**Genetic variants associated with mandibular osteoradionecrosis following radiotherapy for head and neck malignancy**

**Abstract**

Background/Aim

Utilising radiotherapy in the management of head and neck cancer (HNC) often results in long term toxicities. Mandibular osteoradionecrosis (ORN) represents a late toxicity associated with significant morbidity. We aim to identify a panel of common genetic variants which can predict ORN to aid development of personalised radiotherapy protocols.

Method

Single nucleotide polymorphism (SNP) arrays were applied to DNA samples from patients who had prior HNC radiotherapy and minimum two years follow-up. A case cohort of mandibular ORN was compared to a control group of participants recruited to CRUK HOPON clinical trial.  Relevant clinical parameters influencing ORN risk (e.g. smoking/alcohol) were collected. Significant associations from array data were internally validated using polymerase chain reaction (PCR) and pyrosequencing.

Results

Following inclusion of 141 patients in the analysis (52 cases, 89 controls), a model predictive for ORN was developed; after controlling for alcohol consumption, smoking, and age, 4053 SNPs were identified as significant. This was reduced to a representative model of 18 SNPs achieving 92% accuracy. Following internal technical validation, a six SNP model (rs34798038, rs6011731, rs2348569, rs530752, rs7477958, rs1415848) was retained within multivariate regression analysis (ROC AUC 0.859).  Of these, four SNPs (rs34798038 (A/G)(p 0.006), rs6011731 (C/T)(p 0.018), rs530752 (A/G)(p 0.046) and rs2348569 (G/G)(p 0.005)) were significantly associated with the absence of ORN.

Conclusion

This is the first genome wide association study in HNC using ORN as the endpoint and offers new insight into ORN pathogenesis. Subject to validation, these variants may guide patient selection for personalised radiotherapy strategies.

Key Words:

Head and neck cancer, radiotherapy, osteoradionecrosis, mandible, single nucleotide polymorphism, genome wide association studies.[[1]](#footnote-1)

## **Introduction**

Osteoradionecrosis of the jaw (ORN) is a devastating late toxicity of head and neck radiotherapy with reported incidence in modern series ranging from 4-8% [1,2,3]. It is characterised by the presence of non-healing necrotic bone for longer than 3 months in an area previously exposed to radiotherapy without evidence of recurrent tumour [4]. The severity of ORN can vary from a small non healing area within the alveolar margin to pathological fracture and skin fistulae leading to significant compromise on functions associated with normal daily living. It poses a great challenge for clinicians, due to the absence of effective medical management in those with severe forms of the condition leading to technically difficult major reconstructive procedures without guarantee of resolution [5]. The development of this complication is multifactorial with clinical (i.e. cigarette smoking) and dosimetric (volume of irradiated mandible) factors playing an important role and, in addition, functional polymorphisms have been causally linked to its occurrence [6].

Multiple candidate gene studies have taken place investigating the association between ORN and single nucleotide polymorphisms (SNPs) within genes that encode proteins targeting fibrosis pathways, oxidative stress response and DNA repair [7,8,9]. ORN offers an ideal focus for the development of a predictive genetic biomarker panel as it is a dichotomous outcome which is easily diagnosed and quantified, and eliminates subjective interpretation of side effects.

The aim of this study was to discover new common genetic variants predictive of ORN. In doing so we may generate new hypotheses on pathogenesis and contribute to developing a more personalised approach to radiotherapy treatment planning for patients with head and neck cancer.

