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Host Genetic Variation in the TNF and NF- κ B Pathways and Overall Survival in Mantle Cell Lymphoma: A Discovery and Replication Study

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Keywords

mantle cell lymphoma; host genetics; TNF; NK- κ B

Mantle cell lymphoma (MCL) is generally considered to be aggressive and incurable. NF- κ B has been shown to be constitutively activated in MCL cells,^{1,2} due to expression of TNF. ² TNF neutralization inhibited both NF- κ B activation and MCL cell proliferation *in vitro*.² In MCL cells, TRAIL can engage the death receptors DR4 and DR5 and decoy receptors DcR1 and DcR2 to trigger apoptosis. MCL sensitivity to TRAIL was closely linked to NF- κ B activity, and an I κ B kinase inhibitor sensitized MCL to TRAIL-mediated apoptosis.³ Given the importance of TNF and NF- κ B in MCL, we hypothesized that inherited variations in these pathway genes impact MCL survival.

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AUTHOR CONTRIBUTIONS

JRC and TMH conceived and designed the study; JRC provided financial support; TMH, SSW, BKL, ALF, DJI, TEW, WC, NR, SLS and JRC provided study materials, genotyping or participants; YW, MJM, SLS, JRC analyzed and interpreted the data; VS and YA conducted the bioinformatics analysis; YW, TMH, and JRC drafted the manuscript; all authors reviewed and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with the contents of this article.

We conducted a 2-stage pilot study: in stage 1 (discovery) we used existing data to identify candidate gene regions of interest associated with overall survival (OS) and in stage 2 we attempted to replicate top SNPs from those regions (Supplemental Methods). Stage 1 consisted of 39 MCL cases enrolled from 1998–2000 in the population-based NCI-SEER NHL Survival Study. Genotyping data on 487 non-monomorphic SNPs from 41 gene regions (Supplemental Tables 1 and 2) were derived from a previously published project.⁴ Stage 2 consisted of 101 MCL cases with DNA from the Molecular Epidemiology Resource (MER). Of the 97 SNPs from the top gene regions in the NCI-SEER study, we were able to design and genotype a majority (N=69, 71%) on a custom panel.⁵

For stage 1, we used Cox regression to assess the association of each gene region (modeled using principal components) with OS, adjusted for clinical factors. Gene region tests with $P \leq 0.15$ were declared of interest and moved forward for replication. We also calculated the association of each SNP (per allele genetic model) within a gene using Cox regression to estimate Hazard Ratios (HR) and 95% Confidence Intervals (CI). For stage 2, since we did not have all of the same tagging SNPs for all gene regions and thus could not directly replicate the gene region test, we focused on replication at the SNP level. As this was a small and exploratory study, we defined replication as $P \leq 0.10$ for the association of a given SNP with OS from the log-additive model observed in both the discovery and validation datasets with HRs in the same direction. In the MER, we estimated HR and 95% CIs for each SNP using Cox regression, adjusted for a simplified MCL international prognostic index (MIPI) and treatment.

The median age at diagnosis in the NCI-SEER study was 64 years (range 38–74). There were 27 deaths (69.2%) with a median follow-up for living patients of 47 months (range 23–85). Eight gene regions were associated with OS at $P \leq 0.15$ (Supplemental Table 1) and were brought forward for replication. The median age at diagnosis in the MER cohort was 64 years (range 42–88). Through 2009, there were 61 events (60.4%) and 31 (30.7%) deaths with a median follow-up for living patients of 59 months (range 29–90). Because we had the same tagSNPs for only some of the gene regions in both datasets, we focused on replication at the SNP level, which was defined as SNPs with $P \leq 0.10$ and similar HRs from the ordinal model in both datasets; the SNP-level results in the NCI-SEER (discovery) and MER (replication) cohorts are shown in Supplemental Table 2. For *TNFRSF25*, all 7 SNPs in the NCI-SEER study were genotyped in the MER, and rs3138156 replicated ($P=0.087$); for *TRAF5*, all 6 SNPs in the NCI-SEER study were genotyped in the MER, and rs3738199 replicated ($P=0.0092$); for *RELB*, all 6 SNPs in the NCI-SEER study were genotyped in the MER, and rs10424046 replicated ($P=0.065$). None of the other gene regions showed replication at the SNP level by our definition. However, after applying a conservative Bonferroni correction ($P<0.001$), none of these results would meet statistical significance.

We next conducted a meta-analysis of the above SNPs from the three gene regions that replicated. As shown in the Table, the *TNFRSF25* SNP rs3138156 was associated with inferior survival ($P=0.0047$): compared to patients with the AA genotype, those with the AG genotype (HR=3.04, 95%CI 1.41–6.56) had inferior OS; no GG genotype was observed. The *TRAF5* SNP rs3738199 was also associated with inferior OS ($P=0.00086$): compared to patients with the AA genotype, those with the AG (HR=1.81, 95%CI 0.99–3.32) and GG

(HR=4.11, 95%CI 1.86–9.04) genotypes had inferior OS. Finally, the *RELB* SNP rs10424046 was associated with superior OS ($P=0.0062$): compared to patients with the GG genotype, those with the CG (HR=0.45, 95%CI 0.25–0.82) and CC (HR=0.36, 95%CI 0.17–0.78) genotypes had better OS.

We were able to evaluate EFS in the MER. After adjusting for simplified MIPI and treatment, the *TRAF5* SNP rs3738199 was also associated with inferior EFS ($P=0.014$): compared to patients with the AA genotype, those with the AG (HR=1.61, 95%CI 0.89–2.87) and GG (HR=2.49, 95%CI 1.17–5.28) genotypes had inferior EFS. The *RELB* SNP rs10424046 was associated with superior EFS ($P=0.040$): compared to patients with GG genotype, those with the CG (HR=0.49, 95%CI 0.27–0.88) and CC (HR=0.52, 95%CI 0.25–1.04) genotypes had better EFS. In contrast, the *TNFRSF25* SNP rs3138156 was not associated with EFS: compared to patients with the AA genotype, those with the AG genotype (HR=0.87, 95%CI 0.36–2.09) had similar EFS.

We conducted an *in silico* bioinformatics analysis (Supplemental Methods), and found no genotype-expression relationships between all 167 SNPs and *TNFRSF25*, *TRAF5* and *RELB* expressions in lymphoblastoid cell eQTL databases (<http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/>). An additional exploratory eQTL search in the Genotype-Tissue Expression (GTEx) consortium data set found 5 out of 14 SNPs in LD with rs3138156 regulate expression of *TNFRSF25* in the tibial nerve (Supplemental Tables 3 and 4). We also overlapped positions of all 167 SNPs with known genomic functional domains recorded in the USCS Golden Path database in the lymphoblastoid cell line GM12878, and identified multiple SNPs in LD with rs3138156, rs3738199, and rs10424046 located within some potential regulatory regions (Supplemental Table 4). The exact functional roles of these SNPs in *TNFRSF25*, *TRAF5* and *RELB* will need to be evaluated in future research.

All three genes that were implicated play important roles in the TNF-NF- κ B signaling pathway. *TNFRSF25* is a member of the TNF receptor superfamily. It mediates TNF signaling and stimulates NF- κ B activity. *TRAF5* is a member of the TNF receptor-associated factor (TRAF) family, and is a scaffold protein within a multiple protein complex that binds to TNF receptor cytoplasmic domains and mediates TNF-induced signaling transduction. *RELB* is a component of the NF- κ B complex. It dimerizes with NF- κ B2/p100 (inactive form) or NF- κ B2/p52 (active form) and mediates signaling through the noncanonical NF- κ B pathway. The importance of NF- κ B mediated signaling is highlighted by its constitutive activation in MCL cells lines and primary MCL cells,^{1,2} and the sensitivity of MCL cells to a pI κ B α inhibitor and a proteasome inhibitor.¹ Proteasome inhibitors lead to cytoplasmic accumulation of I κ B α and reduced NF- κ B activity. The proteasome inhibitor bortezomib demonstrated activity in MCL in clinical trials and was approved by FDA for treating MCL patients who have received at least one prior therapy. Changes of NF- κ B1/p65 levels were noted to be associated with bortezomib activity in the PINNACLE trial,⁶ further supporting the importance of TNF-NF- κ B signaling in MCL and validating this signaling axis as a key therapeutic target.

Strengths of this study include the two-stage design, defined study populations, high quality genotyping, and adjustment for clinical factors. The major limitations included the small

sample size and limited clinical and treatment data in the discovery cohort from the pre-rituximab era, candidate gene approach, and clear potential for false positives. In summary, host genetic variation in the TNF and NF- κ B genes suggest an association with OS in MCL after accounting for clinical and treatment factors, but need further replication. These novel findings support that the role of the TNF and NF- κ B pathways in the pathogenesis and disease progression of MCL, and suggest targeting these pathways for therapy in MCL should be further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE

Meta-analysis of SNPs with OS in mantle cell lymphoma that replicated in the NCI-SEER and MER cohorts

Gene/SNP	Genotype	NCI-SEER				SPORE MER				Combined (meta-analysis)			
		N	Deaths	HR ^a	95%CI	N	Deaths	HR ^b	95% CI	N	Deaths	HR	95% CI
<i>TNFRSF25</i>	AA	35	23	1.00	reference	90	26	1.00	reference	125	49	1.00	reference
rs3138156	AG	4	4	3.97	1.24–12.6	11	5	2.46	0.87–6.89	15	9	3.04	1.41–6.56
	GG	0	0			0	0			0	0		
		<i>P</i> = 0.02				<i>P</i> = 0.087				<i>P</i> = 0.0047			
<i>TRAF5</i>	AA	16	9	1.00	reference	44	10	1.00	reference	60	19	1.00	reference
rs3738199	AG	19	14	1.53	0.64–3.60	39	13	2.14	0.91–5.01	58	27	1.81	0.99–3.32
	GG	4	4	4.76	1.37–16.4	15	7	3.72	1.33–10.3	19	11	4.11	1.86–9.04
		<i>P</i> = 0.04				<i>P</i> = 0.009				<i>P</i> = 0.00086			
<i>RELB</i>	GG	12	11	1.00	reference	31	13	1.00	reference	43	24	1.00	reference
rs10424046	CG	18	11	0.32	0.13–0.80	46	13	0.57	0.25–1.28	64	24	0.45	0.25–0.82
	CC	9	5	0.33	0.10–1.01	24	5	0.39	0.13–1.12	33	10	0.36	0.17–0.78
		<i>P</i> = 0.04				<i>P</i> = 0.065				<i>P</i> = 0.0062			

^aHR adjusted for clinical risk score.^bHR adjusted for MIPI and treatment.