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Genetic Susceptibility in Head and Neck Squamous Cell Carcinoma in a Spanish Population

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Received: 7 March 2019; Accepted: 4 April 2019; Published: 7 April 2019

Abstract: Despite classical environmental risk factors like tobacco, alcohol or viral infection, not all individuals develop head and neck cancer. Therefore, identification of the genetic susceptibility produced by single nucleotide polymorphisms (SNPs) is an important task. A total of 296 human papillomavirus negative head and neck cancer (HNC) patients (126 laryngeal, 100 pharyngeal and 70 oral cavity) were included in the study, involving 29 candidate SNPs in genes within important carcinogenic pathways (oncogenesis and tumour suppression, DNA repair, inflammation, oxidation and apoptosis). Genotyping was performed using TaqMan probes or restriction fragment length assays in peripheral blood DNA. In addition, 259 paired controls were also evaluated with the same risk factors for each specific location. Nine SNPs in DNA repair (ERCC1 rs11615, ERCC2 rs13181), inflammatory (IL2 rs2069762, IL6 rs1800795), oxidative (NFE2L2 rs13035806 and rs2706110) and apoptotic genes (TP53 rs1042522, MDM2 rs2279744, BCL2 rs2279115) were differently associated with HNSCC susceptibility by location. Some of these SNPs were not described before in this tumour type. In conclusion, we describe several SNPs associated with HNC in a Spanish population.

Keywords: head and neck cancer; single nucleotide polymorphisms; Spanish population
1. Introduction

Head and neck cancer (HNC) includes a set of diverse neoplasms located in the lips, oral cavity, pharynx, larynx, salivary glands and thyroid glands, among others. Most HNC belong to the squamous cell carcinomas group [1]. Approximately 600,000 new cases are diagnosed per year, being the sixth cancer type on incidence worldwide. Treatment of early stages includes surgery and/or radiotherapy, while locally advanced tumours are also treated with chemotherapy and biological therapies [2]. Only 40–50% of patients survive for five years [3] causing an annual death rate of 271,000 patients [4,5].

The oncogenic transformation of normal mucosa into a squamous cell carcinoma of the head and neck is a multifactorial process, associated with a variety of risk factors. At least 75% of head and neck squamous cell carcinomas (HNSCC) are attributable to the combination of cigarette smoking and alcohol drinking, the most classic carcinogens [6,7]. Diverse epidemiological studies have also revealed the existence of other environmental and genetic related factors. Similar to other tumours, viral aetiology has shown an implication in HNSCC development, predominating Epstein–Barr virus (EBV) infection in nasopharynx, and human papillomavirus (HPV), mainly subtype 16, in oral cavity and oropharyngeal tumours [8]. The carcinogenesis process triggered by viral infection defines a different entity to that caused by tobacco and alcohol [8], allowing HNSCC classification into two main prognostic and therapeutic groups, in which HPV-negative tumours are associated with an aggressive course and a worse prognosis than HPV positive ones [9,10].

Despite the defined role of environmental factors, there is also evidence of familial aggregation and increased cancer risk amongst HNSCC relatives [11], suggesting the existence of genetic predisposition factors [12]. However, not all individuals exposed to these carcinogens will develop the disease. In this context, the identification of genetic variants in important signaling pathways could help to define tumour susceptibility, as well as differences in treatment response and toxicity. HNSCC carcinogenesis involves different pathways: carcinogen metabolism, DNA repair, cell cycle, immunity and inflammation [13–15]. Single nucleotide polymorphism (SNP) is the most abundant form of genetic variation, becoming an ideal genetic susceptibility marker [1].

In this study, we aimed to examine polymorphisms in genes involved in relevant oncogenic pathways within a paired population of cases and controls in a large Spanish population.

2. Results

2.1. Characteristics of Groups

After the application of the propensity score method 126 larynx, 100 pharynx and 70 oral cavity squamous cell carcinomas were totally paired with their specific control group. The analysis by location did not show any statistically significant difference between sex, age, tobacco and alcohol intake with respect to the control group (Table 1). Only age was statistically different (p < 0.05) between laryngeal tumour and control group, so this variable was included in the logistic regression as an adjustment variable.

2.2. Candidate Gene Association Study

Nine out of twenty-nine selected SNPs showed a statistically significant result in the distribution between the patient and control groups.

Beginning with DNA repair genes, less common genotypes in ERCC1 rs11615 (p = 0.011, OR = 0.288 (CI 95% = 0.110–0.751) in a recessive model) and ERCC2 rs13181 (p = 0.046, OR = 0.375 (0.143–0.982) in a codominant model) were associated with a lower risk of laryngeal cancer (Table 2 and Table S2).
Table 1. Descriptive characteristics and risk factors of paired patients by location in the case-control study. Data after the propensity score method corroborate the equality between the different locations with their specific controls, except for age in laryngeal tumours (introduced as an adjustment variable in the logistic regression).