## **Methods**

A case-control study of 152 patients was undertaken with ethical approval from North West – Liverpool Central REC (Ref. No. 10/H1002/53) and Greater Manchester Central REC (Ref. No.08/H1008/32). 97 patients took part in the CRUK HOPON clinical trial; a randomised controlled phase III trial examining whether the delivery of hyperbaric oxygen reduced the likelihood of developing ORN following dental procedures in patients who received >50Gy radiotherapy to the head and neck [10]. 93 patients from the prospective HOPON collection did not develop ORN despite being deemed high risk by virtue of post treatment dentoalveolar surgery and blinded clinical/radiological assessment for ORN as its primary outcome measure. In addition to 4 cases of ORN from the HOPON trial, an independent group of 55 patients recruited at University Hospital Aintree NHS Foundation Trust Head and Neck Cancer Unit who similarly received over 50Gy radiotherapy to head and neck were recruited after developing ORN. Thus the case cohort constituted a total of 59 patients and the control cohort of 93 patients. Within the case cohort a diagnosis of ORN was confirmed from clinical notes and review of X-rays, clinical photographs and physical examination with Notani grade noted where specified [11]. Radiotherapy treatment information was obtained via study data collection forms or clinical patient records. Treatment was delivered using either 3D conformal or IMRT depending upon individual treating clinician and centre, with doses ranging 50-70Gy delivered over 20-35 fractions. Information on smoking status, alcohol consumption, post radiotherapy dental procedures, and use of bisphosphonates were also obtained. All patients had at least 2 years of follow up data available at time of analysis. STROGAR guidelines were followed for reporting where possible [12].

### **Genomic Sequencing**

Cell pellets from whole blood collected in ethylenediaminetetraacetic acid (EDTA) containing collection tubes were aliquoted and stored at -80֯C. Genomic DNA was extracted from these cell pellets using QIAGEN QIAamp DNA Blood Midi Kit according to manufacturer handbook instructions (1090244 02/2015, QIAGEN Ltd.). DNA samples were sent for SNP array sequencing at Edinburgh Genomics (Ashworth Laboratories, The University of Edinburgh). Samples were prepared and processed by hybridisation, washing, staining and sequenced using the Infinium Global Screening Array V2.0 (Illumina, Inc.).

### **Bioinformatic analysis**

**Associating SNPs to ORN**

PLINK (v 1.90b6)[13] was utilised to generate genome wide associations. SNPs were extracted for which PLINK returned an empirical p-value based on permutations < 0.01 and were annotated using SNP nexus [14-18,23].

**Identifying a SNP panel predictive of ORN**

To identify a potential panel predictive of ORN outcome, the frequencies of all SNPs within the dataset were inputted into a genetic algorithm based predictive modelling approach (GALGO) [19]. Feature sets were trained within GALGO by applying a random Forest (RF) classifier [20]. The smallest possible model predictive of ORN was obtained using the robustGeneBackwardElimination approach within GALGO. For further details see supplementary methods.

### **Validation**

The ten top highlighted SNPs of interest were internally validated using polymerase chain reaction (PCR) and pyrosequencing. Cognizant of the significant findings in previous publications [8,9,21], SNPs rs1695, rs1042522, rs1047768, rs25487 and rs1800469 were checked for significance within the larger model. We sought to validate the additional SNP rs1800469 (TGFβ1) given this target approached significance (p 0.07) [7].

Primer design for amplification and sequencing was performed using Pyromark Assay Design 2.0 software (QIAGEN Ltd.) and dbSNP NCBI (National Centre for Biotechnology Information)[22]. Following annealing temperature optimisation, DNA samples were genotyped for SNPs outlined in Table S1 using PyroMark PCR Kit (QIAGEN Ltd.) according to manufacturer handbook instructions. PCR products were sequenced with the corresponding primer using PyroMark Q96 ID pyrosequencer (QIAGEN Ltd.) along and PyroMark Gold Q96 reagents (QIAGEN Ltd.). Analysis was performed using PyroMark Q96 ID Software 2.5 (QIAGEN Ltd.).

Validation statistical analysis was completed using IBM SPSS statistics v27. Univariate and multivariate logistic regression validated the association between SNPs within the optimised model and ORN (supplementary methods).

## **Results**

Two patients were excluded from final modelling and validation analysis due to insufficient DNA samples to allow PCR and pyrosequencing.  Of the remaining 150, nine were highlighted as outliers based on the PCA of SNP data generated using the Illumina Infinium Global Array (Figure S1). Of the analysis set of 141 patients, the median age was 56 (range 16-78) and the majority (107/141, 76%) were males. 63 (45%) patients were diagnosed with a primary oropharyngeal tumour and 54 (38%) primary oral cavity tumour; 57% (80/141) having stage 3 or 4 disease. Patients either received adjuvant radiotherapy (83/141, 59%), adjuvant chemoradiotherapy (15/141, 11%), primary radiotherapy (16/141, 11%) or primary chemoradiotherapy (27/141, 19%). A total of 52 patients (including 2 patients from HOPON cohort) out of 141 (37%) developed ORN with no significant association on univariate analysis with sex, smoking, site of tumour, stage or treatment received (Table 1). The HOPON clinical trial excluded any patients taking bisphosphonates and none of the additional patients recruited had a history of taking bisphosphonates.

