Genetic Variants in Inflammation-Related Genes Are Associated with Radiation-Induced Toxicity Following Treatment for Non-Small Cell Lung Cancer

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Abstract

Treatment of non-small cell lung cancer (NSCLC) with radiotherapy or chemoradiotherapy is often accompanied by the development of esophagitis and pneumonitis. Identifying patients who might be at increased risk for normal tissue toxicity would help in determination of the optimal radiation dose to avoid these events. We profiled 59 single nucleotide polymorphisms (SNPs) from 37 inflammation-related genes in 173 NSCLC patients with stage IIIA/IIIB (dry) disease who were treated with definitive radiation or chemoradiation. For esophagitis risk, nine SNPs were associated with a 1.5- to 4-fold increase in risk, including three PTGS2 (COX2) variants: rs20417 (HR:1.93, 95% CI:1.10–3.39), rs5275 (HR:1.58, 95% CI:1.09–2.27), and rs689470 (HR:3.38, 95% CI:1.09–10.49). Significantly increased risk of pneumonitis was observed for patients with genetic variation in the proinflammatory genes IL1A, IL8, TNF, TNFRSF1B, and MIF. In contrast, NOS3:rs1799983 displayed a protective effect with a 45% reduction in pneumonitis risk (HR:0.55, 95% CI:0.31–0.96). Pneumonitis risk was also modulated by polymorphisms in anti-inflammatory genes, including genetic variation in IL13: rs20541 and rs180925 each resulted in increased risk (HR:2.95, 95% CI:1.14–7.63 and HR:3.23, 95% CI:1.09–10.18, respectively). The cumulative effect of these SNPs on risk was dose-dependent, as evidenced by a significantly increased risk of either toxicity with an increasing number of risk genotypes (P<0.001). These results suggest that genetic variations among inflammation pathway genes may modulate the development of radiation-induced toxicity and, ultimately, help in identifying patients who are at an increased likelihood for such events.

Introduction

It was predicted that lung cancer would be diagnosed in over 215,000 individuals in the United States alone in 2008 [1]. Patients with locally advanced stage IIIA and IIIB (dry) disease who are not candidates for surgery are treated with definitive radiation therapy or combination chemoradiation therapy [2]. Unfortunately even with treatment, the overall 5-year survival rate for NSCLC patients is only 10–15% [3].

One of the challenges in lung cancer treatment with radiotherapy is the development of severe dose-limiting side effects. Esophagitis and pneumonitis are common acute radiation-induced normal tissue toxicities occurring in patients within one year following treatment. Presence of these toxicities can also cause a reduction in quality of life and may lead to chronic complications including lung fibrosis [4]. Currently, there are few predictors for the development of these toxicities based on clinical and dosimetric parameters [3–9]. Therefore, the identification of additional reliable markers could help to tailor radiation regimens in order to administer the optimal therapeutic dose while minimizing toxicity.

Inflammation is a physiological response to cellular and tissue damage, including radiation-induced damage. Appropriate response to this damage is tightly regulated through a balance between proinflammatory and anti-inflammatory cytokines and signaling molecules [10,11]. Genetic variation in key inflammation-related genes may cause a shift in balance resulting in deregulation of the inflammatory response and corresponding modulation of susceptibility to radiation-induced normal tissue damage [12]. Previous studies have investigated genetic variation in transforming growth factor-beta 1 (TGF-β1). This important cytokine is up regulated following radiation exposure and common variants located in TGFBI have been found to be associated with late normal tissue complications [13–17].

In this study, we utilized a pathway-based approach to analyze genetic variation from 59 SNPs in 37 inflammation-related genes...
with regard to risk of developing either acute esophagitis or pneumonitis following radiation therapy. We explored the main effects of single SNPs and also the cumulative effect of genetic variation within the inflammation pathway on toxicity risk. These results indicate that an individual’s risk of developing these severe side effects may be modulated by germline variation in inflammation genes and may help to personalize radiation therapy for NSCLC.

### Results

#### Patient Characteristics

A total of 173 non-Hispanic Caucasian patients with stage IIIA (n = 70 or 40.5%) or IIIB (dry) (n = 103 or 59.5%) were included in the analysis (Table 1). Of these patients, 91 (52.6%) were men and 82 (47.4%) were women with a median age of 63.6 years. Most of the patients had a history of smoking with 46.8% (n = 81) being former smokers and 46.2% (n = 80) currently smoking or having quit within a year prior to diagnosis. Sixty-three (36.4%) of the tumors were classified as squamous cell carcinoma, 59 (34.1%) as adenocarcinoma, and 40 (23.1%) as non-small cell carcinoma, with the remainder (11 or 6.4%) as other NSCLC. Twenty-two patients were given a pre-treatment ECOG performance score ≥2. Nearly 80% (n = 138) of the patients received combination chemoradiation therapy, primarily with cisplatin or carboplatin (n = 142). A majority were treated with 3D radiotherapy (n = 72 or 41.6%).