| Group Comparison | LARYNX N = 126 | CONTROL N = 126 | p-Value | PHARYNX N = 100 | CONTROL N = 100 | p-Value | ORAL CAVITY N = 70 | CONTROL N = 70 | p-Value |
|------------------|----------------|-----------------|---------|-----------------|-----------------|---------|-------------------|----------------|---------|
| Characteristics  |                |                 |         |                 |                 |         |                   |                 |         |
| Age (years)      | 63.02 ± 8.566  | 56.30 ± 12.803  | 0.000   | 59.96 ± 8.41    | 59.52 ± 10.044  | 0.742   | 60.92 ± 10.008    | 62.24 ± 8.88   | 0.412   |
| Sex              |                |                 |         |                 |                 |         |                   |                 |         |
| Female           | 13 10.3        | 13 10.3         | 1.000   | 20 20.0         | 22 22.0         | 0.728   | 16 22.9           | 17 22.9       | 1.000   |
| Male             | 113 89.7       | 113 89.7        |         | 80 80.0         | 78 78.0         |         | 54 77.1           | 54 77.1       |         |
| Tobacco smoking  |                |                 |         |                 |                 |         |                   |                 |         |
| Never            | 7 5.5          | 7 5.5           | 0.944   | 7 7.0           | 8 8.0          | 0.943   | 7 10.0            | 7 10.0        | 1.000   |
| <20 PPY          | 20 15.9        | 22 17.5         |         | 22 22.0         | 23 23.0        | 0.943   | 12 17.1           | 12 17.1       | 1.000   |
| >20 PPY          | 99 78.6        | 97 77.0         | 0.944   | 71 71.0         | 69 69.0        | 0.943   | 51 72.9           | 51 72.9       | 1.000   |
| Missing          | 0 0            | 0 0            |         | 0 0             | 0 0            |         | 0 0               | 0 0            |         |
| Alcohol drinking |                |                 |         |                 |                 |         |                   |                 |         |
| Never            | 53 42.1        | 51 40.5         | 0.904   | 26 26.0         | 27 27.0        | 0.985   | 23 32.9           | 23 32.9       | 1.000   |
| <14 SDU/week     | 28 22.2        | 31 24.6         |         | 30 30.0         | 30 30.0        | 0.985   | 19 27.1           | 19 27.1       | 1.000   |
| >14 SDU/week     | 45 35.7        | 44 34.9         | 0.904   | 44 44.0         | 43 43.0        | 0.985   | 28 40.0           | 28 40.0       | 1.000   |
| Missing          | 0 0            | 0 0            |         | 0 0             | 0 0            |         | 0 0               | 0 0            |         |

p-values related to controls. Statistically significant results in bold. PPY: Tobacco packs per year. SDU: Standard unit of alcohol per week.
Table 2. Statistically significant SNPs in laryngeal cancer.

| SNPs       | Genotype | Larynx | Control | \( p \)-Value * | OR (CI 95%)                  |
|------------|----------|--------|---------|-----------------|-------------------------------|
|            |          | N     | %       | N               | %                            |
|            |          |       |         |                 |                              |
| **TP53**   |          |       |         |                 |                              |
| rs1042522  |          |       |         |                 |                              |
|            | GG       | 61    | 48.4    | 62              | 49.2                         | Ref. 1.00                 |
|            | CC       | 54    | 42.9    | 37              | 29.4                         | 0.165 1.050 (0.846–2.677)  |
| Recessive  | GG+GC    | 115   | 91.3    | 99              | 78.6                         | Ref. 1.00                 |
|            | CC       | 11    | 8.7     | 27              | 21.4                         | 0.008 0.319 (0.136–0.745)  |
| Dominant   | GG       | 61    | 48.4    | 62              | 49.2                         | Ref. 1.00                 |
|            | GC+CC    | 65    | 51.6    | 64              | 50.8                         | 0.596 0.986 (0.587–1.654)  |
| **MDM2**   |          |       |         |                 |                              |
| rs2279744  |          |       |         |                 |                              |
|            | TT       | 44    | 34.9    | 62              | 49.2                         | Ref. 1.00                 |
|            | TG       | 57    | 45.2    | 53              | 42.1                         | 0.279 3.778 (2.252–6.222)  |
|            | GG       | 25    | 19.8    | 11              | 8.7                          | 0.015 2.826 (1.219–6.552)  |
| Recessive  | TT+TG    | 101   | 80.2    | 115             | 91.3                         | Ref. 1.00                 |
|            | GG       | 25    | 19.8    | 11              | 8.7                          | 0.029 2.413 (1.094–5.323)  |
| Dominant   | TT       | 44    | 34.9    | 62              | 49.2                         | Ref. 1.00                 |
|            | TG+GG    | 82    | 65.1    | 64              | 50.8                         | 0.073 1.616 (0.953–2.742)  |
| **ERCC1**  |          |       |         |                 |                              |
| rs11615    |          |       |         |                 |                              |
|            | TT       | 53    | 42.1    | 45              | 35.7                         | Ref. 1.00                 |
|            | TC       | 67    | 53.2    | 58              | 46.0                         | 0.872 0.956 (0.550–1.661)  |
|            | CC       | 6     | 4.8     | 23              | 18.3                         | 0.013 0.281 (0.103–0.768)  |
| Recessive  | TT+TC    | 120   | 95.2    | 103             | 81.7                         | Ref. 1.00                 |
|            | CC       | 6     | 4.8     | 23              | 18.3                         | 0.011 0.288 (0.110–0.751)  |
| Dominant   | TT       | 53    | 42.1    | 45              | 35.7                         | Ref. 1.00                 |
|            | TG+GG    | 82    | 65.1    | 64              | 50.8                         | 0.073 1.616 (0.953–2.742)  |
| **ERCC2**  |          |       |         |                 |                              |
| rs13181    |          |       |         |                 |                              |
|            | TT       | 72    | 57.1    | 52              | 41.3                         | Ref. 1.00                 |
|            | TG       | 46    | 36.5    | 58              | 46.0                         | 0.247 0.720 (0.413–1.555)  |
|            | GG       | 8     | 6.3     | 16              | 12.7                         | 0.046 0.375 (0.143–0.982)  |
| Recessive  | TT+TG    | 118   | 93.7    | 110             | 87.3                         | Ref. 1.00                 |
|            | GG       | 8     | 6.3     | 16              | 12.7                         | 0.079 0.433 (0.170–1.102)  |
| Dominant   | TT       | 72    | 57.1    | 52              | 41.3                         | Ref. 1.00                 |
|            | TG+GG    | 54    | 42.9    | 74              | 58.7                         | 0.093 0.638 (0.377–1.078)  |
| **IL6**    |          |       |         |                 |                              |
| rs1800795  |          |       |         |                 |                              |
|            | CC       | 43    | 34.1    | 62              | 50.8                         | Ref. 1.00                 |
|            | CG       | 64    | 50.8    | 46              | 37.7                         | 0.003 2.471 (1.372–4.552)  |
|            | GG       | 19    | 15.1    | 14              | 11.5                         | 0.070 2.164 (0.938–4.991)  |
| Recessive  | CC+CG    | 107   | 84.9    | 108             | 88.5                         | Ref. 1.00                 |
|            | GG       | 19    | 15.1    | 14              | 11.5                         | 0.444 1.351 (0.625–2.921)  |
| Dominant   | CC       | 43    | 34.1    | 62              | 50.8                         | Ref. 1.00                 |
|            | CG+GG    | 83    | 65.9    | 60              | 49.2                         | 0.002 2.394 (1.376–4.163)  |
| **NRF2**   |          |       |         |                 |                              |
| rs1303566  |          |       |         |                 |                              |
|            | GG       | 109   | 87.2    | 95              | 76.0                         | Ref. 1.00                 |
|            | GA       | 14    | 11.2    | 29              | 23.2                         | 0.019 0.424 (0.207–0.869)  |
|            | AA       | 2     | 1.6     | 1               | 0.8                          | 0.520 2.235 (0.193–25.903) |
| Recessive  | GG+GA    | 123   | 98.4    | 124             | 99.2                         | Ref. 1.00                 |
|            | AA       | 2     | 1.6     | 1               | 0.8                          | 0.444 2.600 (0.225–30.064) |
| Dominant   | GG       | 109   | 87.2    | 95              | 76.0                         | Ref. 1.00                 |
|            | GA+AA    | 16    | 12.8    | 30              | 24.0                         | 0.035 0.478 (0.240–0.949)  |
| **NRF2**   |          |       |         |                 |                              |
| rs2706110  |          |       |         |                 |                              |
|            | CC       | 92    | 73.6    | 72              | 57.1                         | Ref. 1.00                 |
|            | CT       | 24    | 19.2    | 47              | 37.3                         | 0.005 0.425 (0.233–0.775)  |
|            | TT       | 9     | 7.2     | 7               | 5.6                          | 0.732 1.207 (0.411–3.541)  |
| Recessive  | CC+CT    | 116   | 92.8    | 119             | 94.4                         | Ref. 1.00                 |
|            | TT       | 9     | 7.2     | 7               | 5.6                          | 0.403 1.574 (0.544–4.560)  |
| Dominant   | CC       | 92    | 73.6    | 72              | 57.1                         | Ref. 1.00                 |
|            | CT+TT    | 33    | 26.4    | 54              | 42.9                         | 0.020 0.518 (0.299–0.900)  |