A PCA analysis of the dataset showed no specific separation between ORN positive and negative patients. As shown in Supplementary Figure 1A, gender defined the two groups within the dataset based on the amount of missing data representing the Y chromosome specific SNPs on the array. For identification of significantly different SNPs between ORN positive and negative cases, four additional covariates were included; gender, age, alcohol consumption, and smoking status. This identified a total of 4053 SNPs (Table S2).

The significant SNP lists were compared with the data within the 1000 Genomes Project [23] and HapMap [24], as expected, controls clustered closely together with the European population within the 1000 Genome Project (Figure S2).

Annotation of the SNPs identified 1764 overlapped genes and 483 genes within 10000 bases of their position. To better understand the genes the SNPs might have affected, separate functional enrichment analyses of the directly overlapped and nearest genes took place.

Functional enrichment of the directly overlapping geneset (based on the 1764 genes) highlighted cell junction organization, parasympathetic nervous system development, synapse organisation and neuron differentiation (Figure S3 and Table S2).

Genes within 10000bp to SNPs without direct gene overlap enriched again in nervous system development, bone remodelling, and immune system relevant functions such as CD4+/CD25+ T cell differentiation.

To test whether the allele frequencies could predict ORN, feature selection methodology was applied to the data. First the coded allele usage for each sample was extracted and used in a genetic algorithm based feature selection methodology (GALGO). In conjunction with a RF classifier, small sets of SNPs able to predict ORN within this patient cohort were identified. GALGO uses a dual training and test strategy linked with a cross-validation methodology to overcome overtraining of the models. The first training split is further split into a second level training/test split which is then used to generate a set of models. To ensure we covered a large enough search space we developed 5000 models. All 5000 models generated reached an accuracy of least 92% within the second level split. To develop the most robust and representative predictive model, a forward selection approach on the initial training and test split was applied (Figure 1). The resulting model highlighted that while the control samples were well predicted, ORN cases could be predicted with approximately 70% accuracy (Figure 1A). Here the best performing model reached an overall accuracy of 82%. To test whether this model could be optimised, the backward selection procedure within GALGO removed excess features from the representative model. This resulted in a list of 18 SNPs that within the initial training and test split achieved 92% accuracy (Table S3). The model was tested against numerous first splits within GALGO and confirmed a good prediction for the majority of control samples (Figure 1B). Several ORN cases, however, were misclassified.

In order to internally validate optimised 18 SNP model, both univariate (UVA) and multivariate (MVA) regression analysis took place on a representative selection of ten targets, using pyrosequencing data from our set of 141 patients (Table S3 and Table S4). When significant variables on UVA were incorporated into MVA, the model yielding the lowest AIC (117.5) excluded rs2105042 and rs11605273 whilst retaining rs34798038, rs6011731, rs2348569, rs530752, rs7477958, rs1415848.   Significant genotypes were heterozygote rs34798038 (A/G) (p 0.006), rs6011731 (C/T) (p 0.018) and rs530752 (A/G) (p 0.046) along with the rarer variant homozygote genotype for rs2348569 (G/G) (p 0.005) (Table 2). This model produced ROC with AUC 0.859 and was a good fit to the data (H-L test p>0.05). To further test model performance these steps were repeated with the inclusion of the nine patients removed during the SNP array PCA. This resulted in a marginally smaller AUC of 0.853.

## The SNP rs1800469 (TGF-β1) approached significance (p 0.07) within the wider model [7], however the presence of the rarer C/T or T/T allele were not found to be significant on UVA during validation (Table S4).  Other SNPs previously reported within candidate gene studies to be associated with ORN [8,9,21] did not retain significance; rs1695 within GSTP1 (p 0.258), rs1042522 pro allele of TP53 (p 0.557), rs1047768 within ERCC5 (p 0.146), and rs25487 within XRCC1 (p 0.307).

