/nkover.htm	Donor selection and GVHD prophylaxis
Aplastic anemia, marrow failure syndromes, prior thoraco-abdominal XRT; use of ATG, anti-T-cell Ab, or TBI; older age and CSA-treated cGVHD	Secondary cancers	*HFE* (see above)	Monitor iron stores, chelation, avoidance of iron post transplant
Renal insufficiency before HSCT ± planned TBI	Renal failure	*ACE* (D/I) www.genetests.org	?Early use of ACE inhibitor

Specific genetic tests and interventions need further investigation and cannot be routinely recommended.[130–137] HVOD = hepatic veno-occlusive disease; ALI = acute lung injury; D_LCO = pulmonary diffusing capacity for carbon monoxide; XRT = radiation; MTX = methotrexate; TBI = total-body irradiation; ATG = anti-thymocyte globulin; Ab = antibody; CSA = cyclosporine A; aGVHD/cGVHD = acute or chronic graft-versus-host disease. *Genes: HFE*, hemochromatosis; *MBL2*, mannose-binding lectin 2; *CPSI*, carbamyl-phosphate synthetase I; *MTHFR*, methylene-tetrahydrofolate reductase; *ACE*, angiotensin-converting enzyme; *KIR*, killer immunoglobulin receptor; *HLA-C*, human leukocyte antigen group C.
[a]Measurement of protein levels (MBL), rather than genotyping, is performed.
[b]Measurement of CPSI enzymatic activity and genetic testing are available.

SNPs, hold the key to understanding genetically determined variability in risk, and due to the statistical limitations of multilocus genetic analyses, large-scale and carefully designed studies are needed to address these issues. Currently, the only solution to the problems with gene–disease association studies is to prospectively validate multivariable predictive models that are generated from these studies in independent patient cohorts. An important first step is for transplant clinicians to recognize this new opportunity for clinical investigation and to initiate multicenter collaborations to develop large clinical/DNA databases for such studies. Haplotype- or SNP-based analyses in patients with well-characterized responses (phenotypes) to standardized regimens, including patients who participate in cooperative-group trials, may ultimately enable investigators to identify panels of genes that modulate responses to and toxicity from chemotherapy and determine the risk of specific complications. To be successful, these efforts will require interdisciplinary collaboration among transplant clinicians, molecular epidemiologists, statistical geneticists, and informatics specialists, a novel paradigm in clinical research. Further research to identify at-risk genetic haplotypes will enable clinicians to predict with far greater accuracy than before the likelihood of complications from HSCT and to individualize treatment approaches. As in other areas of clinical medicine in which genetic studies are gaining momentum, the expectation is that an individualized approach to HSCT that incorporates genetic data will ultimately lead to reduced transplant toxicity, reduced cost of care, and improved outcomes.

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