* \( p \)-values adjusted by age. Statistically significant results in bold.
Secondly, pro-inflammatory *IL6* rs1800795 polymorphism was related to a higher risk of laryngeal cancer in a dominant model (*p* = 0.002, OR = 2.394 (1.376–4.163)) (Table 2 and Table S2), similar to the association found in CG+GG variants with increased oral cavity susceptibility (*p* = 0.018, OR = 2.265 (1.148–4.467)). Moreover, another SNP in the inflammatory gene *IL2* rs2069762 G variant was associated with a lower risk of oral cavity cancer (GG *p* = 0.039, OR = 0.300 (0.096–0.940)) (Table 3 and Table S3).

### Table 3. Statistically significant SNPs in oral cavity carcinoma.

| SNPs       | Genotype | Oral Cavity | Control |  |  |  |
|------------|----------|-------------|---------|---|---|---|
| **IL2 rs2069762** | TT       | 43          | 61.4    | 31 | 44.3 | /  |
|            | TG       | 22          | 31.4    | 27 | 38.6 | 0.152 |
|            | GG       | 5           | 7.1     | 12 | 17.1 | **0.039** |
|            | GG       | 5           | 7.1     | 12 | 17.1 | 0.078 |
|            | TT+TG    | 65          | 92.9    | 58 | 82.9 | /  |
|            | GG       | 5           | 7.1     | 12 | 17.1 | 0.039 |
| **Dominant** | TT       | 43          | 61.4    | 31 | 44.3 | /  |
|            | TG+GG    | 27          | 38.6    | 39 | 55.7 | **0.043** |
|            | GG       | 5           | 7.1     | 12 | 17.1 | 0.078 |
| **IL6 rs1800795** | CC       | 25          | 35.7    | 39 | 55.7 | /  |
|            | CG       | 33          | 47.1    | 23 | 32.9 | **0.031** |
|            | GG       | 12          | 17.1    | 8  | 11.4 | 0.104 |
|            | GG       | 12          | 17.1    | 8  | 11.4 | 0.337 |
|            | CC+CG    | 58          | 82.9    | 62 | 88.6 | /  |
|            | CC       | 25          | 35.7    | 39 | 55.7 | /  |
|            | CG+GG    | 45          | 64.3    | 31 | 44.3 | **0.018** |
| **BCL2 rs2279115** | CC       | 13          | 18.6    | 27 | 38.6 | /  |
|            | CA       | 43          | 61.4    | 30 | 42.9 | **0.008** |
|            | AA       | 14          | 20.0    | 13 | 18.6 | 0.116 |
|            | AA       | 14          | 20.0    | 13 | 18.6 | 0.830 |
|            | CC+CA    | 56          | 80.0    | 57 | 81.4 | /  |
|            | AA       | 14          | 20.0    | 13 | 18.6 | 0.830 |
|            | CC       | 13          | 18.6    | 27 | 38.6 | /  |
|            | CA+AA    | 57          | 81.4    | 43 | 61.4 | **0.010** |

Statistically significant results in bold.

In relation to apoptotic genes, three SNPs in apoptotic genes were associated with different susceptibility in all HNSCC locations. The *TP53* rs1042522 mutant allele in the recessive model was associated with a decreased risk of developing laryngeal cancer (*p* = 0.002, OR = 0.286 (0.119–0.607)) (see Table 2 and Table S2); and pharyngeal cancer (*p* = 0.001, OR = 0.124 (0.035–0.476)) (see Table 4 and Table S4). Further, variant allele in *MDM2* rs2279744 was associated with higher risk of laryngeal cancer (*p* = 0.029 OR = 2.413 (1.094–5.323)) in a recessive model (Table 2 and Table S2). Meanwhile CA+AA genotypes in *BCL2* rs2279115 were related with a higher risk of developing oral carcinoma (*p* = 0.010, OR = 2.753 (1.273–5.952)) in a dominant model (Table 3 and Table S3).

Finally, an association between antioxidative SNPs and laryngeal and pharyngeal cancer was found. Variant genotypes rs1303586 GA+AA and rs2706110 CT+TT, both in the *NRF2* gene, were associated with a lower risk of laryngeal carcinoma (*p* = 0.035, OR = 0.478 (0.240–0.949) and *p* = 0.518, OR = 0.518 (0.299–0.900), respectively) (Table 2 and Table S2). On the other hand, in pharyngeal cancer, only *NRF2* rs2706110 less common allele genotypes CC+CT were related with a lower risk of developing pharyngeal carcinoma (*p* = 0.043, OR = 0.552 (0.311–0.982)) (Table 4 and Table S4).
Table 4. Statistically significant SNPs in pharyngeal cancer.

| SNPs   | Genotype | Pharynx | Control | p-Value | OR (CI 95%) |
|--------|----------|---------|---------|---------|-------------|
|        |          | N (%)   | N (%)   |         |             |
| TP53   | rs1042522|         |         |         |             |
|        | GG       | 53  | 53.0  | 47  | 47.0 | Ref. | 1.00 |
|        | GC       | 44  | 44.0  | 33  | 33.0 | 0.583 | 1.182 (0.650–2.151) |
|        | CC       | 3   | 3.0   | 20  | 20.0 | 0.002 | 0.133 (0.037–0.476) |
| Recessive | GG+GC  | 97  | 97.0  | 80  | 80.0 | Ref. | 1.00 |
|        | CC       | 3   | 3.0   | 20  | 20.0 | 0.001 | 0.124 (0.035–0.431) |
| Dominant | GG       | 53  | 53.0  | 47  | 47.0 | Ref. | 1.00 |
|        | GC+CC    | 47  | 47.0  | 53  | 53.0 | 0.396 | 0.786 (0.451–1.370) |
| NRF2   | rs2706110|         |         |         |             |
|        | CC       | 68  | 68.0  | 54  | 54.0 | Ref. | 1.00 |
|        | CT       | 25  | 25.0  | 41  | 41.0 | 0.020 | 0.484 (0.262–0.893) |
|        | TT       | 7   | 7.0   | 5   | 5.0  | 0.863 | 1.112 (0.334–3.698) |
| Recessive | CC+CT  | 93  | 93.0  | 95  | 95.0 | Ref. | 1.00 |
|        | TT       | 7   | 7.0   | 5   | 5.0  | 0.553 | 1.430 (0.438–4.667) |
| Dominant | CC       | 68  | 68.0  | 54  | 54.0 | Ref. | 1.00 |
|        | CT+TT    | 32  | 32.0  | 46  | 46.0 | 0.043 | 0.552 (0.311–0.982) |

Statistically significant results in bold.

3. Discussion

Not all individuals exposed to the same classical carcinogens (tobacco and alcohol) develop HNSCC. Although several susceptibility studies have identified SNPs in carcinogenesis-related pathways, their results are controversial due to an inadequate control group. In this multicentre case-control study, we examined the association between some polymorphisms and HNSCC susceptibility in a Spanish cohort with a control group totally paired by their risk factors, avoiding confounder variables.

Analysis of laryngeal squamous cell carcinoma showed an association with lower susceptibility risk in ERCC1 rs11615 and ERCC2 rs13181 SNPs. Indeed, these genotypes have also been associated with a better response and longer survival in patients treated with platinum [16] due to an increase in DNA damage and induction of cell death, providing a potential explanation of our results.

Inflammation has been considered an important factor in the pathogenesis of human cancer [17–19], with a special interest in the context of oral cancer [20,21]. The rs1800795-174C variant in the promoter of the IL6 gene is related to a lower level of serum proteins, while -174G corresponds to a higher expression, increasing the inflammatory response [22]. Our study shows an association between the G allele and a higher risk of developing laryngeal and oral tumours, probably related to the carcinogenesis induced by inflammation. Moreover, cytokine IL-2 plays a role in the proliferation of activated T-lymphocytes and in the activation of phagocytes. The G allele in the -330G>T (rs2069762) SNP increases the IL2 gene expression, whereas the T allele is associated with a decreased IL2 expression skewing the Th1/Th2 immune balance towards Th2 [23]. In our study, the IL2 rs2069762 GG genotype was associated with lower oral cavity risk, in contrast to previous reported associations [23] in another tumour types with different risk factors and ethnic background. This result could be explained by the main role of IL-2 in the elimination of self-reactive cells [24], decreasing the antitumour response produced by the immune system.

Mdm2 attenuates the tumour suppressor protein p53 through proteasomal degradation via ubiquitinylation, while p53 induces MDM2 transcription in response to genotoxic stress [25]. SNP rs2277144-410T>G, located in the P2 promoter, increases MDM2 expression by improving the binding affinity with the Sp1 transcription factor, attenuating the TP53 suppressor pathway [26]. Our data is in line with previous reports [27], demonstrating a higher risk of laryngeal cancer in those patients with the GG genotype.
The polymorphism c.215C>G (Pro72Arg) in the exon 4 of TP53 is found in an essential domain in the apoptotic response and carcinogenesis inhibition. The arginine allele is a more powerful apoptotic inductor than the proline one [28,29]. Some studies have associated the Pro72Arg polymorphism with an increased risk of developing gastric, oesophageal and bladder cancer [30,31], but little data has been reported regarding HNSCC [32,33]. Our results show a lower susceptibility of developing pharyngeal and laryngeal cancer for the variant alleles. While this could be due to its association with longer survival or modifications at cell cycyle and the maintenance of DNA integrity [28,34], we have also shown this protective association in stroke [35] and other ischemic processes (Cruz-González et al., data not published), possibly being a case selection bias.

In addition, we found a statistically significant association between the anti-apoptotic gene BCL2 SNP and oral cavity cancer susceptibility. BCL2-938C>A (rs2279115) polymorphism is found in P2 gene promoter, acting as a negative regulator element, decreasing P1 promoter activity [36]. The presence of C allele highly reduces the activity of P1 and Bcl-2 protein expression, increasing apoptosis. Our results showed similar results to those reported in breast cancer and acute myeloid leukaemia [37] where the presence of the A allele (CA+AA) increased tumour susceptibility due to an anti-apoptotic effect [38].

Finally, NFE2L2 gene codes for a transcription factor protein (Nrf2) that induce many antioxidative genes under oxidative stress. SNPs in this gene have been associated with cancer risk [39]. In our series, NFE2L2 rs2706110 and rs1303586 less common genotypes were linked with lower risk of developing laryngeal cancer, while in pharyngeal cancer, only rs1303586 was associated. Functional analyses of these SNPs have not yet been described but our hypothesis is that these changes could increase antioxidative gene induction under stress produced at high levels in HNSCC by tobacco and alcohol consumption.

4. Material and Methods

4.1. Study Population

TTCC-2010-05 was an observational multicentre study conducted in 19 Spanish centres, all of them belonging to the Spanish Head and Neck Cancer Treatment Group (TTCC) coordinated by the Medical Oncology Department of the University Hospital of Salamanca, between January 2012 and December 2014. Epidemiological and clinicopathological details have been previously described [40].

Cases inclusion criterion was: histologically confirmed HPV-negative HNSCC patients from larynx, oro/hypopharynx and oral cavity carcinomas. They were recruited in Oncology, Radiotherapy and Otorhinolaryngology departments. Controls were follow-up individuals with minor issues and without a tumour history and paired by age, sex, smoking and alcoholic habit with HNSCC cases. They were captured in Pneumology, Radiotherapy, Otorhinolaryngology and Internal Medicine departments. Only the Spanish population were permitted, avoiding ethnicity bias.

Considering HNSCC incidence in Spain, 10% of possible losses and duration of the study, initial calculations of recruitment were of 440 individuals in patient and control group. Finally, a total of 459 patients and 259 controls were included.

In this study, the variables were polymorphisms in oncogenes, tumour suppressor genes, genes implicated in DNA reparation, inflammation, carcinogen metabolism and apoptosis, together with some risk factors collected in the socio-demographic (6 questions) and the data informed by patients (19 questions) questionnaires. The information of both questionnaires was collected via auto-application, being supervised by the members of the research team. Clinico-pathologic data, response and specific toxicity to treatment were collected by oncologists in the case report form questionnaire (CRF).

The study was approved by the University Hospital of Salamanca and the local ethics committees in accordance with the 1964 Helsinki declaration and its later amendments. All participants were previously informed and signed the provided informed consent. All data were treated with the
security measures established in compliance with the Protection of Personal Data Organic Law 15/1999, December 13, and safe-keeping by the University Hospital of Salamanca in its specific hospital server. This study was supported by the Ministry of Economy and Competitiveness under the identification code PI11/0059.

4.2. Selection of Polymorphism

Candidate SNPs selection was done according to at least two of the following criteria: >5% allele frequency in Caucasian/European population, previously defined association with HNSCC susceptibility and earlier related different response or toxicity to chemotherapy or radiotherapy. At the initial stages of the project design, a huge search was performed in available databases using keywords such as SNPs, susceptibility, HNSCC, response and toxicity, selecting only those with statistically significant results in other populations [14,15,32,41–43]. SNPs with some published evidence of functionality were preferably selected Table 5.

Table 5. SNPs selected in the study. Candidate SNPs were selected in oncogenes and tumour suppressor genes, DNA repair (either BER, NER and DSB), inflammatory, apoptotic and carcinogen metabolism genes, as described in Material and Methods.

| FUNCTION                                   | GENE   | RS     | ID               | Change   |
|--------------------------------------------|--------|--------|------------------|----------|
| Oncogenes and tumour suppressor genes      | TP53   | 1042522| C_2403545_10     | Pro72Arg |
|                                            | MDM2   | 2279744| PCR-RFLP        | -410T>G  |
|                                            | KRAS-LC6 | 6176430| PCR-custom probe | 3'-UTR   |
|                                            | EGFR   | 2227983| C_16170352_20   | Lys521Arg|
|                                            |        | 25487  | C_622564_10     | Gln399Arg|
|                                            | APEX   | 1799782| C_11463404_10   | Arg194Trp|
|                                            |        | 1799798| C_8921503_10    | Asp148Glu|
|                                            | ERCC1  | 13181  | C_3145033_10    | Lys751Gln|
|                                            |        | 11615  | C_2532959_10    | Asn118Asn|
|                                            | XPC    | 2228000| C_16018061_10   | Ala499Val|
|                                            |        | 261539  | C_8901525_10    | Thr241Met|
|                                            |        | 1799794| C_2983904_10    | -316A>G  |
|                                            |        | 2267437| C_15872242_20   | -731C>G  |
|                                            |        | 16944  | C_1839943_10    | -511T>C  |
|                                            |        | 2069762| C_15859930_10   | -330T>G  |
|                                            |        | 1800795| C_1839697_20    | -174C>G  |
|                                            |        | 1800872| C_1747363_10    | -592C>A  |
|                                            |        | 361525  | C_2215707_10    | -238A>C  |
|                                            | NOD2   | 2066844| C_11717468_20   | Arg702Trp|
|                                            |        | 2066845| C_11717466_20   | Arg908Gly|
|                                            | BAX    | 4645878| C_27848291_10   | -248G>A  |
|                                            |        | 2279115| C_3044428_30    | -938C>A  |
|                                            |        | 776746  | C_26201809_30   | 698A>G   |
|                                            | GSTP1  | 1695   | C_3237198_20    | Ile105Val|
|                                            | GSTT1  | N/A    | PCR             | Null/present|
|                                            | GSTT1  | N/A    | PCR             | Null/present|
|                                            | NFE2L2 (NRF2) | 13035806| C_11745134_10  | 3'-UTR   |
|                                            |        | 2706110| C_11745133_10   | 3'-UTR   |
|                                            | KEAPI  | 1048290| C_9323035_10    | Leu471Leu|
4.3. DNA Isolation and Genotyping

DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method. Genotyping was performed using the TaqMan® Allelic Discrimination Assay [44] (Applied Biosystems, Foster, CA, USA) in those SNPs where the probes were available. A concentration of 40 ng/µL of DNA samples were added to 6.25 µL of Taqman® Universal PCR Master Mix and it was combined with specific forward and reverse primers, and allele-specific VIC (allele 1) and FAM (allele 2) labelled probes. The assay was performed in a 96 well plate and the detection was measured in the Step One Plus Real-Time PCR System Thermal Cycling Block (Applied Biosystems, Foster, CA, USA). Negative and positive controls were always added. A total of 5% of random samples were re-genotyped to ensure the reproducibility.

In those candidate SNPs in which TaqMan® probes were not available, genotyping was analysed using polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP). Specific oligonucleotides were designed to amplify the polymorphic sequences and digestion was made via the specific restriction enzymes. The PCR products were run on 3% Syber-safe stained agarose gel and visualized under UV light. GSTT1 and GSTM1 null/present SNPs were analysed using PCR with β-actin as an endogenous control. Finally, for KRAS-LC6 rs61764370, a custom probe was specifically designed. Sequences and type of assays are shown in Table S1.

4.4. Statistical Analysis

The statistical analysis to associate the relation between the different clinical and molecular variables was analysed using cross tabs and the $\chi^2$ test of Pearson. The odds ratios (OR) and 95% confidence intervals were calculated using logistic regression analysis. The quantitative variable distribution was analysed using the ANOVA test in those examples where the sample followed a parametric distribution ($p > 0.05$ in Levene’s test), while in those with a non-parametric distribution, a Mann–Whitney $U$ test was applied. Hardy–Weinberg equilibrium (HWE) was tested in a control population using a $\chi^2$ test. Statistically significant differences were considered to exist when the two-sided $p$-value was <0.05. Only TP53 rs1042522 and APEX rs1130409 were in disequilibrium ($p_{HWE} < 0.05$).

Because of the lower inclusion of matched controls, the statistical analysis was realised when matching the group via the propensity score method (PS). This allowed us to equate groups in a cohort study through a logistic regression introducing the confounders as predictive variables [45–47]. Groups were matched according to: packs per year consumed (PPY): no smokers, <20PPY and >20PPY, standard unit of alcohol per week (SDU/week): <14SDU/week and >14SDU/week, and sex. Quantitative age was not included in the PS and it was introduced in the logistic regression as adjustment variable only in laryngeal cancer where the age between both groups was statistically significant.

These analyses were performed with the statistical software SPSS v.21.0 (IBM-SPSS Inc., Chicago, IL, USA).

5. Conclusions

This study shows the association between several polymorphisms in genes involved in DNA repair, inflammation, antioxidative and apoptotic pathways with susceptibility to developing HPV-negative HNSCC. The characteristics of the control group positively indicates that these results are caused by the genetic background, avoiding confounder variables. Likewise, the differences found in this association study according to the location corroborate the heterogeneity in these tumours included under the same term of head and neck squamous cell carcinoma. It is important to mention that this study could provide evidence to define the consideration of different genetic entities within HNSCC and the necessity of using a matched control population by their risk factors in future case-control studies. Larger studies should be performed and would be necessary to confirm these results.
Acknowledgments: The authors thank the patients who have participated in this study and their families.

Author Contributions: Conceptualization, J.J.C.-H. and R.G.-S.; methodology, J.F.-M.; validation, J.J.C.-H. and R.G.-S.; formal analysis, J.F.-M. and R.S.-T.; investigation, J.F.-M.; resources, J.J.C.-H. and R.G.-S.; data curation, J.C.A.K., M.P.B., E.F.-R., R.M., E.d.B., C.S.C., A.R.D., A.R.D., J.C.D., E.F.R.; writing—original draft preparation, J.F.-M., R.S.-T., J.C.A.K., A.O., R.G.-S., J.J.C.-H.; writing—review and editing, All authors; visualization, J.F.-M., R.S.-T., J.J.C.-H.; supervision, J.J.C.-H. and R.G.-S.; project administration, J.J.C.-H.; funding acquisition, J.J.C.-H.

Funding: This study was supported by the health research program of the “Instituto de Salud Carlos III” (Spanish Ministry of Economy and Competitiveness, PI11/00519, PI13/01741 and PIE14/00066) co-funded with FEDER founds and by the Health Regional Management of the Junta de Castilla y León (GRS630/A11). J. Fernández-Mateos was partially supported by a predoctoral research grant from the Consejería de Educación—Junta de Castilla y León and the European Social Fund to CC-B (EDU/1084/2012).

Acknowledgments: The authors thank the patients who have participated in this study and their families. Moreover, we are grateful for the great effort from all the collaborators of the Spanish Head and Neck Cancer Cooperative Group (TTCC) that are not included in this manuscript and the support from the Institute of Biomedical Research of Salamanca (IBSAL).

Conflicts of Interest: R.M. declares advisory role by Merck Kga, MSD, BMS, AZ, Nanobiotix, Roche and conferences with fee by Merck Kga, BMS, MSD, Roche; E.d.B declares conflict of interest by BMS, MSD, AstraZeneca; A.R.D declares advisory role by Roche, Bristol-Myers Squibb, Merck Serono, MSD, Takeda, Novartis and honoraria by Roche, Bristol-Myers Squibb and Merck Serono; J.C.D declares conferences and courses by Merck and Takeda; J.J.C.-H declares advisory role by Merck, MSD, BMS, Novartis and conferences with fee by Merck, BMS, MSD, Roche, AstraZeneca and Novartis. AO declares conflict of interest by Merck, Entremech, Daiichi Sankyo and Servier. The rest of the authors declare no conflict of interest.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6694/11/4/493/s1, Table S1: Primer sequences and genotyping assays in those SNPs defined in Table 5 without specific TaqMan® probes, Table S2: Distribution between studied SNPs in different genes in laryngeal tumours (Ca) and controls (Co)*, Table S3: Distribution between studied SNPs in different genes in tumours from oral cavity (Ca) and controls (Co), Table S4: Distribution between studied SNPs in different genes in pharyngeal tumours (Ca) and controls (Co).

References
1. Ganci, F.; Sacconi, A.; Manciocco, V.; Covello, R.; Spriano, G.; Fontemaggi, G.; Blandino, G. Molecular Genetics and Biology of Head and Neck Squamous Cell Carcinoma: Implications for Diagnosis, Prognosis and Treatment; Agulnik, M., Ed.; In Tech: Rijeka, Croatia, 2012.
2. Argiris, A.; Karamouzis, M.V.; Raben, D.; Ferris, R.L. Head and neck cancer. Lancet 2008, 371, 1695–1709. [CrossRef]
3. Leemans, C.R.; Braakhuis, B.J.M.; Brakenhoff, R.H. The molecular biology of head and neck cancer. Nat. Rev. Cancer 2011, 11, 9–22. [CrossRef] [PubMed]
4. Perez-Ordoñez, B.; Beauchemin, M.; Jordan, R.C.K. Molecular biology of squamous cell carcinoma of the head and neck. J. Clin. Pathol. 2006, 59, 445–453. [CrossRef] [PubMed]
5. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. CA Cancer J. Clin. 2015, 65, 87–108.
6. Hashibe, M.; Brennan, P.; Benhamou, S.; Castellsague, X.; Chen, C.; Curado, M.P.; Maso, L.D.; Daudt, A.W.; Fabianova, E.; Wünsch-Filho, V.; et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: Pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J. Natl. Cancer Inst. 2007, 99, 777–789. [CrossRef] [PubMed]
7. Hashibe, M.; Brennan, P.; Chuang, S.C.; Boccia, S.; Castellsague, X.; Chen, C.; Curado, M.P.; Dal Maso, L.; Daudt, A.W.; Fabianova, E.; et al. Interaction between Tobacco and Alcohol Use and the Risk of Head and Neck Cancer: Pooled Analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol. Biomark. Prev. 2009, 18, 541–550. [CrossRef] [PubMed]
8. Sturgis, E.M.; Wei, Q.; Spitz, M.R. Descriptive epidemiology and risk factors for head and neck cancer. Semin. Oncol. 2004, 31, 726–733. [CrossRef]
9. Ragin, C.C.R.; Modugno, F.; Gollin, S.M. The Epidemiology and Risk Factors of Head and Neck Cancer: A Focus on Human Papillomavirus. J. Dent. Res. 2007, 86, 104–114. [PubMed]
10. Cardesa, A.; Nadal, A. Carcinoma of the head and neck in the HPV era. Acta Dermatovenerol. APA 2011, 20, 161–173.
11. Jefferies, S.; Eeles, R.; Goldgar, D.; A’Hern, R.; Henkk, J.M.; Gore, M. The role of genetic factors in predisposition to squamous cell cancer of the head and neck. Br. J. Cancer 1999, 79, 865–867. [CrossRef] [PubMed]

12. Cloos, J.; Spitz, M.R.; Schantz, S.P.; Hsu, T.C.; Zhang, Z.F.; Tobi, H.; Braakhuis, B.J.; Snow, G.B. Genetic Susceptibility to Head and Neck Squamous Cell Carcinoma. Int. J. Radiat. Oncol. 2014, 89, 38–48. [CrossRef] [PubMed]

13. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. Cell 2011, 144, 646–674. [CrossRef]

14. Azad, A.K.; Bairati, I.; Samson, E.; Cheng, D.; Cheng, L.; Mirshams, M.; Savas, S.; Waldron, J.; Wang, C.; Goldstein, D.; et al. Genetic sequence variants and the development of secondary primary cancers in patients with head and neck cancers. Cancer 2012, 118, 1554–1565. [CrossRef]

15. Hiyama, T.; Yoshihara, M.; Tanaka, S.; Chayama, K. Genetic polymorphisms and head and neck cancer risk (Review). Int. J. Oncol. 2008, 32, 945–973. [CrossRef]

16. Hoyer, K.K.; Dooms, H.; Barron, L.; Abbas, A.K. Interleukin-2 in the development and control of inflammatory disease. Immunol. Rev. 2008, 226, 19–28. [CrossRef] [PubMed]

17. Gansmo, L.B.; Vatten, L.; Romundstad, P.; Hveem, K.; Ryan, B.M.; Harris, C.C.; Knappskog, S.; Lønning, P.E. Associations between the MDM2 promoter P1 polymorphism del1518 (rs3730485) and incidence of cancer of the breast, lung, colon and prostate. Oncotarget 2016, 7, 28637–28646. [CrossRef]

18. Grivennikov, S.I.; Greten, F.R.; Karin, M. Immunity, Inflammation, and Cancer. Cell 2010, 140, 883–899. [CrossRef]

19. Kundu, J.K.; Surh, Y.-J. Inflammation: Gearing the journey to cancer. Mutat. Res. 2008, 659, 15–30. [CrossRef] [PubMed]

20. Degenhardt, K.; Mathew, R.; Beaudoin, B.; Bray, K.; Anderson, D.; Chen, G.; Mukherjee, C.; Shi, Y.; Gélinas, C.; Fan, Y.; et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006, 10, 51–64. [CrossRef]

21. He, B.; Zhang, Y.; Pan, Y.; Xu, Y.; Gu, L.; Chen, L.; Wang, S. Interleukin 1β (IL1B) promoter polymorphism and cancer risk: Evidence from 47 published studies. Mutageneis 2011, 26, 637–642. [CrossRef] [PubMed]

22. Wu, J.; Lu, Y.; Ding, Y.B.; Ke, Q.; Hu, Z.B.; Yan, Z.G.; Xue, Y.; Zhou, Y.; Hua, Z.L.; Shu, Y.Q.; et al. Promoter polymorphisms of IL2, IL4, and risk of gastric cancer in a high-risk Chinese population. Mol. Carcinog. 2009, 48, 626–632. [CrossRef] [PubMed]

23. Serefoglou, Z.; Yapijakis, C.; Nkenke, E.; Vairaktaris, E. Genetic association of cytokine DNA polymorphisms with head and neck cancer. Oral Oncol. 2008, 44, 1093–1099. [CrossRef] [PubMed]

24. Hsiung, C.; Fan, Y.; et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006, 10, 51–64. [CrossRef] [PubMed]

25. He, B.; Zhang, Y.; Pan, Y.; Xu, Y.; Gu, L.; Chen, L.; Wang, S. Interleukin 1β (IL1B) promoter polymorphism and cancer risk: Evidence from 47 published studies. Mutageneis 2011, 26, 637–642. [CrossRef] [PubMed]

26. Yang, X.L.; Zhu, Y.; Ye, D.; Liu, Y.; Sun, H.; Ruan, M.; Liu, W. Association of MDM2 promoter T309G polymorphism with oral cancer risk: A meta-analysis of 3536 subjects. Mol. Carcinog. 2009, 48, 626–632. [CrossRef] [PubMed]

27. Bojesen, S.E.; Nordestgaard, B.G. The common germline Arg72Pro polymorphism of p53 and increased longevity in humans. Cell Cycle 2014, 7, 158–163. [CrossRef] [PubMed]

28. Naccarati, A.; Pardini, B.; Polakova, V.; Smerhovsky, Z.; Vodickova, L.; Soucek, P.; Vrana, D.; Holcataova, I.; Ryska, M.; Vodicka, P. Genotype and haplotype analysis of TP53 gene and the risk of pancreatic cancer: An association study in the Czech Republic. Carcinogenesis 2010, 31, 666–670. [CrossRef]

29. Khan, M.H.; Khalil, A.; Rashid, H. Evaluation of the C-reactive protein and its association with cancer risk: A HuGE review and meta-analysis. Genet. Res. 2015, 97, e7. [CrossRef] [PubMed]

30. Brunotto, M.; Zarate, A.M.; Berra, S. Risk genes in head and neck cancer: A systematic review and meta-analysis of last 5 years. Oral Oncol. 2014, 50, 178–188. [CrossRef]

31. Ryska, M.; Vodicka, P. Genetic polymorphisms and head and neck cancer risk: A HuGE review and meta-analysis. Genet. Res. 2010, 97, e7. [CrossRef] [PubMed]

32. Sourvinos, G.; Rizos, E.; Spandidos, D.A. p53 codon 72 polymorphism is linked to the development and not the progression of benign and malignant laryngeal tumours. Oral Oncol. 2001, 37, 572–578. [CrossRef]
34. Siddique, M.; Sabapathy, K. Trp53-dependent DNA-repair is affected by the codon 72 polymorphism. *Oncogene* **2006**, *25*, 3489–3500. [CrossRef] [PubMed]

35. Gomez-Sanchez, J.C.; Delgado-Esteban, M.; Rodriguez-Hernandez, I.; Sobrino, T.; de la Ossa, N.P.; Reverte, S.; Bolaños, J.P.; Gonzalez-Sarmiento, R.; Castillo, J.; Almeida, A. The human Trp53 Arg72Pro polymorphism explains different functional prognosis in stroke. *J. Exp. Med.* **2011**, *208*, 429–437. [CrossRef] [PubMed]

36. Lehnerdt, G.F.; Franz, P.; Bankfalvi, A.; Grehl, S.; Kela, A.; Nückel, H.; Lang, S.; Schmid, K.W.; Siffert, W.; Bachmann, H.S. The regulatory BCL2 promoter polymorphism (-938C>A) is associated with relapse and survival of patients with oropharyngeal squamous cell carcinoma. *Ann. Oncol.* **2009**, *20*, 1094–1099. [CrossRef] [PubMed]

37. Cingeetham, A.; Vuree, S.; Dunna, N.R.; Gorre, M.; Nanchari, S.R.; Edathara, P.M.; Meka, P.; Annamaneni, S.; Digumarthi, R.; Sinha, S.; et al. Influence of BCL2-938C>A and BAX-248G>A promoter polymorphisms in the development of AML: Case-control study from South India. *Tumor Biol.* **2015**, *36*, 7967–7976. [CrossRef] [PubMed]

38. Chen, K.; Hu, Z.; Wang, L.E.; Sturgis, E.M.; El-Naggar, A.K.; Zhang, W.; Wei, Q. Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of BAX and BCL2 genes and risk of squamous cell carcinoma of the head and neck. *Carcinogenesis* **2007**, *28*, 2008–2012. [CrossRef]

39. Jaramillo, M.C.; Zhang, D.D. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev.* **2013**, *27*, 2179–2191. [CrossRef]

40. Seijas-Tamayo, R.; Fernández-Mateos, J.; Klain, J.A.; Borgoño, M.P.; Pérez-Ruiz, E.; Fernández, S.V.; Coloma, C.S.; Domínguez, A.R.; Taberna, M.; et al. Epidemiological characteristics of a Spanish cohort of patients diagnosed with squamous cell carcinoma of head and neck: Distribution of risk factors by tumor location. *Clin. Transl. Oncol.* **2016**, *18*, 1114–1122. [CrossRef]

41. Hashibe, M.; Brennan, P.; Strange, R.C.; Bhisey, R.; Casper, I.; Lazarus, P.; Ophuis, M.B.O.; Benhamou, S.; Foulkes, W.D.; Katoh, T.; et al. Meta- and Pooled Analyses of GSTM1, GSTT1, GSTP1, and CYP1A1 Genotypes and Risk of Head and Neck Cancer. *Cancer Epidemiol. Biomark. Prev.* **2003**, *12*, 1509–1517.

42. Canova, C.; Hashibe, M.; Simonato, L.; Nelis, M.; Metspalu, A.; Lagiou, P.; Trichopoulos, D.; Ahrens, W.; Pigeot, I.; Merletti, F.; et al. Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 european countries: The ARCAGE project. *Cancer Res.* **2009**, *69*, 2956–2965. [CrossRef] [PubMed]

43. Chuang, S.C.; Agudo, A.; Ahrens, W.; Anantharaman, D.; Benhamou, S.; Boccia, S.; Chen, C.; Conway, D.; Fabianova, E.; Hayes, R.B.; et al. Sequence Variants and the Risk of Head and Neck Cancer: Pooled Analysis in the INHANCE Consortium. *Front. Oncol.* **2011**, *1*, 13. [CrossRef]

44. Schleinitz, D.; Distefano, J.K.; Kovacs, P. Targeted SNP genotyping using the TaqMan® assay. *Methods Mol. Biol.* **2011**, *700*, 77–87.

45. Rosenbaum, B.Y.P.; Rubin, D.B. The central role of the propensity score in observational studies for causal effects. *Biometrika* **1983**, *70*, 41–55. [CrossRef]

46. Streiner, D.L.; Norman, G.R. The pros and cons of propensity scores. *Chest* **2012**, *142*, 1380–1382. [CrossRef]

47. D’Agostino, R.B. Propensity scores in cardiovascular research. *Circulation* **2007**, *115*, 2340–2343. [CrossRef] [PubMed]

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