## **Discussion:**

We set out to discover novel genetic variants which may affect the probability of developing ORN following head and neck radiotherapy. Our optimised predictive model encapsulates 18 SNPs which distinguish control and ORN cases. When validated, six SNPs were retained within the model and four genotypes significantly reduced the likelihood of developing ORN; rs2348569 (G/G), rs34798038 (A/G), rs6011731 (C/T) and rs530752 (A/G) (Table 2).

To our knowledge this is the first GWAS experiment investigating ORN as a long term radiotherapy toxicity outcome. The bioresource obtained from patients recruited to the CRUK HOPON study provided a uniquely well characterised and relevant control cohort and the means to investigate a binary outcome with very few confounding factors. In maintaining such unrivalled clean cohorts with blinded assessment of ORN, we have avoided the ‘noise’ of data pooling/multiple outcomes seen in other radiogenomic GWAS experiments.

The pathophysiology of ORN is poorly understood and theories have evolved from infection following trauma to treatment induced cellular and vascular damage leading to hypoxia and tissue breakdown [25,26]. More recently, reactive oxygen species (induced through the acute inflammatory response) are thought to dysregulate collagen and fibrotic pathways [27]. The resultant promotion of fibrosis and prevention of effective vascularisation has been confirmed through the examination of dento-alveolar bone cores following irradiation; a reduction in microcapillaries correlating to increasing doses [28]. The heterozygote variant (C/T) SNP rs6011731 (p 0.018, OR 0.172) included within validation is located within COL20A1 (collagen type XX alpha 1 chain). This gene encodes pro-alpha 1 chain of type 1 collagen which plays a key role in assembly of collagen fibrils, organisation of extracellular matrix and tissue repair [29,30]. It is also a participant in the integrin pathway which ultimately results in inhibition of angiogenesis via thrombospondin 1 (TSP1) [31]. Similarly the heterozygote (A/G) rs34798038 was seen to reduce the odds of developing ORN (p 0.006, OR 0.048). This SNP lies within transducin like enhancer of split 4 (TLE 4) which is a member of TLE family of transcription repressor genes involved in regulating a number of pathways, including expression of WNT signalling and Runx2/Cbfa1 (that codes for a protein essential for osteoblast differentiation) [32,33]. The knock out of this gene in murine models has led to defective bone mineralisation and cortical bone thinning [34]. The SNP rs2348569 (p 0.005, OR 0.086) lies within an uncharacterised genomic region, close to non-coding transcripts. Long non-coding RNAs are known to be differentially expressed following tumour irradiation in murine models, however it is currently unclear as to whether SNPs near to these non-coding transcripts may play a role in modulating normal tissue radiosensitivity [35]. A number of our validated genetic variants seem to relate to the described pathogenesis of ORN and may contribute to altered tolerance of the mandible to radiation through defective collagen assembly, angiogenesis and bone mineralisation.

4053 SNPs were highlighted as significant in distinguishing ORN cases from controls. Subsequent functional enrichment of overlapping genes highlighted parasympathetic nervous system development, synapse organisation, and neuron differentiation pathways. Bone remodelling was previously thought to be regulated by hormones (i.e. parathyroid hormone and insulin like growth factor 1) however more recent evidence supports a complex interplay between endocrine and neural control. Central control of bone mass is modulated by a variety of neurotransmitters which can exert an inhibitory or excitatory effect through both beta 2 receptors possessed by osteoblasts and osteoclast nicotinic receptors [36]. Murine models demonstrate osteoclast apoptosis in response to cholinergic signalling promoting increased bone mass, whereas sympathetic signalling has the opposite effect [37]. Irradiating osteoblasts in culture led to elevated levels of acetylcholinesterase and this was confirmed in murine models whereby mouse limb bud irradiation promoted bone formation, osteoblast differentiation and a cholinergic phenotype presumably encouraging repair and remodelling [38]. Interestingly, the validated SNP rs530752 (p 0.046, OR 0.163) lies approximately 8000 base pairs from cholinergic receptor muscarinic 1 (CHRM1) coding for muscarinic acetylcholine receptor M1. This receptor centrally regulates haematopoietic stem cell differentiation within the bone marrow via G-CSF [39]. Variants close to this gene may influence the expression of CHRM1, parasympathetic innervation of mandibular bone and augmentation of osteoblast function [40,41] thus altering repair and remodelling in response to radiotherapy.

Previously identified variants from candidate gene studies examining long term radiation toxicity in HNC did not prove to be significantly associated with ORN in our study [8,9,21]. RS1800469 (TGF-β1) was of particular interest given the evidence reported across a variety of solid tumours [7]. TGF-β proteins have a wide range of reported functions; with abnormal bone remodelling, increased fibroblast proliferation and abnormal accumulation of extracellular matrix originally thought to be responsible for poor healing following radiotherapy [42]. TGF-β is also a modulator of inflammation and promotes Th17 cell differentiation from CD4+/CD25+ T cells [43]. Enrichment analysis highlighted SNPs without direct overlap within these immune functions, and although rs1800469 was not significantly associated with ORN during validation analysis, it remains likely that it could have an additive effect within this multi-loci model.

Our study has limitations, specifically the small sample size, however through internally validating single targets we have confirmed our conclusions. In addition, as with all GWAS experiments, there is the potential of missing low frequency loci with small effect sizes and as a result it will be necessary to further confirm our findings with larger external validation cohorts. A further limitation is the absence of comprehensive dosimetric data; unfortunately this reflects a pooling of patients from multiple units and time points, as follows the recruitment to the respective clinical trials, along with historic changes in planning software. It would be of interest to determine if the site and extent of ORN correlated with critical dose/volume threshold of mandibular exposure, or were dominated by mandibular blood supply and other anatomical features such as the muscle insertions of mentalis, masseter, medial pterygoid. Future investigations would be strengthened by the inclusion and utilisation of validated normal tissue complication probability models (e.g. percentage volume of mandible receiving 35Gy [44]).

During QC of the array data, nine patients were excluded based on their positioning within a PCA.  One could argue these individuals may represent a phenotype influenced by an alternative mechanism of interest; however, the study sample size limited further exploration. Interestingly, validation via pyrosequencing with inclusion of the nine outlier patients did not significantly decrease the AUC of the model, suggesting that technical differences in the array influenced their PCA placement.

With the incidence of HNC steadily increasing over the last three decades and survival outcomes improving through the use of new anticancer agents, more patients are living with devastating consequences of ORN [45]. It is vitally important we expand our understanding of this condition, develop biomarkers to prevent its occurrence and improve therapeutic options. If our findings are confirmed in larger, external validation cohorts our polygenic risk model may contribute to a pre-treatment risk prediction tool and facilitate much needed tailored radiotherapy strategies.

**Acknowledgements:**

The authors would like to thank the Aintree Head and Neck Cancer Patient Research Forum for funding this work and the Liverpool Clinical Trials Centre for HOPON clinical trial data acquisition.

**Funding:**

This work was funded by Aintree Head and Neck Cancer Patient Research Forum, Aintree Hospital, Liverpool, UK. The HOPON Trial was funded by Cancer Research UK (CRUK CTAAC: C23033/A12122 & FSC: C23033/A9397). Dr R.C. Brooker is supported though a clinical research fellowship awarded by the Clatterbridge Cancer Charity.

**Conflict of Interest Statement:**

Conflict of Interest: None

## **References**

1. Shaw RJ, Butterworth CJ, Silcocks P, et al. HOPON (Hyperbaric Oxygen for the Prevention of Osteoradionecrosis): a randomised controlled trial of hyperbaric oxygen to prevent osteoradionecrosis of the irradiated mandible after dentoalveolar surgery. *Int J Radiat Oncol*. 2019;104(3):530-539 doi:10.1016/j.ijrobp.2019.02.044

2. Owosho AA, Tsai CJ, Lee RS, et al. The prevalence and risk factors associated with osteoradionecrosis of the jaw in oral and oropharyngeal cancer patients treated with intensity modulated radiation therapy (IMRT): The Memorial Sloan Kettering Cancer Centre experience. *Oral Oncology* 2017; 64: 44-51. doi:10.1016/j.oraloncology.2016.11.015

3. Tsai CJ, Hofstede TM, Sturgis EM, et al. Osteoradionecrosis and radiation dose to the mandible in patients with oropharyngeal cancer. *Int J Radiat Oncol Biol Phys*. 2013;85:415-420. doi:10.1016/j.ijrobp.2012.05.032

4. Harris M. The conservative management of osteoradionecrosis of the mandible with ultrasound therapy. *Br J Oral Maxillofac Surg*. 1992;30(5):313-318. doi:10.1016/0266-4356(92)90181-H

5. Rice N, Polyzois I, Ekanayake K, Omer O, Stassen LFA. The management of osteoradionecrosis of the jaws - A review. *Surg J R Coll Surg E*. 2015;13(2):101-109. doi:10.1016/j.surge.2014.07.003

6. Raguse JD, Hossamo J, Tinhofer I, et al. Patient and treatment-related risk factors for osteoradionecrosis of the jaw in patients with head and neck cancer. *Or Surg Or Med Or Pa*. 2016;121(3):215-221.e1. doi:10.1016/j.oooo.2015.10.006

7. Lyons AJ, West CM, Risk JM, et al. Osteoradionecrosis in head-and-neck cancer has a distinct genotype-dependent cause. *Int J Radiat Oncol Biol Phys*. 2012;82(4):1479-1484. doi:10.1016/j.ijrobp.2011.05.016

8. Danielsson D, Brehwens K, Halle M, et al. Influence of genetic background and oxidative stress response on risk of mandibular osteoradionecrosis after radiotherapy of head and neck cancer. *Head Neck-J Sci Spec*. 2016;38(3):387-393. doi: 10.1002/hed.23903

9. Borchiellini D, Etienne-Grimaldi MC, Bensadoun RJ, et al. Candidate apoptotic and DNA repair gene approach confirms involvement of ERCC1, ERCC5, TP53 and MDM2 in radiation-induced toxicity in head and neck cancer. *Oral Oncol*. 2017;67:70-76. doi:10.1016/j.oraloncology.2017.02.003

10. Shaw R, Butterworth C, Tesfaye B, et al. HOPON (Hyperbaric Oxygen for the Prevention of Osteoradionecrosis): A randomised controlled trial of hyperbaric oxygen to prevent osteoradionecrosis of the irradiated mandible: Study protocol for a randomised controlled trial. *Trials*. 2018;19(1):1-10. doi:10.1186/s13063-017-2376-7

11. Shaw R, Tesfaye B, Bickerstaff M, Silcocks P, Butterworth C. Refining the definition of mandibular osteoradionecrosis in clinical trials: The cancer research UK HOPON trial (Hyperbaric Oxygen for the Prevention of Osteoradionecrosis). *Oral Oncol*. 2017;64:73-77. doi:10.1016/j.oraloncology.2016.12.002

12. Kerns SL, Ruysscher D De, Andreassen CN, et al. STROGAR - STrengthening the Reporting of Genetic Association studies in Radiogenomics. *Radiother Oncol*. 2014;110(1):182-188. doi:10.1016/j.radonc.2013.07.011

13. Chang CC, Chow CC, Tellier LCAM, et al. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4(1):1-16. doi:10.1186/s13742-015-0047-8

14. Oscanoa J, Sivapalan L, Gadaleta E, et al. SNPnexus: a web server for functional annotation of human genome sequence variation (2020 update). *Nucleic Acids Res*. 2020; 48(W1):W185-W192.

15. Dayem Ullah AZ, Oscanoa J, Wang J, et al. SNPnexus: assessing the functional relevance of genetic variation to facilitate the promise of precision medicine. *Nucleic Acids Res*. 2018; 46(W1):W109-W113.

16. Dayem Ullah AZ, Lemoine NR, Chelala C. A practical guide for the functional annotation of genetic variations using SNPnexus. *Briefings in Bioinformatics.* 2013; 14(4):437-

17. Dayem Ullah AZ, Lemoine NR, Chelala C. SNPnexus: a web server for functional annotation of novel and publicly known genetic variants (2012 update). *Nucleic Acids Res*. 2012; 40(W1):W65-W70.

18. Chelala C, Khan A, Lemoine NR. SNPnexus: A web database for functional annotation of newly discovered and public domain Single Nucleotide Polymorphisms. *Bioinformatics.* 2009; 25(5):655-61.1.

19. Trevino V, Falciani F. GALGO: An R package for multivariate variable selection using genetic algorithms. *Bioinformatics*. 2006;22(9):1154-1156. doi:10.1093/bioinformatics/btl0741.

20. Pavlov YL. Random forests. *Random for*. 2019:1-122. doi:10.1201/9780429469275-8.

21. Alsbeih G, Al-Harbi N, Al-Hadyan K, El-Sebaie M, Al-Rajhi N. Association between Normal Tissue Complications after Radiotherapy and Polymorphic Variations in TGFB1 and XRCC1 Genes. *Radiat Res*. 2010;*173*(4),505–511. [doi:10.1667/rr1769.1](https://doi.org/10.1667/rr1769.1)

22. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. [*Nucleic Acids Res*. 2001 Jan 1; 29(1):308-11](https://www.ncbi.nlm.nih.gov/pubmed/11125122).

23. <https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>

24. <https://ftp.ncbi.nlm.nih.gov/hapmap/>

25. Meyer I. Infectious diseases of the jaws. *J Oral Surg.* 1970;28(17e26)*.*

26. Marx, R. E. A new concept in the treatment of osteoradionecrosis. *J Oral Maxillofac Surg.* 1983;41(351e357).

27. Delanian S, Lefaix JL. The radiation-induced fibroatrophic process: Therapeutic perspective via the antioxidant pathway. *Radiother Oncol*. 2004;73(2):119–131. [doi:10.1016/j.radonc.2004.08.021](https://doi.org/10.1016/j.radonc.2004.08.021)

28. Patel V, Di Silvio L, Kwok J, et al. The impact of intensity-modulated radiation treatment on dento-alveolar microvasculature in pharyngeal cancer implant patients. J Oral Rehabil. 2020;47(11):1411-1421. doi:10.1111/joor.13084

29. Yates AD, Achuthan P, Akanni W, et al. Ensembl 2020 (release 103). PubMed PMID: 31691826. [doi:10.1093/nar/gkz966](https://doi.org/10.1093/nar/gkz966)

30. Sayers EW, Beck J, Bolton EE, et al. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*. 2021;49(D1):D10-D17. doi:10.1093/nar/gkaa892<https://www.ncbi.nlm.nih.gov/snp/>

31. Belinky F, Nativ N, Stelzer G, et al. PathCards: Multi-source consolidation of human biological pathways. *Database*. 2015;2015(2):1-13. doi:10.1093/database/bav006

32. Zaidi SK, Javed A, Choi JY, et al. A specific targeting signal directs Runx2/Cbfa1 to subnuclear domains and contributes to transactivation of the osteocalcin gene. *J Cell Sci*. 2001;114(17):3093-3102.

33. Milili M, Gauthier L, Veran J, Mattei MG, Schiff C. A new Groucho TLE4 protein may regulate the repressive activity of Pax5 in human B lymphocytes. *Immunology*. 2002;106(4):447-455. doi:10.1046/j.1365-2567.2002.01456.x

34. Wheat JC, Krause DS, Shin TH, et al. The corepressor Tle4 is a novel regulator of murine hematopoiesis and bone development. *PLoS One*. 2014;9(8). doi:10.1371/journal.pone.0105557

35. Tang J, Huang Y, Nguyen DH, Costes SV, Snijders AM, Mao JH. Genetic background modulates lncRNA-coordinated tissue response to low dose ionizing radiation. Int J Genomics. 2015;2015. doi:10.1155/2015/4610381.

36. Huang S, Li Z, Liu Y, et al. Neural regulation of bone remodeling: Identifying novel neural molecules and pathways between brain and bone. J Cell Physiol. 2019;234(5):5466-5477. doi:10.1002/jcp.265021.

37. Spieker J, Frieß JL, Sperling L, Thangaraj G, Vogel-Höpker A, Layer PG. Cholinergic control of bone development and beyond. Int Immunopharmacol. 2020;83(January):106405. doi:10.1016/j.intimp.2020.106405

38. