Influence of Genetic Variants in Type I Interferon Genes on Melanoma Survival and Therapy

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Abstract

Melanoma is an immunogenic tumor; however, the efficacy of immune-therapy shows large inter-individual variation with possible influence of background genetic variation. In this study we report the influence of genetic polymorphisms in the type I interferon gene cluster on chromosome 9p22 on melanoma survival. We genotyped 625 melanoma patients recruited in an oncology center in Germany for 44 polymorphisms located on chromosome 9p22 that were informative for 299 polymorphisms and spanned 15 type I interferon genes. Our results showed associations between time to metastasis/survival and two linked (r² = 0.76) polymorphisms, rs10964859 (C>G) and rs10964862 (C>A). The rs10964859 polymorphism was located at 3’UTR and rs10964862 was 9.40 Kb towards 5’UTR of IFNW1 gene. The carriers of the variant alleles of the rs10964859 and rs10964862 polymorphisms were associated with a reduced disease-free survival. The validation of data in an independent group of 710 patients from Spain showed that the direction of the effect was similar. Stratification based on therapy showed that the adverse effect on metastasis development was statistically significant in the patients from Spain who did not receive any treatment and were homozygous for variant allele of rs10964862 (HR = 2.52, 95% CI 1.07–5.90; P = 0.03). Patients homozygous for rs10964859 (HR = 2.01, 95% CI 1.17–3.44; P = 0.01) and rs10964862 (HR 1.84, 95%CI 1.03–3.27, P = 0.04) were associated to increased risk of death following metastasis. The rs10964859 polymorphism was located at 3’UTR and rs10964862 was 9.40 Kb towards 5’UTR of IFNW1 gene. The carriers of the variant alleles of the rs10964859 and rs10964862 polymorphisms were associated with a reduced disease-free survival. The validation of data in an independent group of 710 patients from Spain showed that the direction of the effect was similar. Stratification based on therapy showed that the adverse effect on metastasis development was statistically significant in the patients from Spain who did not receive any treatment and were homozygous for variant allele of rs10964862 (HR = 2.52, 95% CI 1.07–5.90; P = 0.03). Patients homozygous for rs10964859 (HR = 2.01, 95% CI 1.17–3.44; P = 0.01) and rs10964862 (HR 1.84, 95%CI 1.03–3.27, P = 0.04) were associated to increased risk of death following metastasis. GTCGACAA haplotype, found in 8.8% of the patients, was associated with an increased risk of death (HR 1.94, 95%CI 1.16–3.26, P = 0.01). In conclusion, our results identified genetic variants in interferon genes that influence melanoma progression and survival with modulation of effect due to treatment status.

Introduction

Melanoma is considered a highly immunogenic tumor [1–3]. Strong evidence of the interaction of melanoma with the immune system has been supported by spontaneous remissions of growing melanomas [4], presence of tumor-infiltrated lymphocytes and development of vitiligo, which is associated with improved prognosis [5]. An increased incidence of melanoma tumors in transplant recipients [6] and evidence of immunoevasion are also common occurrences [7]. Immunotherapy has been one of the standard treatments in combating metastasized disease, however, the response rate remains poor [8,9]. In order to increase humoral and cell mediated immunity against melanoma, therapies that include cytokines are used particularly in the adjuvant setting. Interferon-alpha (IFNA), a type I IFN has been reportedly shown to affect melanoma behavior in humans and despite side effects it has been associated with prolonged relapse-free survival [10–13]. A clear effect on disease free and distant metastasis free survival but not on the overall survival was observed in high risk patients [14,15]. Evidence suggested that the level of responsive-ness to IFN treatment varies among individuals. Among the different possible causes for these inter-subject variations are the genetic polymorphisms. Associations of genetic variants in several type I IFN genes with different diseases, including melanoma have been reported in several studies [16–21].

Human type I IFN genes are located on chromosome 9p and, with an exception of IFN kappa, form a cluster upstream of the CDKN2A and CDKN2B tumor-suppressor genes and the noncoding antisense RNA encoded by CDKN2BAS. The region is frequently mutated and deleted in a wide variety of tumors and associated with melanoma [22,23]. In this work, we aimed at surveying an entire set of variants spanning a 342 kb region with type I IFN genes with focus on the cluster on chromosome 9p22. The role of genetic variants in the type I IFN genes was investigated for association with melanoma survival and therapy in melanoma patients recruited in Germany. The results from the investigation of these polymorphisms were additionally confirmed in an independent group of patients recruited in Spain.
Table 1. Characteristics of the melanoma patients from Germany and Spain.

|                     | GERMANY                      | SPAIN                      |
|---------------------|------------------------------|----------------------------|
|                     | All patients with skin melanoma | Patients with AJCC stage 0, I or II at first diagnosis (FD) | All patients with skin melanoma | Patients with AJCC stage 0, I or II at first diagnosis (FD) |
| Number of patients  | 752                          | 541                        | 837                          | 638                        |
| Gender              |                              | 118                        |                              | 127                        |
| Male                | 412 (54.8%)                  | 301 (55.6%)                | 379 (45.3%)                  | 309 (43.5%)                |
| Female              | 340 (45.2%)                  | 240 (44.4%)                | 458 (54.7%)                  | 401 (56.5%)                |
| Age at FD           |                              |                            |                              |                            |
| Median              | 55                            | 55                         | 53                           | 52                         |
| Mean                | 54.0                          | 54.4                       | 51.5                         | 50.8                       |
| Standard deviation  | 16.0                          | 15.7                       | 16.0                         | 16.0                       |
| Breslow thickness (mm) |                          |                            |                              |                            |
| Median              | –                             | 1.2                        | –                            | 1.0                        |
| Mean (95%CI)        | –                             | 1.8 (1.6–1.9)              | –                            | 1.6 (1.5–1.8)              |
| Standard deviation  | –                             | 1.8                        | –                            | 1.9                        |
| Range (minimum, maximum) | –                         | 14.0 (0.0, 14.0)           | –                            | 17.9 (0.1, 18.0)           |
| AJCC stages at FD   |                              |                            |                              |                            |
| 0                   | 10 (1.3%)                    | 6 (1.1%)                   | 70 (8.4%)                    | 4 (0.6%)                   |
| I                   | 400 (53.2%)                  | 338 (62.5%)                | 451 (53.9%)                  | 446 (69.9%)                |
| II                  | 215 (28.6%)                  | 197 (36.4%)                | 189 (22.6%)                  | 188 (29.3%)                |
| III                 | 111 (14.8%)                  | –                          | 114 (13.6%)                  | –                          |
| IV                  | 11 (1.5%)                    | –                          | 4 (0.9%)                     | –                          |
| unknown             | 5 (0.7%)                     | –                          | 10 (1.2%)                    | –                          |
| Total number of metastasis | 379 (50.4%)              | 257 (41.1%)                | 146 (17.4%)                  | 92 (12.9%)                 |
| Total number of deaths | 238 (31.6%)               | 174 (27.8%)                | 146 (27.0%)                  | 45 (6.3%)                  | 45 (7.1%)                  |

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Materials and Methods

Study Population

The study was carried out on 752 German cutaneous melanoma patients. Survival analysis included 625 patients diagnosed at AJCC stage 0, I and II; complete data for age, gender and Breslow thickness were available for 541 patients (Table 1). Melanoma cases were referred to the Skin Cancer Unit, German Cancer Research Center Heidelberg, at the University Hospital Mannheim. For validation purposes, 837 patients from Spain were added to the study, which included 797 patients with tumors classified with AJCC stage 0, I or II and and 725 patients had complete information for age, gender and Breslow thickness (Table 1). Spanish melanoma patients were recruited at the Department of Dermatology, Instituto Valenciano de Oncología, a referral skin cancer centre for the provinces of Valencia, Alicante, and Castellón, with a catchment population of ~5 million people. Blood samples from melanoma patients were collected between 2000 and 2007 and diagnoses were confirmed by histopathology. The ethical approval for the study was granted by Ethics Commission of the Faculty of Clinical Medicine of University of Heidelberg and written informed consent was obtained from all study participants. All the patients were of European ethnicity.

Selection of Polymorphisms in Interferon Gene Cluster on Chromosome 9p22

We selected 44 SNPs using tagging approach, which encompassed 15 genes and represented 299 SNPs tagged with a $r^2 \geq 0.8$ (Table S1). We aimed to evaluate an entire set of variants within a 342 kb region that contained a cluster of type I interferon genes on chromosome 9p22. IFNW1, located 24 kb farther from IFNA21, and IFNE, located at a distance of 40 kb from IFNA1, defined the limits of the locus. We included SNPs within the KLH9 gene because of its location within the type I interferon cluster (Figure 1). The inclusion criteria also included a minor allele frequency (MAF) of 5% or more in Caucasian population based on HapMap data (release #28).

Genotyping

Genotyping was carried out using an allelic discrimination method (Kaspar Assay from KBiosciences). PCR was carried out in 384-well format in a total volume of 4µl using 5 ng of DNA.
template, 0.11μl of assay mix (100 nM of two allele specific forward primers and common reverse primer, final concentration), 4μl reaction mix (Kbioticsiences) and MgCl₂. The reactions were performed using standard optimized conditions. Underdetermined samples were genotyped again separately in a 96-well plate format. For quality control, ~5% of samples were randomly selected and included as replicates.

Statistical Analysis

Survival analyses were performed for the polymorphisms within the interferon genes in order to investigate the influence of the genotypes on disease free survival (DFS), time from metastasis to death (MD) and overall survival (OS). DFS was defined as the time (in years) from diagnosis of primary melanoma until the first metastasis (either regional or distant), MD survival was defined as the time from first metastasis to death or last patient contact. For MD analysis we only considered those patients who developed metastasis during the follow up time. The OS was the time from date of first diagnosis until death or last patient contact. The associations of the genotypes with DFS, MD and OS were estimated for 10 years using Kaplan-Meier methods and log-rank test was used to compare difference between the survival curves. Haplotype frequencies were inferred using the expectation-maximization algorithm (PROC HAPLOTYPE, SAS/Genetics Software). For the genotypes and inferred haplotypes associations were estimated as hazard ratio (HR) based on Cox regression, adjusted for gender, age and Breslow thickness (PROC PHREG, SAS 9.2). The analysis was carried out on data from the German and Spanish patients without metastasis at first diagnosis (AJCC stages 0, I or II) with adjustment for age, gender and Breslow thickness (Tables S2 and S3). Ulceration status was not included in the multivariate analysis due to unavailability of complete data.

Determination of the Effect of Genotypes on Melanoma Therapy

Genotype data were also analyzed after stratification of both German and Spanish melanoma patients on the basis of administered therapy; survival analyses were performed on different subgroups of patients, separately. Start time and duration of therapy were considered to explore the possible interaction between therapy and genotypes. The treatment was taken into account in statistics analysis as a time-dependent variable. The variability in treatment in terms of dosage and frequency could not be evaluated due to lack of information.

German melanoma patients included those who did not receive any therapy or those who received different kinds of therapies including chemotherapy, radiotherapy and/or immunotherapy. Within the immune-treated group there were those patients that received interferon as adjuvant therapy, and those patients that received different types of immune-treatment in stage IV as part of an chemoinmunotherapy regimen (Figure 2 A). We analyzed data after stratification into groups, those who received interferon, alone or in combination with some other kind of treatment (chemotherapy and/or radiotherapy), and the rest of the patients that never received interferon. We called these two groups “with interferon” and “without interferon”, respectively (Figure 2 B and C). We also compared a subgroup of 84 patients that received only interferon and no other kind of treatment with the total set of the patients without therapy and those that went under different therapies without only IFN. We called these groups “with only interferon” and “without only interferon”, respectively (Figure 2 D and E).

Spanish melanoma patient group was comprised of cases those received interferon as adjuvant treatment and those who did not receive any kind of treatment. These patients were analyzed under similar criteria of “with only interferon” and “without only interferon”, with the difference being that, unlike the patients from Germany, in the “without only interferon” group, the Spanish melanoma patients received no treatment at all.

Prediction of Functional Effects of Human SNPs Located at the 5’ and 3’UTR

Prediction models were used for the role in putative miRNA-mRNA interactions of those polymorphisms that were associated with different melanoma survival parameters and were located within and near 3’UTRs of the genes. Different algorithms were used to predict the ability of the variant to affect miRNA binding sites, which included PITA (http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html) [24], PolymiRTS (Polymorphism in microRNA Target Site) Database 2.0 (http://compbio.uthsc.edu/miRSNP/search.php) [25–27], and miRanda (http://www.microrna.org/microrna/getGeneForm.do) [27,28]. Similarly, in order to identify putative transcription factor binding sites at sequence motifs containing melanoma survival associated SNPs, located near 5’UTRs of the genes, we used the TESS (Transcription Element Search System) web-based software tool (http://www.cbil.upenn.edu/cgi-bin/tess) [29].

Results

Results of Genotype Analysis of Polymorphisms in Interferon Genes

We genotyped 625 melanoma patients recruited in an oncology center in Germany for 44 polymorphisms located on chromosome 9p22 that were informative for 299 polymorphisms and spanned 15 type I interferon genes. Data analysis showed statistical significant association between variants alleles of three polymorphisms, rs10964859 (3’UTR of IFNW1), rs10964862 and rs597408, in more than one survival parameter (Table 2). The three polymorphisms were additionally genotyped in an independent set of 797 melanoma patients recruited in Valencia, Spain, and the data confirmed the association between the rs10964859 and rs10964862 polymorphisms and different survival parameters similar to that in German patients. The association observed for the rs597408 polymorphism in German melanoma patients did not replicate in Spanish patients.

Data analysis showed that the carriers of the variant allele of the rs10964859 polymorphism were associated with a shorter time to the develop metastasis than the non-carriers (7.0 years versus 9.3 years; Kaplan-Meier survival log rank test P = 0.03; Figure 3 A). Multivariate Cox regression showed associated HR 1.33 for DFS in the carriers (95%CI 0.99–1.74; P = 0.06; Table S4). The patients, homozygote for minor G-allele, had a median survival of 2.0 years from metastasis to death compared to 4.2 years for patients that were homozygote for major C-allele (data not shown). The 3’utR of IFNW1 polymorphism was associated with reduced DFS (HR 1.61, 95% CI 0.99–2.62; P = 0.05) in the group of patients that never received only IFN (Table S5, Figure 2 E). 78% of the patients with GG genotype had died within the
The group “with IFN” (HR = 3.08, 95% CI 1.48–6.40; P = 0.003; Table S6, Figure 2 B) compared to 53% with the CC homozygous genotype. The effect of the minor allele GG genotype on the risk of death was higher with than without therapy based stratification. The same group of patients with GG genotype showed association with OS (HR = 1.84, 95% CI 1.04–3.26; P = 0.04; Table S5). We observed that the detrimental effect of GG genotype on OS was mainly visible in the group that received either IFN therapy alone or in combination with other therapies (HR = 2.38, 95% CI 1.13–5.02; P = 0.02; Table S6, Figure 2 B “with IFN”). Within that group 57% of the patients with the GG homozygous genotype had died compared to 30% with the CC homozygous genotype.

Figure 2. A, Number of melanoma patients from Germany and therapy types. B, “with IFN”, patients that received IFN alone or combined with other treatments. C, “without IFN”, patients that either did not receive any therapy or that received different kinds of therapies but never IFN. D, “with only IFN”, patients that received only IFN as therapy. E, “without only IFN”, patients that either did not receive any therapy or that received different kinds of therapies combined or not with IFN. IFN, interferon; C, chemotherapy; R, radiotherapy; INI, immunotherapy no IFN; NT, no treatment.

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Table 2. Estimated 10 years survival analysis performed on the patients from Germany with first metastasis at diagnosis adjusted for age, gender and Breslow thickness.

| SNP          | genotype | n D % | HR CI | P       | n M % | HR CI | P       | n D % | HR CI | P       | Disease free progression |
|--------------|----------|-------|-------|---------|-------|-------|---------|-------|-------|---------|-------------------------|
| rs1424860    | TT       | 385   | 90    | 1       | 0.01  | 155   | 31        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | TC       | 138   | 26    | 66.00   | 0.00  | 138   | 26        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | CC       | 135   | 28    | 18.80   | 0.21  | 135   | 28        | 1.00  | 1.00  | 0.39  | Overall survival        |
| rs10964859   | CC       | 232   | 43    | 18.50   | 1.00  | 232   | 43        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | CG       | 257   | 55    | 22.40   | 1.00  | 257   | 55        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | GG       | 7     | -     | -       | -     | 7     | -         | -     | -     | -       | Overall survival        |
| rs10511694   | CC       | 267   | 45    | 24.30   | 1.00  | 267   | 45        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | TC       | 219   | 43    | 24.30   | 1.00  | 219   | 43        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | CC       | 126   | 22    | 18.20   | 0.21  | 126   | 22        | 1.00  | 1.00  | 0.39  | Overall survival        |
| rs10811482   | AA       | 396   | 81    | 20.50   | 1.00  | 396   | 81        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | AG       | 124   | 25    | 19.80   | 0.21  | 124   | 25        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | GG       | 18    | -     | -       | -     | 18    | -         | -     | -     | -       | Overall survival        |
| rs2081381    | CC       | 197   | 44    | 22.30   | 1.00  | 197   | 44        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | CG       | 264   | 59    | 22.30   | 1.00  | 264   | 59        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | GG       | 65    | 13    | 20.00   | 0.21  | 65    | 13        | 1.00  | 1.00  | 0.39  | Overall survival        |
| rs10081742   | AA       | 396   | 81    | 20.50   | 1.00  | 396   | 81        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | AG       | 124   | 25    | 19.80   | 0.21  | 124   | 25        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | GG       | 7     | -     | -       | -     | 7     | -         | -     | -     | -       | Overall survival        |
| rs10964862   | CC       | 231   | 43    | 18.60   | 1.00  | 231   | 43        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | CG       | 253   | 55    | 22.40   | 1.00  | 253   | 55        | 1.00  | 1.00  | 0.39  | Overall survival        |
|              | GG       | 49    | 10    | 22.40   | 1.00  | 49    | 10        | 1.00  | 1.00  | 0.39  | Overall survival        |
| rs10383852   | AA       | 18    | 4     | -       | -     | 18    | -         | -     | -     | -       | Overall survival        |
|              | AG       | 18    | 4     | -       | -     | 18    | -         | -     | -     | -       | Overall survival        |

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Variants in Interferon Genes and Melanoma
| SNP          | Overall survival | Disease free progression | Metastasis to death |
|--------------|------------------|--------------------------|---------------------|
|              | genotype         | n | D | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
|              | SNP genotype     | n | M | %  | HR | CI | P    | n | M | %  | HR | CI | P    |
| SNP     | genotype | n   | D | %   | HR CI         | P     | n   | M | %   | HR CI         | P     |
|---------|----------|-----|---|-----|---------------|-------|-----|---|-----|---------------|-------|
| TA      | AA       | 158 | 36| 22.80| 1.03 (0.69 - 1.53)| 0.89  | 158 | 55 | 34.80| 0.93 (0.68 - 1.27)| 0.65  |
|         | AG       | 405 | 89 | 22.00| 1.00 (referent)| -     | 405 | 154| 38.00| 1.00 (referent)| -     |
|         | AA       | 11  | 2  | 18.20| 0.75 (0.18 - 3.05)| 0.68  | 11  | 4  | 36.40| 0.99 (0.63 - 1.57)| 0.99  |
|         | AG       | 8   | 2  | 16.20| 0.69 (0.15 - 2.89)| 0.33  | 8   | 4  | 50.00| 0.95 (0.62 - 1.44)| 0.70  |
| rs913931| TA + AA  | 169 | 38 | 22.50| 1.01 (0.68 - 1.49)| 0.97  | 169 | 54 | 31.30| 0.85 (0.59 - 1.23)| 0.44  |
|         | GG       | 60  | 38 | 63.30| 0.93 (0.63 - 1.37)| 0.71  | 60  | 38 | 63.30| 0.93 (0.63 - 1.37)| 0.71  |
|         | AG-GG    | 120 | 38 | 25.00| 0.75 (0.45 - 1.28)| 0.31  | 120 | 38 | 25.00| 0.75 (0.45 - 1.28)| 0.31  |
|         | GG       | 14  | 4  | 28.60| 1.27 (0.75 - 2.16)| 0.37  | 14  | 4  | 28.60| 1.27 (0.75 - 2.16)| 0.37  |
| rs597408 | AA       | 466 | 101| 21.70| 1.00 (referent)| -     | 466 | 169| 36.30| 1.00 (referent)| -     |
|         | AG       | 50  | 9  | 18.00| 0.89 (0.45 - 1.77)| 0.50  | 50  | 9  | 18.00| 0.89 (0.45 - 1.77)| 0.50  |
|         | GG       | 14  | 7  | 50.00| 2.75 (1.26 - 6.00)| 0.01  | 14  | 7  | 50.00| 2.75 (1.26 - 6.00)| 0.01  |
| rs10448208| AA       | 405 | 89 | 22.00| 1.00 (referent)| -     | 405 | 154| 38.00| 1.00 (referent)| -     |
|         | AG       | 50  | 9  | 18.00| 0.89 (0.45 - 1.77)| 0.50  | 50  | 9  | 18.00| 0.89 (0.45 - 1.77)| 0.50  |
|         | GG       | 14  | 7  | 50.00| 2.75 (1.26 - 6.00)| 0.01  | 14  | 7  | 50.00| 2.75 (1.26 - 6.00)| 0.01  |
| rs647167 | TT       | 382 | 85 | 23.50| 0.96 (0.67 - 1.38)| 0.55  | 382 | 85 | 23.50| 0.96 (0.67 - 1.38)| 0.55  |
|         | TG       | 382 | 85 | 23.50| 0.96 (0.67 - 1.38)| 0.55  | 382 | 85 | 23.50| 0.96 (0.67 - 1.38)| 0.55  |
| rs151544 | AA       | 203 | 45 | 22.20| 0.97 (0.62 - 1.52)| 0.70  | 203 | 45 | 22.20| 0.97 (0.62 - 1.52)| 0.70  |
|         | CA       | 70  | 20 | 28.60| 1.27 (0.75 - 2.16)| 0.37  | 70  | 20 | 28.60| 1.27 (0.75 - 2.16)| 0.37  |
| SNP     | Overall survival | | Disease free progression | | Metastasis to death |
|---------|-----------------|-----------------|-----------------|-----------------|
|         | genotype        | n   | D | % | HR CI | P   | n   | M | % | HR CI | P   | n   | D | % | HR CI |
| TC      | TT              | 339 | 127 | 39.00 | 1.23 (0.92 - 1.66) | 0.24 | 68 | 44 | 64.70 | 0.98 (0.68 - 1.42) | 0.03 |
| rs4978113 | TT            | 339 | 127 | 39.00 | 1.23 (0.92 - 1.66) | 0.24 | 68 | 44 | 64.70 | 0.98 (0.68 - 1.42) | 0.03 |
| TC      | CC              | 103 | 37 | 35.90 | 0.83 (0.55 - 1.25) | 0.38 | 42 | 24 | 57.10 | 0.92 (0.55 - 1.55) | 0.77 |
| rs1330322 | AA            | 177 | 70 | 39.50 | 1.00 (referent) | - | 107 | 69 | 64.50 | 1.17 (0.80 - 1.72) | 0.42 |
| rs7025006 | CC            | 156 | 61 | 39.10 | 1.00 (referent) | - | 64 | 40 | 62.50 | 1.00 (referent) | - |
| rs2104880 | CC            | 479 | 179 | 37.40 | 1.00 (referent) | - | 198 | 125 | 63.10 | 1.00 (referent) | - |
| rs1332179 | TC            | 428 | 160 | 37.40 | 1.00 (referent) | - | 177 | 113 | 63.80 | 1.00 (referent) | - |
| rs1591032 | AA            | 352 | 130 | 36.90 | 1.00 (referent) | - | 145 | 92 | 63.40 | 1.00 (referent) | - |
| rs7871767 | GA            | 178 | 63 | 35.40 | 0.92 (0.68 - 1.24) | 0.59 | 68 | 39 | 57.40 | 0.77 (0.53 - 1.12) | 0.17 |
| rs7043990 | CC            | 446 | 165 | 37.00 | 1.00 (referent) | - | 182 | 118 | 64.80 | 1.00 (referent) | - |

Table 2. Cont.
### Table 2. Cont.

| SNP         | Overall survival | Disease free progression | Metastasis to death |
|-------------|------------------|--------------------------|---------------------|
|             | n    | D % | HR CI                  | n    | M % | HR CI                  | n    | D % | HR CI                  |
| CT          | 81   | 13  | 16.00 (0.77 - 1.13)    | 81   | 30  | 37.00 (1.14)           | 33   | 15  | 45.50 (0.65)           |
| TT          | 2    | -   | -                      | 3     | 2   | -                      | 15   | 2   | -                      |
| CT + TT     | 83   | 13  | 15.70 (0.76 - 1.15)    | 83   | 30  | 36.10 (1.12)           | 33   | 15  | 45.50 (0.65)           |
| rs1332190   | TT   | 266  | 62  | 23.30 (0.38)           | 266  | 95  | 23.50 (1.00)           | 107  | 69  | 46.05 (1.00)           |
| TC          | 277  | 50  | 22.00 (0.86)           | 277  | 89  | 39.20 (1.02)           | 97   | 59  | 60.80 (0.84)           |
| CC          | 45   | 6   | 13.30 (0.50)           | 45   | 12  | 26.70 (0.69)           | 12   | 5   | 41.70 (0.56)           |
| TC + CC     | 272  | 56  | 20.60 (0.80)           | 272  | 101 | 37.10 (0.96)           | 109  | 64  | 58.70 (0.81)           |
| rs7864960   | GG   | 131  | 36  | 27.50 (1.00)           | 131  | 46  | 35.10 (1.00)           | 51   | 32  | 62.70 (1.00)           |
| GA          | 274  | 53  | 19.30 (0.58)           | 274  | 101 | 36.90 (0.89)           | 113  | 66  | 58.40 (0.99)           |
| AA          | 126  | 27  | 21.40 (0.70)           | 126  | 46  | 36.50 (1.03)           | 49   | 33  | 60.80 (0.84)           |
| GA + AA     | 400  | 80  | 20.00 (0.62)           | 400  | 147 | 36.80 (0.90)           | 162  | 99  | 61.10 (1.01)           |
| rs6475535   | CC   | 356  | 77  | 21.60 (1.00)           | 356  | 132 | 37.10 (1.00)           | 146  | 87  | 59.60 (1.00)           |
| GG          | 168  | 40  | 23.80 (1.00)           | 168  | 62  | 36.90 (0.92)           | 94   | 52  | 55.30 (0.96)           |
| AA          | 127  | 27  | 21.40 (0.70)           | 127  | 46  | 36.50 (1.03)           | 51   | 32  | 62.70 (1.00)           |
| GA + AA     | 400  | 80  | 20.00 (0.62)           | 400  | 147 | 36.80 (0.90)           | 162  | 99  | 61.10 (1.01)           |
| rs10491569  | CC   | 266  | 65  | 24.40 (1.00)           | 266  | 96  | 36.10 (1.00)           | 103  | 67  | 65.00 (1.00)           |
| CT          | 221  | 42  | 19.00 (0.80)           | 221  | 83  | 37.60 (1.03)           | 94   | 52  | 55.30 (0.96)           |
| TT          | 41   | 11  | 26.80 (1.12)           | 41   | 15  | 36.60 (0.91)           | 17   | 14  | 82.40 (1.01)           |
| CT + TT     | 262  | 53  | 20.20 (0.85)           | 262  | 98  | 37.40 (1.01)           | 111  | 66  | 59.50 (1.05)           |
| rs1888888   | GG   | 472  | 100 | 21.20 (1.00)           | 472  | 171 | 36.20 (1.00)           | 186  | 114 | 61.30 (1.00)           |
| GA          | 62   | 17  | 27.40 (1.12)           | 62   | 22  | 35.50 (1.06)           | 27   | 18  | 66.70 (1.04)           |
| AA          | 181  | 40  | 22.10 (1.12)           | 181  | 23  | 36.50 (1.06)           | 75   | 49  | 65.00 (1.04)           |
| AG + AA     | 266  | 53  | 19.90 (0.80)           | 266  | 92  | 36.90 (1.03)           | 94   | 52  | 55.30 (0.96)           |
| rs2383192   | TT   | 132  | 28  | 21.20 (1.00)           | 132  | 53  | 37.60 (1.00)           | 75   | 49  | 65.00 (1.00)           |
| TC          | 284  | 59  | 20.80 (0.74)           | 284  | 99  | 34.90 (0.93)           | 110  | 58  | 56.90 (0.94)           |
| CC          | 106  | 28  | 26.40 (1.08)           | 106  | 39  | 36.80 (0.85)           | 43   | 30  | 69.80 (1.09)           |
| AC          | 469  | 99  | 21.10 (0.74)           | 469  | 171 | 36.50 (1.00)           | 188  | 112 | 59.60 (1.00)           |
| CC          | 2    | -   | -                      | 2    | -   | -                      | 15   | 2   | -                      |

Variants in Interferon Genes and Melanoma

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The analysis of data from Spanish melanoma patients showed that carriers of the variant G-allele of the rs10964859 polymorphism were associated with an early development of metastasis compared to non-carriers (log rank test $P = 0.08$). The carriers of GG-genotype showed reduced time for DFS, reduced time for MD and decreased OS, but associations were not statistically significant. Detrimental effect of the variant allele was more pronounced in the carrier patients and who never received IFN therapy (HR 1.88; 95% CI 0.97–3.65; $P = 0.06$; Table S7, Figure 3 B).

The prediction analysis using PITA showed an intermediate ddG energy score for binding of hsa-mir-26a to the sequence motif containing the rs10964859 polymorphism. PolymiRTS algorithm predicted that CG nucleotide transition could influence the putative binding site of the hsa-miR-4503. It also indicated that the derived allele disrupts a conserved miRNA binding site.

The rs10964859 polymorphism was in linkage with 11 kb apart polymorphism rs10964862 with an $r^2$ of 0.76. Melanoma patients with the variant A-allele of the rs10964862 polymorphism were associated with reduced time for DFS with 9.3 years versus 7.0 years (log rank test $P = 0.03$; Figure 3 C) with multivariate HR 1.42 (95% CI 1.05–1.91; $P = 0.02$). The effect was statistically significant in heterozygote as well as homozygote carriers (Table 3). We also observed that the carriers of the minor allele of rs10964862 in the group of patients “without IFN” and “without only IFN” were associated with statistically significant increase in DFS rate (“without IFN” HR 2.52, 95% CI 1.07–5.90; $P = 0.03$; Table S9). The minor allele AA genotype for rs10964862 was also statistically significantly associated with decreased MD survival only in the group “without only IFN”, (HR of 2.63, 95% CI 1.20–5.76; $P = 0.02$; Table S8, Figure 2 B). The validation of results in patients from Spain showed the association of the carriers of the variant allele for rs10964862 with a reduced DFS; though association after adjustment was not statistically significant (HR 1.32, 95% CI 0.83–2.10; $P = 0.24$; Table 3).

The patients with homozygous genotype for the variant G-allele for the rs597408 polymorphism were associated with DFS (HR 2.16, 95% CI 1.03–4.43; $P = 0.04$) and reduced OS (HR 2.75, 95% CI 1.26–6.00; $P = 0.01$; Table S11). After stratification, we also observed that detrimental effect was statistically significant for the minor allele genotype in the group “without only IFN” for the rs10964862 polymorphism with a reduced DFS; though association after adjustment was not statistically significant (HR 1.32, 95% CI 0.83–2.10; $P = 0.24$; Table 3). The effect of variant genotypes on OS and MD was also not statistically significant. In the Spanish patients, for rs10964862 polymorphism, we observed that detrimental effect was statistically significant for the minor allele homozygous genotype for “without only IFN” patients (HR 2.52, 95% CI 1.07–5.90; $P = 0.03$; Table S10).

The patients with homozygous genotype for the variant G-allele for the rs597408 polymorphism were associated with DFS (HR 2.16, 95% CI 1.03–4.43; $P = 0.04$) and reduced OS (HR 2.75, 95% CI 1.26–6.00; $P = 0.01$; Table S11). After stratification, we also observed that detrimental effect was statistically significant for the minor allele genotype in the group “without only IFN” for the DFS (HR 2.09, 95% CI 1.01–4.29; $P = 0.03$) and OS analysis (HR 2.63, 95% CI 1.20–5.76; $P = 0.02$; Table S12). Though, results did not replicate in Spanish patients; however, the direction of the effect was similar. Due to lack of sufficient events in Spanish patients with GG-genotype, the analysis for OS and MD could not be performed (Table S11). We also observed other polymorphisms, rs10511694 (near gene-3 IFNW1), rs10081742 (7.20 kb from 3' IFNW1) rs7038852 (near gene-3 INFg2), rs632941, rs7043990 and rs7864960 were associated with one of the survival parameters (Table 2).

| Table 2. Cont. |
|----------------|
| Overall survival |
| Disease free progression |
| Metastasis to death |
| SNP | genotype | n | M | D | % | HR | CI | P | n | M | D | % | HR | CI | P |
| AC | CC | 66 | 18 | 115 | 37.30 | 1.15 (0.69 - 1.91) | 0.58 | 66 | 25 | 79.00 | 1.02 (0.69 - 1.51) | 0.96 | 27 | 20 | 74.10 | 1.29 (0.79 - 2.10) | 0.30 |
| AA | GG | 290 | 57 | 35.60 | 0.87 (0.57 - 1.32) | 0.51 | 289 | 103 | 35.60 | 0.79 (0.55 - 1.17) | 0.25 | 112 | 62 | 55.40 | 0.79 (0.53 - 1.21) | 0.26 |
| AG | GG | 90 | 23 | 34.40 | 1.00 (0.64 - 1.56) | 1.00 | 90 | 31 | 34.40 | 1.00 (0.64 - 1.56) | 1.00 | 35 | 26 | 74.10 | 0.96 (0.58 - 1.56) | 0.34 |
| rs10811561 | AA | 152 | 37 | 24.30 | 1.00 (referent) | - | 152 | 58 | 38.20 | 1.00 (referent) | - | 64 | 43 | 67.20 | 1.00 (referent) | - |
| AG | GG | 289 | 57 | 19.70 | 0.87 (0.57 - 1.32) | 0.51 | 289 | 103 | 35.60 | 0.79 (0.55 - 1.17) | 0.25 | 112 | 62 | 55.40 | 0.79 (0.53 - 1.21) | 0.26 |
| GG | GG | 90 | 23 | 34.40 | 1.00 (0.64 - 1.56) | 1.00 | 90 | 31 | 34.40 | 1.00 (0.64 - 1.56) | 1.00 | 35 | 26 | 74.10 | 0.96 (0.58 - 1.56) | 0.34 |

n, number of cases; M, number of metastasis; D, number of deaths; HR, hazard ratio; CI, confidence interval.
Haplotype Analysis

Haplotypes were inferred for the polymorphisms genotyped located within a 26 kb region encompassing IFNW1 and IFNA21 on chromosome 9p22 that contained five SNPs that individually showed association with survival parameters. The analysis resulted in the inference of 40 haplotypes for the DFS and OS analysis and 31 haplotypes for the MD analysis for the polymorphisms rs10964859, rs105111694, rs2081381, rs10964859, rs10964862, rs10964863, rs10811482 and rs7038852. The most frequent haplotype taken as a reference was the one without any variant allele. GTCGACAA haplotype, found in 8.8% melanoma patients, showed an association with an increased risk MD (HR 1.94, 95%CI 1.16–3.26, P = 0.01) (Table 4). This haplotype included the minor alleles G and A of the rs10964859 and rs10964862 polymorphisms respectively, which were individually associated with an increased risk of MD. Other haplotypes were associated with worse prognosis in the DFS, OS and MD analysis; however, those occurred with a frequency ≤0.7 in the investigated patients (data not shown).

Discussion

Polymorphisms in type I IFN genes have been reported to be associated with different diseases [17–21]. In this study we observed that, of all the type I IFN variants evaluated, two linked polymorphisms rs10964862 and rs10964859, located at 3'UTR of IFNW1, were associated with detrimental effects on survival of melanoma patients. Those associations with poor survival were confirmed in an independent population. One of the haplotypes within the region that contained variant alleles of the both rs10964859 and rs10964862 polymorphisms, was associated with an increased risk of death. The prediction analysis showed that the rs10964862 variant is contained in a sequence with a potential binding site for the GATA transcription factors family, GT-IIA, NF-1 and NP-TCII and the variant allele results in loss of those
The location of the cluster of type I IFN genes on chromosome 9p22 is in contiguity with a region associated with melanoma pathogenesis [30]. In addition to the known tumor suppressor genes CDKN2A and CDKN2B, it was suggested that other genes and loci in this region may also be involved in melanoma and cutaneous nevi development [31–33]. An earlier report showed location of deletion breakpoints within the IFN gene cluster in primary leukemia cells that resulted in partial loss of the IFN genes on the short arm of chromosome 9p. It was suggested that, in addition to the classic two-hit tumor suppressor gene model, the loss of the IFN genes, when it occurs, may play an additional role in the progression of these tumors [34]. Moreover, the possibility that genetic variants in the region 9p21 exert effects through altering the expression of CDKN2A and/or genes in the region in addition to their own functional relevance has also been suggested [16]. Variants associated with coronary artery disease, located in an enhancer interval on 9p21 locus, physically interact with CDKN2A/B and MTAP genes and with another interval downstream of IFNX2. Interestingly, this interval coincides with the region where we found the strongest associated variants with melanoma survival. In addition, it has been observed that the long-range interactions as well as the transcriptional regulation of the 9p21 locus were affected by IFN-α and IFN-γ treatment [35]. Based on our observations, we hypothesize that the region of 26 kb encompassing IFNA1 and IFNA21 could be a regulatory region in chromosome 9p22, containing natural genetic variants, which might play a role in disease outcome in melanoma. The variants rs10964859 and rs10964862 or others in linkage might be regulatory genetic variants and probably those have some influence on IFNA1 gene through disruption of miRNA or transcription binding sites.

Melanoma therapy with IFNA has shown limited clinical efficacy, however remarkable survival response has been reported for a small group of patients, who also were predisposed to autoimmunity [36, 37]. It has been suggested that the genetic sites. The rs10964862 polymorphism has been previously reported to be associated with reduced tanning ability in melanoma-prone families [16].

| Table 3. Variation rs10964862 for the OS, DFS and MD analysis for the patients from Germany and Spain adjusted for the covariates age, gender and Breslow thickness. |
|-------------------------------|-----------------|-----|---|---|---|
| rs10964862 genotype cases n % HR CI P | | | | | |
| **OS GERMAN** | | | | | |
| CC 231 43 18.6 1.00 (referent) – | | | | | |
| CA 253 60 23.7 1.12 (0.75–1.69) 0.58 | | | | | |
| AA 49 14 28.6 1.67 (0.91–3.07) 0.10 | | | | | |
| CA + AA 302 74 24.5 1.20 (0.82–1.78) 0.35 | | | | | |
| **OS SPANISH** | | | | | |
| CC 271 13 4.8 1.00 (referent) – | | | | | |
| CA 295 20 6.8 1.39 (0.69–2.80) 0.36 | | | | | |
| AA 67 7 10.4 2.13 (0.85–5.35) 0.11 | | | | | |
| CA + AA 362 27 7.5 1.53 (0.79–2.97) 0.21 | | | | | |
| **DFS GERMAN** | | | | | |
| CC 231 69 29.9 1.00 (referent) – | | | | | |
| CA 253 104 41.1 1.38 (1.00–1.88) 0.04 | | | | | |
| AA 49 23 46.9 1.63 (1.01–2.62) 0.04 | | | | | |
| CA + AA 302 127 42.1 1.42 (1.05–1.91) 0.02 | | | | | |
| **DFS SPANISH** | | | | | |
| CC 271 28 10.3 1.00 (referent) – | | | | | |
| CA 295 38 12.9 1.24 (0.76–2.02) 0.39 | | | | | |
| AA 67 11 16.4 1.69 (0.84–3.40) 0.14 | | | | | |
| CA + AA 362 49 13.5 1.32 (0.83–2.10) 0.24 | | | | | |
| **MD GERMAN** | | | | | |
| CC 80 47 58.8 1.00 (referent) – | | | | | |
| CA 112 69 61.6 1.04 (0.71–1.52) 0.85 | | | | | |
| AA 24 17 70.8 1.84 (1.03–3.27) 0.04 | | | | | |
| CA + AA 136 86 63.2 1.14 (0.79–1.64) 0.50 | | | | | |
| **MD SPANISH** | | | | | |
| CC 31 16 51.6 1.00 (referent) – | | | | | |
| CA 44 20 45.5 1.02 (0.52–2.00) 0.97 | | | | | |
| AA 12 7 58.3 1.36 (0.55–3.34) 0.51 | | | | | |
| CA + AA 56 27 48.2 1.09 (0.58–2.06) 0.79 | | | | | |

n, number of deaths for OS and MD analysis or number of metastases for DFS analysis.
OS, overall survival; DFS, disease free progression; MD, metastasis to death.
HR, Hazard Ratio; CI, Confidence Interval.
doi:10.1371/journal.pone.0050692.t003

| Table 4. MD haplotype analysis for the SNPs rs10964859 rs10511694 rs2081381 rs10081742 rs10964862 rs10964863 rs10811482 rs7038852 adjusted for the covariates age, gender and Breslow thickness. |
|-------------------------------|-----------------|-----|---|---|---|
| haplotype n % HR CI P | | | | | |
| C-C-C-A-C-C-A-A 82 19.0 1.00 (referent) – | | | | | |
| C-T-G-A-C-T-A-G 70 16.2 1.42 (0.92–2.20) 0.11 | | | | | |
| G-T-C-A-C-G-A 60 13.9 1.49 (0.94–2.36) 0.09 | | | | | |
| C-T-G-A-C-T-A-A 51 11.8 1.05 (0.62–1.79) 0.86 | | | | | |
| C-T-G-A-C-A-A 38 8.8 1.94 (1.16–3.26) 0.01 | | | | | |
| G-T-C-A-C-G-G 37 8.6 1.38 (0.77–2.47) 0.28 | | | | | |
| C-C-C-A-C-A-G 23 5.3 0.90 (0.46–1.76) 0.77 | | | | | |
| C-T-G-G-C-C-A-G 10 2.3 1.07 (0.45–2.59) 0.87 | | | | | |
| C-T-G-A-C-A-G 9 2.1 1.48 (0.60–3.63) 0.39 | | | | | |
| G-T-A-C-T-A-G 7 1.6 1.45 (0.55–3.84) 0.45 | | | | | |
| C-T-G-A-C-A-A 6 1.4 1.55 (0.60–4.04) 0.37 | | | | | |
| G-T-C-A-C-G-A 6 1.4 1.09 (0.33–3.62) 0.88 | | | | | |
| C-T-G-A-C-A-A 4 0.9 0.38 (0.08–1.77) 0.22 | | | | | |
| C-T-G-A-C-A-G 3 0.7 0.48 (0.06–3.62) 0.47 | | | | | |
| C-G-A-A-C-G-G 3 0.7 0.35 (0.05–2.56) 0.30 | | | | | |
| G-C-C-A-C-C-A-A 3 0.7 19.56 (4.67–82.0) <.0001 | | | | | |

n = number of haplotypes in the population.
HR, Hazard Ratio; CI, Confidence Interval.
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background of the individuals could be partly the reason for the observed variation in the treatment response [30]. Our data showed that variation in the effect of genotypes on the disease outcome was dependent on whether the patients were treated with IFNA.

In accordance with published reports, the beneficial effects of IFNA in the treatment of melanoma patients were observed at the beginning of the disease; however, with advancement of the disease, the effects of IFN treatment become less pronounced [39,40]. IFN seems to be active in the eradication of micrometastasis and in the prevention of relapse. According to our data, melanoma patients who were carriers of the minor allele genotypes for rs10964859 and rs10964862 had an increased risk of metastasis, if they did not receive IFN therapy. However, their risk of death was augmented under treatment with IFN.

Our study shows that genetic variants in type I IFN genes play a role in melanoma disease and have an influence in the therapy outcome. Though it can be speculated, the effect being driven through variants in regulatory regions like 5'UTR or 3'UTR of IFN/1 gene, the effect through some other functional linked SNP or gene beyond the region we studied cannot be ruled out. Polymorphisms in type I interferon genes that affect the biology of melanoma might be potentially used as predictors of survival and progression in the early stages of the disease. Genetic variants, which affect the efficacy of IFNA treatment, can also be useful for identification of patients for treatment. However, to achieve those levels of applications, it is imperative the findings reported in this study are confirmed further in large studies. It may, however, be pointed out that none of the associations between different polymorphisms and survival outcomes in melanoma patients remained significant after correction for multiple hypothesis testing. Nevertheless, the observed associations between the rs10964862 and rs10964859 polymorphisms and survival parameters were uniform in two independent groups of patients.

Supporting Information

Table S1 Polymorphisms within interferon gene cluster selected for genotyping. (DOCX)

Table S2 Detailed information about metastatic events in the German patients within and after the 10 years (10y) follow up. (DOCX)

Table S3 Detailed information about death events in the German patients within and after the 10 years (10y) follow up. (DOCX)

Table S4 Variation rs10964859 for the OS, DFS and MD analysis for the patients from Germany and Spain adjusted for the covariates age, gender and Breslow thickness. (DOCX)

Table S5 Estimated 10 years DFS, OS and MD survival analysis for the group of patients from Germany “with only IFN” (Figure 2 D) and “without only IFN” (Figure 2 E) for the SNP rs10964859. (DOCX)

Table S6 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Germany “with IFN” (Figure 2 B) and “without IFN” (Figure 2 C) for the SNP rs10964859. (DOCX)

Table S7 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Spain “with only IFN” and “without only IFN” for the SNP rs10964859. (DOCX)

Table S8 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Germany “with only IFN” (Figure 2 B) and “without only IFN” (Figure 2 C) for the SNP rs10964862. (DOCX)

Table S9 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Germany “with only IFN” (Figure 2 D) and “without only IFN” (Figure 2 E) for the SNP rs10964862. (DOCX)

Table S10 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Spain “with only IFN” and “without only IFN” for the SNP rs10964862. (DOCX)

Table S11 Variation rs597408 for the OS, DFS and MD analysis for the patients from Germany and Spain adjusted for the covariates age, gender and Breslow thickness. (DOCX)

Table S12 Estimated 10 years OS, DFS and MD survival analysis for the group of patients from Germany “with only IFN” (Figure 2 D) and “without only IFN” (Figure 2 E) for the SNP rs597408. (DOCX)

Author Contributions

Conceived and designed the experiments: RL EN KH DS RK. Performed the experiments: RL MB AB JLB RK. Contributed reagents/materials/analysis tools: JLB KH RK DS EN AS IM DP CR. Wrote the paper: RL EN KH DS RK. Suggestions and final approval of the manuscript: RL MB AB JLB KH RK DS EN AS IM DP CR. Wrote the paper: RL EN KH DS RK. Suggestions and final approval of the manuscript: RL MB AB JLB KH RK DS RK.

References

1. Zeuthen J, Dhandhzhugayan K, Hansen MR, Karkin AF (1998) The immunogenic properties of human melanomas and melanoma-associated antigens recognized by cytotoxic T lymphocytes. Bratisl Lek Listy 99: 426–434.
2. Karkin AF, Dhandhzhugayan K, Zeuthen J (1998) Melanoma-associated antigens recognized by cytotoxic T lymphocytes. AIMIS 106: 665–679.
3. Karkin AF, Dhandhzhugayan K, Zeuthen J (1998) The immunogenic properties of melanoma-associated antigens recognized by cytotoxic T lymphocytes. Exp Clin Imunogenet 15: 19–32.
4. Barnettson RS, Halliday GM (1997) Regression in skin tumours: a common phenomenon. Australas J Dermatol 38 Suppl 1: S61–65.
5. Kiniwa Y, Fujita T, Akada M, Ito K, Shofuda T, et al. (2003) Tumor antigens isolated from a patient with vitiligo and T-cell-infiltrated melanoma. Cancer Res 63: 7900–7907.
6. Stoff B, Salisbury C, Parker D, O’Reilly Zwald F (2010) Dermatopathology of skin cancer in solid organ transplant recipients. Transplant Rev (Orlando) 24: 172–189.
7. Gajewski TF (2007) Failure at the effector phase: immune barriers at the level of the melanoma tumor microenvironment. Clin Cancer Res 13: 5256–5261.
8. Russ K, Hassel JC (2009) Chemotherapeutics, chemoresistance, and the management of melanoma. G Ital Dermatol Venereol 144: 61–78.
9. Szno M (2011) Molecular markers of response to treatment for melanoma. Cancer J 17: 127–133.
24. Kertesz M, Iovino N, Unnerstall U, Gaul U, Segal E (2007) The role of site accessibility in microRNA target recognition. Nat Genet 39: 1278–1284.

25. Bao L, Zhou M, Wu L, Lu L, Goldowitz D, et al. (2007) PolymiRTS Database: linking polymorphisms in microRNA target sites with complex traits. Nucleic Acids Res 35: D51–54.

26. Sethupathy P, Collins FS (2006) MicroRNA target site polymorphisms and human disease. Trends Genet 24: 489–497.

27. John B, Enright AJ, Aravin A, Tuschl T, Sander C, et al. (2004) Human MicroRNA targets. PLoS Biol 2: e363.

28. Betel D, Wilson M, Gubov A, Marks DS, Sander C (2008) The microRNA.org resource: targets and expression. Nucleic Acids Res 36: D149–153.

29. Schug J (2008) Using TESS to predict transcription factor binding sites in DNA sequence. Curr Protoc Bioinformatics Chapter 2: Unit 2.6.

30. Rakosy Z, Viukeleti L, Erceti S, Begany A, Emri G, et al. (2008) Characterization of 9p21 copy number alterations in human melanoma by fluorescence in situ hybridization. Cancer Genet Cytogenet 182: 116–121.

31. Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, et al. (2009) Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet 41: 920–925.

32. Falchi M, Bataille V, Hayward NK, Duffy DL, Bishop JA, et al. (2009) Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nat Genet 41: 915–919.

33. Chatzinasiou F, Lill CM, Kypros K, Stefanaki I, Nicolaou V, et al. (2011) Comprehensive field synopsis and systematic meta-analyses of genetic association studies in cutaneous melanoma. J Natl Cancer Inst 103: 1227–1235.

34. Olopade OI, Jenkins RB, Ransom DT, Malik K, Pomykala H, et al. (1992) Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res 52: 2523–2529.

35. Harisimendy O, Notani D, Song X, Rahim NG, Tanasa B, et al. (2011) 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response. Nature 470: 264–268.

36. Gogas H, Ioannovich J, Dafni U, Stavropoulou-Giakas C, Frangia K, et al. (2006) Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med 354: 709–718.

37. Mellman I, Coukos G, Dranoff G (2011) Cancer immunotherapy comes of age. Nature 480: 480–489.

38. Alexandrescu DT, Ichim TE, Riotton NA, Marincola FM, Di Nardo A, et al. (2010) Immunotherapy for melanoma: current status and perspectives. J Immunother 33: 570–590.

39. Petrella T, Verma S, Spithoff K, Qvist I, McGready D (2012) Adjuvant Interferon Therapy for Patients at High Risk for Recurrent Melanoma: An Updated Systematic Review and Practice Guideline. Clin Oncol (R Coll Radiol).

40. Larkin JM, Fisher RA, Gore ME (2012) Adjuvant Interferon Therapy for Patients at High Risk for Recurrent Melanoma: An Updated Systematic Review. Clin Oncol (R Coll Radiol).