| Table 1. Patient characteristics. |
|----------------------------------|
| **Gender**                       |
| Male                             |
| Female                           |
| Total                            |
| **Age, mean(SD)**                |
| Never                            |
| Former                           |
| Current & Recent Quitter         |
| Total                            |
| **Packyr, mean(SD)**             |
| **Histology**                    |
| Adenocarcinoma                   |
| Squamous Cell Carcinoma          |
| Non-small Cell Carcinoma         |
| Other NSCLC                      |
| Total                            |
| **Clinical stage**               |
| Stage IIIA                       |
| Stage IIIB (dry)                 |
| Total                            |
| **Performance status**           |
| 0                                |
| 1                                |
| 2–4                              |
| Total                            |
| **Treatment**                    |
| Radiation                        |
| Chemoradiation                   |
| Total                            |
| **Radiation type**               |
| 2D                               |
| 3D                               |
| IMRT                             |
| Total                            |
| **Radiation dose, mean (SD)**    |

![](https://www.plosone.org/figure/Table1)
Inflammation-related SNPs and Risk of Esophagitis

Among the 59 SNPs studied, a total of nine inflammation-related SNPs were found to be significantly associated with risk of esophagitis following radiation treatment (Table 2). All of these variants remained significant at an FDR level of 10%. In addition, because esophagitis typically presents 4–6 weeks following initiation of radiation therapy, we also analyzed the effect of these variants using logistic regression. The results are similar to those from the Cox regression analysis (data not shown).

### Proinflammatory Genes

Of these nine SNPs, six were among genes involved in the proinflammatory response: IL6, IL16, TNF, and PTGS2 (COX2). Interleukin 6 (IL6):rs1800795 resulted in a 2.16-fold increased risk (95% CI:1.18–3.94) under the recessive model. A similar effect was observed for IL16:rs11556218 (HR:2.28, 95% CI:1.16–4.47). Patients with at least one tumor necrosis factor-α (TNF) variant rs1799724 had a nearly 2-fold increased risk (HR:1.97, 95% CI:1.10–3.50). Three SNPs in PTGS2 modulated esophagitis risk in our patient population: rs20417, rs5273, and rs689470. PTGS2:rs5273 was associated with an increased risk (P for trend = 0.014). For rs20417 and rs689470, carriers of at least one variant allele were at an increased risk (HR:1.93, 95% CI:1.10–3.39 and HR:3.38, 95% CI:1.09–10.49, respectively).

### Anti-inflammatory Genes

SNPs in the IL4 receptor, IL10, and the alpha subunit of the IL10 receptor were found to be significantly associated with increased esophagitis risk. The IL10:rs1800872 and IL10RA:rs3135932 variants were both associated with significantly increased risks under the additive

#### Table 2. Inflammation-related SNPs and risk of esophagitis.

|                      | Grade <2 n(%) | Grade ≥2 n(%) | *HR | 95% CI | P value | Q value | Grade <2 n(%) | Grade ≥2 n(%) | *HR | 95% CI | P value | Q value |
|----------------------|--------------|--------------|-----|--------|---------|---------|--------------|--------------|-----|--------|---------|---------|
| **Proinflammatory cytokines, receptors, and related molecules** |              |              |     |        |         |         |              |              |     |        |         |         |
| IL6:rs1800795        | 94           | 76           |     |        |         |         | 94           | 76           |     |        |         |         |
| CC                   | 32(34.0)     | 30(39.5)     | 1.00|        |         |         | GG           | 82(87.2)     | 54(71.1)| 1.00   |         |         |
| CG                   | 53(56.4)     | 27(35.5)     | 0.67| 0.38 to| 1.18   | 0.162   | GC           | 12(12.8)     | 20(26.3)| 1.90   | 1.07 to| 3.39   | 0.029   |
| GG                   | 9(9.6)       | 19(25.0)     | 1.70| 0.87 to| 3.35   | 0.123   | CC           | 0(0.0)       | 2(2.6)  |       |         |         |
| CC+GG vs. GG         | 85           | 57           | 2.16| 1.18 to| 3.94   | 0.013   | 0.052        | GC+CC        | 12      | 22     | 1.93   | 1.10 to| 3.39   | 0.029   | 0.052   |
| IL16:rs11556218      | 96           | 75           |     |        |         |         | 92           | 75           |     |        |         |         |
| CC                   | 58(60.4)     | 41(54.7)     | 1.00|        |         |         | TT           | 51(55.4)     | 32(42.7)| 1.00   |         |         |
| CG                   | 32(33.3)     | 22(29.3)     | 0.86| 0.49 to| 1.53   | 0.615   | TG           | 39(42.4)     | 33(44.0)| 1.43   | 0.85 to| 2.39   | 0.178   |
| GG                   | 6(6.3)       | 12(16.0)     | 2.14| 1.05 to| 4.36   | 0.035   | GC           | 2(2.2)       | 10(13.3)| 2.71   | 1.25 to| 5.88   | 0.011   |
| TT+TG vs. GG         | 90           | 63           | 2.28| 1.16 to| 4.47   | 0.017   | 0.052        | TT+TG        | 4        | 4      | 3.38   | 1.09 to| 10.49  | 0.035   |
| **TNF:rs1799724**    | 92           | 71           |     |        |         |         | 96           | 74           |     |        |         |         |
| CC                   | 74(80.4)     | 50(70.4)     | 1.00|        |         |         | CT           | 92(72.3)     | 70(94.6)| 1.00   |         |         |
| CG                   | 12(13.0)     | 20(28.2)     | 2.13| 1.17 to| 3.86   | 0.013   | CT           | 4(4.2)       | 3(4.1)  | 2.67   | 0.73 to| 9.96   | 0.136   | 0.072   |
| GG                   | 6(6.5)       | 1(1.4)       | 0.91| 0.12 to| 6.99   | 0.928   | TT           | 0(0.0)       | 1(1.4)  |       |         |         |
| CT+TT                | 18           | 21           | 1.97| 1.10 to| 3.50   | 0.022   | 0.052        | CT+TT        | 4        | 4      | 3.38   | 1.09 to| 10.49  | 0.035   |
| **Anti-inflammatory cytokines, receptors, and related molecules** |              |              |     |        |         |         |              |              |     |        |         |         |
| IL4:rs1801275        | 94           | 75           |     |        |         |         | 94           | 75           |     |        |         |         |
| AA                   | 60(63.8)     | 40(53.3)     | 1.00|        |         |         | AA           | 68(71.6)     | 43(56.6)| 1.00   |         |         |
| AG                   | 34(36.2)     | 28(37.3)     | 0.99| 0.58 to| 1.68   | 0.973   | AG           | 24(25.3)     | 28(36.8)| 1.38   | 0.83 to| 2.28   | 0.217   |
| GG                   | 0(0.0)       | 7(9.3)       |     |        |         |         | GG           | 3(3.2)       | 5(6.6)  | 2.60   | 0.99 to| 9.83   | 0.053   |
| AA+AG vs. GG         | 94           | 68           | 4.12| 1.60 to| 10.59  | 0.003   | 0.052        | AA+AG        | 1.49     | 1.01   | 2.20   | 0.046  | 0.083   |
| IL10:rs1800872       | 94           | 75           |     |        |         |         | 2(2.1)       | 6(8.0)       | 2.88   | 1.15 to| 7.22   | 0.024   |

*adjusted for age, gender, pack years, clinical stage, performance status, treatment regimen, radiation type, and radiation dosage.
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model with HRs of 1.65 (95% CI: 1.11–2.45) and 1.49 (95% CI: 1.01–2.20), respectively. IL4R rs1801275 resulted in over a 4-fold increased risk (HR: 4.12, 95% CI: 1.60–10.59).

**Joint Analysis of Esophagitis Risk Alleles.** To understand the cumulative effect of unfavorable genotypes on risk of esophagitis, we performed a combined analysis. We included all significant SNPs identified from our individual SNP analysis and an additional seven SNPs reaching borderline significance at p<0.10 (Table 3). Patients with four unfavorable genotypes had a 3.71-fold increased risk (95% CI: 1.53–8.99) compared to those with 0–3 unfavorable genotypes. This risk increased to 8.35 (95% CI: 1.49–18.68) for patients with five or more unfavorable genotypes. Furthermore, patients with an increasing number of unfavorable genotypes developed esophagitis significantly quicker following initiation of radiation therapy (Figure 1A). Carriers of five or more unfavorable genotypes had a median time to event of only 1.1 months compared to over 12 months for those with three or less unfavorable genotypes (P<0.0001).

**Inflammation-related SNPs and Risk of Pneumonitis**

A different set of inflammation-related SNPs was found to be significantly associated with risk of developing pneumonitis following radiation therapy and remained so at an FDR of 10% (Table 4). Only one of the 12 SNPs identified were also associated with esophagitis risk – TNF rs1799724. Patients homozygous for this variant exhibited a 5.96-fold increased risk (95% CI: 1.33–26.58) of pneumonitis. This risk is similar for esophagitis risk in patients carrying at least one variant allele (Table 2).

**Proinflammatory Genes.** Other significant genetic variants associated with pneumonitis included six SNPs in proinflammatory genes, including IL1A, IL1B, TNFRSF1B, MIF, and NOS3. Two SNPs in IL1A – rs1800587 and rs17561 – are in strong linkage disequilibrium and each resulted in a more than doubling of risk with HRs of 2.90 (95% CI: 1.34–6.25) and 2.51 (95% CI: 1.19–5.27), respectively. The risk associated with IL8 rs4073 was similar at 3.16-fold (95% CI: 1.54–6.48). Under the additive model, TNFRSF1B:rs1061622 resulted in a 2.12-fold increase in pneumonitis risk (95% CI: 1.01–4.03). Patients with four unfavorable genotypes had a 13.30-fold increased risk (95% CI: 1.72–102.94) compared to those with 0 to 2 unfavorable genotypes (P=0.013). This risk was dramatically increased for the group of patients with four or more unfavorable genotypes (P<0.0001). These high risk individuals also had a shorter duration between start of treatment and development of pneumonitis of only 5.33 months compared to over 12 months for those with 0 to 2 unfavorable genotypes (Figure 1B).

**Table 3. Cumulative effect of unfavorable genotypes and radiation-induced toxicity risk.**

| Number of Unfavorable Genotypes | Grade <2 n | Grade ≥2 n | *HR | 95% CI | P value |
|--------------------------------|-----------|-----------|-----|--------|---------|
| **Esophagitis**                |           |           |     |        |         |
| 0–3                            | 49        | 11        | 1.00|        |         |
| 4                              | 23        | 14        | 3.71| 1.53 to 8.99 | 0.004 |
| ≥5                             | 16        | 42        | 8.85| 4.19 to 18.68 | <0.0001 |
| **P trend**                     |           |           |     |        | <0.0001 |
| **Pneumonitis**                |           |           |     |        |         |
| 0–2                            | 41        | 1         | 1.00|        |         |
| 3                              | 58        | 17        | 13.30| 1.72 to 102.94 | 0.013 |
| ≥4                             | 15        | 20        | 69.42| 8.62 to 558.91 | <0.0001 |
| **P trend**                     |           |           |     |        | <0.0001 |

*adjusted for age, gender, pack years, clinical stage, performance status, treatment regimen, radiation type, and radiation dosage.

1 unfavorable genotypes: IL6 rs1800795, IL16 rs11566218, TNF rs1799724, PTG52 rs20417, PTG22 rs1275, PTG22 rs2283470, IL8 rs1800587, IL10 rs8904, IL10A rs10933, IL1B rs16944, IL28B rs228942, IL10R rs4073, IL10RB rs2834167, IL13 rs180925, NOS2 rs2297518.

In contrast, genetic variation in NOS3 was associated with a 50% decrease in pneumonitis risk (HR:0.55, 95% CI:0.31–0.96). This was the only SNP in our analysis to be significantly associated with a reduction in risk.

**Anti-inflammatory Genes.** IL4 and IL13 share a common receptor and have many of the same anti-inflammatory functions. In our population, we found that genetic variations in both of these interleukins were associated with increased risks of developing pneumonitis. The two IL4 SNPs each resulted in increased risk with HRs of 2.54 (95% CI: 1.27–5.08) and 3.05 (95% CI: 1.50–6.22), respectively. IL13 polymorphisms had a similar effect on pneumonitis risk. Patients with two variant alleles or either rs20541 or rs180925 were approximately 3-times more likely to develop pneumonitis compared to those with wild-type or heterozygous genotypes (HR:2.95, 95% CI:1.14–7.63 and HR:3.23, 95% CI:1.03–10.18). The signaling molecule IkappaB-alpha (NFKBIA) inhibits the inflammatory response by blocking NFkappaB-mediated transcription of proinflammatory genes. NFKBIA rs8904 resulted in a 2.02-fold increased pneumonitis risk (95% CI: 1.01–4.03).

**Joint Analysis of Pneumonitis Risk Alleles.** In combined analysis, the significant SNPs together with an additional borderline significant variant – IL1R rs1801275 (P=0.053) – showed an increase in pneumonitis risk as the number of unfavorable genotypes increased (Table 3). The increased risk for carrying three unfavorable genotypes was 13.30-fold compared to patients with 0 to 2 risk genotypes (P=0.013). This risk was dramatically increased for the group of patients with four or more unfavorable genotypes (P<0.0001). These high risk individuals also had a shorter duration between start of treatment and development of pneumonitis of only 5.33 months compared to over 12 months for those with 0 to 2 unfavorable genotypes (Figure 1B).

**Inflammation-related SNPs and Overall Survival**

The development of toxicity and survival are often related since patients who develop toxicity are those who are responding to treatment. Therefore, we determined if any of the variants identified as toxicity risk factors were also associated with survival
over three years. We found that patients with at least one variant allele of IL10:rs1800872 had a 1.74-fold increased risk of esophagitis, but a 40% decreased risk of dying when compared to patients with wild-type genotypes (HR: 0.62, 95% CI: 0.40–0.97). Figure 2A illustrates the time to esophagitis for patients with IL10:rs1800872 genotypes. Although not significant, patients with wild-type genotypes had median time to event of greater than 12 months contrasted with only 1.8 months for those with at least one variant of rs1800872. For survival (Figure 2B), there was a non-significant survival advantage of nearly four months for carriers with a median survival time of 16.1 months compared to only 12.4 months for patients with wild-type genotypes.

Discussion

In this study, we systematically analyzed 59 common genetic variations in inflammation-related genes for association with risk of developing acute esophagitis or pneumonitis following radiation treatment in NSCLC patients. Multiple individual SNPs in important pro- and anti-inflammatory genes were identified as modulating risk for both normal tissue toxicities. Furthermore, the cumulative effect of these SNPs was dose-dependent with individuals carrying multiple unfavorable alleles having a corresponding increase in risk.

Nine genetic variants were identified as significantly associated with esophagitis risk, and of those, six were in proinflammatory genes (Table 2). We found that rs1800795 in IL6 resulted in a 2.16-fold increase in esophagitis. This polymorphism is located within the 5′-untranslated region of IL6 and has been functionally studied with conflicting results of the effect on gene expression and response to stimulation [18,19]. However, a recent meta-analysis of over 5,500 patients was not able to demonstrate a relationship between this variant and IL6 serum levels [20]. Gao et al. demonstrated that IL16:rs11556218 was significantly associated with colorectal and gastric cancer, but did not observe a correlation between IL16 serum levels measured in these patients and rs11556218 [21]. PTGS2:rs20417 was also associated with increased risk of esophagitis. This promoter variant disrupts a Sp1/Sp3 transcription factor binding site and causes a decrease in transcriptional activity in lung fibroblast cells [22,23]. Decreased expression of COX2 would suggest a decrease in inflammation signaling. However, this same variant, while altering the Sp1/Sp3 site, also introduces a binding site for another transcription factor, Egr-1, although the consequences are unknown [22]. The other two significant variants (rs5275 and rs689470) are located in the 3′-UTR and regulate PSTGS2 mRNA levels. Our results suggest that these SNPs are linked with an increase in pro-inflammatory...
activity leading to esophagitis. Further functional analysis is warranted to understand the underlying mechanisms [24].

For anti-inflammatory molecules and esophagitis risk, Khurana Hershey et al. demonstrated that IL4R:rs1801275 resulted in enhanced IL4 signaling and the induction of high levels of the IgE receptor CD23 [25]. IL10:rs1800872 and IL10RA:rs3135932 have been shown to decrease IL10 signaling by decreasing serum levels and altering IL10-IL10RA interactions, respectively [26,27]. The reported functions of these three SNPs would be in agreement with our findings of an increased risk of esophagitis by decreasing the anti-inflammatory response.

Twelve common polymorphisms were found to be significantly associated with risk of pneumonitis. The two IL1A variants are in linkage disequilibrium and were found to increase risk by nearly 3-fold. IL1A:rs1800587 has been shown to contribute to an increase in IL1-\(\alpha\) promoter activity, mRNA levels and protein levels [28]. IL1A:rs17561 is a non-synonymous SNP and increases processing of the IL1-\(\alpha\) precursor resulting in an increase in the levels of active IL1-\(\alpha\) [29]. The variant of IL8:rs4073, which was found to increase pneumonitis risk 3-fold, has been associated with increased secretion of the proinflammatory cytokine IL8 [30]. IL4 and IL13 work together to regulate the inflammatory response.

### Table 4. Inflammation-related SNPs and risk of pneumonitis.

| Proinflammatory cytokines, receptors, and related molecules | Grade \(<2\) n(%) | Grade \(\geq 2\) n(%) | HR | 95% CI | P value | Q value | Grade \(<2\) n(%) | Grade \(\geq 2\) n(%) | HR | 95% CI | P value | Q value |
|---------------------------------------------------------------|----------------|----------------|----|-------|--------|--------|----------------|----------------|----|-------|--------|--------|
| IL1A:rs1800587                                               | 128            | 43             |     |       |        |        | 128            | 42             |    |       |        |        |
| CC                                                            | 65(50.8)       | 11(25.6)       | 1.00 |       |        |        | 72(57.1)       | 17(40.5)       | 1.00 |       |        |        |
| CT                                                            | 51(39.8)       | 30(69.8)       | 3.66 | 1.66 to 8.07 | 0.001 |        | 50(39.7)       | 22(52.4)       | 1.84 | 0.90 to 3.79 | 0.096 |        |
| TT                                                            | 12(9.4)        | 2(4.7)         | 0.89 | 0.19 to 4.23 | 0.885 |        | 4(3.2)         | 3(7.1)         | 5.88 | 1.50 to 23.09 | 0.011 |        |
| CT+TT                                                         | 63             | 32             | 2.90 | 1.34 to 6.25 | 0.007 | 0.021 | 2.12           | 1.18 to 3.79   | 0.012 |        | 0.023 |        |
| IL1A:rs17561                                                 | 128            | 43             |     |       |        |        | 126            | 42             |    |       |        |        |
| CC                                                            | 65(50.8)       | 12(27.9)       | 1.00 |       |        |        | 89(70.6)       | 26(60.5)       | 1.00 |       |        |        |
| GT                                                            | 52(40.6)       | 29(67.4)       | 3.11 | 1.44 to 6.72 | 0.004 |        | 32(25.4)       | 12(27.9)       | 1.49 | 0.69 to 3.24 | 0.312 |        |
| TT                                                            | 11(8.6)        | 2(4.7)         | 0.85 | 0.18 to 4.01 | 0.836 |        | 5(4.0)         | 5(11.6)        | 4.49 | 1.14 to 17.66 | 0.031 |        |
| CT+TT                                                         | 63             | 31             | 2.51 | 1.19 to 5.27 | 0.015 | 0.024 | 121            | 38             | 3.96 | 1.04 to 15.12 | 0.044 | 0.038 |
| IL8:rs4073                                                   | 128            | 41             |     |       |        |        | 126            | 42             |    |       |        |        |
| CC                                                            | 65(50.8)       | 12(27.9)       | 1.00 |       |        |        | 89(70.6)       | 26(60.5)       | 1.00 |       |        |        |
| GT                                                            | 52(40.6)       | 29(67.4)       | 3.11 | 1.44 to 6.72 | 0.004 |        | 32(25.4)       | 12(27.9)       | 1.49 | 0.69 to 3.24 | 0.312 |        |
| TT                                                            | 11(8.6)        | 2(4.7)         | 0.85 | 0.18 to 4.01 | 0.836 |        | 5(4.0)         | 5(11.6)        | 4.49 | 1.14 to 17.66 | 0.031 |        |
| CT+TT                                                         | 63             | 31             | 2.51 | 1.19 to 5.27 | 0.015 | 0.024 | 121            | 38             | 3.96 | 1.04 to 15.12 | 0.044 | 0.038 |
| TNFRSF1B:rs1061622                                           | 128            | 43             |     |       |        |        | 126            | 42             |    |       |        |        |
| CC                                                            | 65(50.8)       | 12(27.9)       | 1.00 |       |        |        | 89(70.6)       | 26(60.5)       | 1.00 |       |        |        |
| GT                                                            | 52(40.6)       | 29(67.4)       | 3.11 | 1.44 to 6.72 | 0.004 |        | 32(25.4)       | 12(27.9)       | 1.49 | 0.69 to 3.24 | 0.312 |        |
| TT                                                            | 11(8.6)        | 2(4.7)         | 0.85 | 0.18 to 4.01 | 0.836 |        | 5(4.0)         | 5(11.6)        | 4.49 | 1.14 to 17.66 | 0.031 |        |
| CT+TT                                                         | 63             | 31             | 2.51 | 1.19 to 5.27 | 0.015 | 0.024 | 121            | 38             | 3.96 | 1.04 to 15.12 | 0.044 | 0.038 |
| NOS3:rs1799983                                               | 129            | 42             |     |       |        |        | 126            | 42             |    |       |        |        |
| CC                                                            | 65(50.8)       | 12(27.9)       | 1.00 |       |        |        | 89(70.6)       | 26(60.5)       | 1.00 |       |        |        |
| GT                                                            | 52(40.6)       | 29(67.4)       | 3.11 | 1.44 to 6.72 | 0.004 |        | 32(25.4)       | 12(27.9)       | 1.49 | 0.69 to 3.24 | 0.312 |        |
| TT                                                            | 11(8.6)        | 2(4.7)         | 0.85 | 0.18 to 4.01 | 0.836 |        | 5(4.0)         | 5(11.6)        | 4.49 | 1.14 to 17.66 | 0.031 |        |
| CT+TT                                                         | 63             | 31             | 2.51 | 1.19 to 5.27 | 0.015 | 0.024 | 121            | 38             | 3.96 | 1.04 to 15.12 | 0.044 | 0.038 |

*adjusted for age, gender, pack years, clinical stage, performance status, treatment regimen, radiation type, and radiation dosage.

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NSCLC Radiation Toxicity

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response. Four genetic variants in these two genes were associated with ~3-fold increased risk of pneumonitis. Studies have demonstrated increased IgE production for IL4:rs2070874 and rs2243250 [31] and increased IL13 activity for IL13:rs20541 and rs180925 [32,33].

Genetic variation in TNF and the receptor TNFRSF1B were also associated with increased risk of pneumonitis. TNF-α signaling is an important modulator of the inflammatory response. The TNF:rs1799724 variant is located within the promoter region of the gene and thought to influence gene expression by creating an OCT-1 transcription factor binding site [34]. The effect of this differential binding on TNF-α signaling is not clear. Some studies have shown an increase in TNF-α production [35,36,37], while others have shown the opposite effect [38,39,40]. For TNFRSF1B, the non-synonymous variant Met196Arg (rs1061622) does not alter TNF-α binding affinity, but results in intensified TNF-α signaling [41] and decreased NF-kB signaling [42].

Only one genetic variant was found to confer a protective effect following radiotherapy. This variant, rs1799983, in NOS3 was associated with a 70% reduction in risk of pneumonitis. Functional studies have demonstrated that this variant results in production of a variant allozyme with reduced enzyme activity [43] resulting in a reduction in nitric oxide production [44]. These observations support our findings of decreased pneumonitis due to decreased inflammatory signaling.

In all, the functional consequences of the variants identified as strongly associated with increased risk of normal tissue toxicity following radiation exposure suggest a high biological plausibility for our findings. However, little to no information is known about how these variants specifically alter pneumonitis and esophagitis risk. The inflammatory response is complex and many prototypic “proinflammatory” molecules have anti-inflammatory attributes under specific conditions, and vice versa. Further studies are warranted to elucidate the specific function of these SNPs in target tissues following exposure to radiation.

Interestingly, we observed several variants with a trend towards a relationship between toxicity and overall survival, and only one SNP was identified as being associated with both. This result...
| dbSNP ID | Alleles | Gene Symbol | Gene Name | SNP Location* |
|----------|---------|-------------|-----------|---------------|
| rs180872 | C/A     | IL10        | interleukin 10 | 5'-FR         |
| rs180896 | G/A     | IL10        | interleukin 10 | 5'-FR         |
| rs190871 | A/C     | IL10        | interleukin 10 | 5'-FR         |
| rs313592 | A/G     | IL10RA      | interleukin 10 receptor, alpha | Ser159Gly     |
| rs2834167| A/G     | IL10RB      | interleukin 10 receptor, beta | Lys47Glu      |
| rs1800925| C/T     | IL13        | interleukin 13 | 5'-FR         |
| rs20541  | C/T     | IL13        | interleukin 13 | Arg130Gln     |
| rs2070874| C/T     | IL4         | interleukin 4  | 5'-UTR        |
| rs2243250| C/T     | IL4         | interleukin 4  | 5'-FR         |
| rs1801275| A/G     | IL4R        | interleukin 4 receptor | Gln576Arg     |
| rs1805010| A/G     | IL4R        | interleukin 4 receptor | Ile75Val      |
| rs1805011| A/C     | IL4R        | interleukin 4 receptor | Glu400Ala     |
| rs1805015| T/C     | IL4R        | interleukin 4 receptor | Ser503Pro     |
| rs1805016| T/G     | IL4R        | interleukin 4 receptor | Ser752Ala     |
| rs2069812| C/T     | IL5         | interleukin 5 receptor | 5'-FR        |
| rs2233409| C/T     | NFKBIA      | IkB alpha    | 5'-FR         |
| rs8904   | C/T     | NFKBIA      | IkB alpha    | 3'-UTR        |
| rs1800206| C/G     | PPARA       | peroxisome proliferator-activated receptor alpha | Leu162Val     |
| rs2016520| A/G     | PPARD       | peroxisome proliferator-activated receptor delta | 5'-UTR       |
| rs1801282| C/G     | PPARG       | peroxisome proliferator-activated receptor gamma | Pro12Ala      |
| rs1024611| T/C     | CCL2        | chemokine (C-C motif) ligand 2 | 5'-FR        |
| rs2069614| C/T     | CSF2        | colony stimulating factor 2 (granulocyte-macrophage) | 5'-FR        |
| rs25882  | T/C     | CSF2        | colony stimulating factor 2 (granulocyte-macrophage) | Ile117Thr     |
| rs2257167| G/C     | IFNAR1      | interferon (alpha, beta and omega) receptor 1 | Val168Leu    |
| rs1051393| T/G     | IFNAR2      | interferon (alpha, beta and omega) receptor 2 | Phe10Val     |
| rs2069705| T/C     | IFNG        | interferon, gamma | 5'-FR       |
| rs2430561| A/T     | IFNG        | interferon, gamma | intron       |
| rs3212227| A/C     | IL12B       | interleukin 12B | 3'-UTR      |
| rs375947 | A/G     | IL12RB      | interleukin 12 receptor, beta 1 | Met365Thr     |
| rs11556218| T/G    | IL16        | interleukin 16 | Asn446Lys    |
| rs4778889| T/C     | IL16        | interleukin 16 | 5'-FR         |
| rs17561  | G/T     | IL1A        | interleukin 1, alpha | Ala114Ser     |
| rs1800587| C/T     | IL1A        | interleukin 1, alpha | 5'-FR       |
| rs1143627| T/C     | IL1B        | interleukin 1, beta | 5'-FR       |
| rs1143634| C/T     | IL1B        | interleukin 1, beta | Phe105Phe    |
| rs16944  | C/T     | IL1B        | interleukin 1, beta | 5'-FR       |
| rs2228139| C/G     | IL1R1       | interleukin 1 receptor, type 1 | Ala124Gly     |
| rs2069762| T/G     | IL2         | interleukin 2 | 5'-FR         |
| rs228942 | C/A     | IL2RB       | interleukin 2 receptor, beta | Asp391Glu     |
| rs1800795| C/G     | IL6         | interleukin 6 (interferon, beta 2) | 5'-FR        |
| rs2228145| A/C     | IL6R        | interleukin 6 receptor | Asp358Ala     |
| rs4073   | T/A     | IL8         | interleukin 8 | 5'-FR         |
| rs234671 | G/C     | IL8RA       | interleukin 8 receptor, alpha | Ser276Thr     |
| rs2229092| A/C     | LTA         | lymphotoxin alpha | His15Pro      |
| rs2229094| T/C     | LTA         | lymphotoxin alpha | Arg13Cy      |
| rs75562  | C/G     | MIF         | macrophage migration inhibitory factor | 5'-FR        |
| rs1799724| C/T     | TNF         | tumor necrosis factor | 5'-FR        |
| rs1799964| T/C     | TNF         | tumor necrosis factor | 5'-FR        |
| rs1800629| G/A     | TNF         | tumor necrosis factor | 5'-FR        |
| rs361525 | G/A     | TNF         | tumor necrosis factor | 5'-FR        |
suggestions that these patients who are developing acute normal tissue toxicity are responding well to therapy with longer survival times. Unfortunately, these side effects are dose limiting and often result in cessation of treatment. It may be that these select patients would receive the most benefit from the inclusion of radioprotective agents such as amifostine and glutamine in their treatment regimen. Both work by decreasing the levels of reactive oxygen species in the exposed normal tissue and, thus, potentially avoiding the development of inflammation. It would be of interest to test the species in the exposed normal tissue and, thus, potentially avoiding the development of inflammation. It would be of interest to test the significant SNPs identified in this study within the framework of the development of inflammation. It would be of interest to test the significant SNPs identified in this study within the framework of the development of inflammation.

Our study has several advantages, including the patient population with availability of comprehensive clinical and epidemiological information. To our knowledge, no study has systematically investigated the effect of genetic variations within inflammation-related genes and risk of normal tissue toxicity due to radiation therapy. This pathway-based approach allowed us to comprehensively elucidate the cumulative effects of multiple adverse alleles on toxicity risk. Since a patient’s genome can contain several of these risk associated genetic variants in both proinflammatory and anti-inflammatory pathways, this approach is much more powerful in detecting the effect of these SNPs on a patient’s risk of developing esophagitis or pneumonitis. The variants included in this study were candidate SNPs based on known or predicted effects on gene function. A candidate-gene approach has the advantage of being anchored by known biological plausibility, but there is a possibility that this study has missed additional risk alleles or detected a variant in linkage disequilibrium with the true causative SNP. In addition, we were not able to include additional variables that may also impact toxicity, including radiation field size, dose to organ at risk (esophagus and lung), treatment volume, and tumor location.

In conclusion, we identified several biologically plausible associations between genetic variants in important inflammation-related genes and risk of developing esophagitis and pneumonitis. We also demonstrated a dose-effect of inflammation SNPs as evidenced by the dramatic increases in risk with increases in number of unfavorable genotypes. Furthermore, we identified one variant in IL10 that is associated with increased risk of esophagitis, but a decreased risk of dying. Since radiotherapy is a mainstay of lung cancer treatment, having the ability to screen patients prior to initiation of treatment would potentially minimize these acute toxicity events while allowing for higher doses of radiation for those who are not at increased risk in order to improve local control. With validation, these results, together with clinical and dosimetric predictors, could serve to increase the overall benefit of radiation therapy in NSCLC patients.

Methods

Ethics Statement

Participants gave written informed consent and the study was approved by The University of Texas MD Anderson Cancer Center’s Institutional Review Board.

Patient Population

The study included non-Hispanic Caucasian subjects who were newly diagnosed, histologically confirmed stage IIA or IIB without a malignant effusion (dry) NSCLC patients receiving definitive thoracic radiation or chemoradiation therapy at The University of Texas MD Anderson Cancer Center. All of the patients were enrolled in an ongoing epidemiology lung cancer study between 1995 and 2007.

Epidemiological and Clinical Data Collection

Epidemiologic data were collected during an in-person interview using a structured questionnaire to determine demographic characteristics, medical history, and smoking history. Clinical and follow-up information was abstracted from medical records. Pre-treatment performance status was determined based on the Eastern Cooperative Oncology Group scale. Radiation-induced esophagitis was characterized by documentation of new-onset pain on swallowing occurring during treatment. Pneumonitis was detected by roentgenographic or CT scan abnormalities and often associated with nonproductive cough and/or fever. Severity of pneumonitis or esophagitis was scored by the physician according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0) guidelines [45]. For this study, an event was considered the occurrence of grade ≥2 toxicity.

SNP Selection and Genotyping

Blood was drawn from each participant following the in-person interview. These samples were used to extract genomic DNA from peripheral blood lymphocytes using the Human Whole Blood Genomic DNA Extraction Kit (Qiagen, Valencia, CA). A total of 59 candidate SNPs (Table 5) were selected from 37 known inflammation-related genes as previously described [46]. Briefly, candidate SNPs were selected if they had a minor allele frequency

| dbSNP ID | Alleles | Gene Symbol | Gene Name | SNP Location* |
|----------|---------|-------------|-----------|---------------|
| rs4149570 | G/T | TNFRSF1A | tumor necrosis factor receptor superfamily, member 1A | 5’-FR |
| rs4149584 | G/A | TNFRSF1A | tumor necrosis factor receptor superfamily, member 1A | Arg121Gln |
| rs1061622 | T/G | TNFRSF1B | tumor necrosis factor receptor superfamily, member 1B | Met196Arg |
| rs5746026 | G/A | TNFRSF1B | tumor necrosis factor receptor superfamily, member 1B | Glu232Lys |
| rs2297518 | G/A | NOS2 | nitric oxide synthase 2, inducible | Leu608Ser |
| rs1799983 | G/T | NOS3 | nitric oxide synthase 3 (endothelial cell) | Glu298Asp |
| rs20417 | G/C | PTGS2 | prostaglandin-endoperoxide synthase 2 | 5’-FR |
| rs5275 | T/C | PTGS2 | prostaglandin-endoperoxide synthase 2 | 3’-UTR |
| rs689470 | C/T | PTGS2 | prostaglandin-endoperoxide synthase 2 | 3’-UTR |

*FR: flanking region, UTR: untranslated region.
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Table 5. Cont.
greater than 5% and were located in a putative functional region of the gene (promoter, untranslated regions (UTR) or exons) or had previously been reported as associated with cancer or an inflammatory disorder. Genotyping was performed using the SNPlex assay following manufacturer’s instructions (Applied Biosystems). For toxicity, CA with analysis on an Applied Biosystems 3730 DNA Analyzer. SNP genotypes were called using the GeneMapper software (Applied Biosystems). Three SNPs: IL8RArs2249671, LTACrs2290902 and IL4Rrs1805011 were removed because of excessive missing genotypes (>20%). All genotyping was completed blinded with regard to toxicity status.

Statistical Analysis

Time to event (grade ≥2 pneumonitis or esophagitis) was based on the duration from start of radiation treatment to occurrence of toxicity. Three-year survival was also defined as time from start of radiation treatment to the date of death or the date of last follow-up during the three year period. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for each individual SNP and endpoint combination were estimated by fitting the Cox proportional hazard model while adjusting for age, gender, clinical stage, pack years of smoking, pre-treatment performance status, treatment regimen (radiotherapy or chemoradiotherapy), radiation type, and radiation dosage. Kaplan-Meier curves and log-rank tests were used to assess differences in time to event and overall survival rate. Combined effects of unfavorable genotypes were based on the main effect analysis of individual SNPs and included those with significant (P<0.05) and borderline significant (P<0.10) associations. STATA software (version 10, STATA Corp., College Station, TX) was used for statistical analyses. Q-values were calculated to control for multiple comparisons based on an FDR value of 10% [47].

Author Contributions

Conceived and designed the experiments: MATH XW. Performed the experiments: JG. Analyzed the data: MATH YW. Contributed reagents/materials/analysis tools: RK ZL JG YJ SL JDC WRK MRS XW. Wrote the paper: MATH XW.

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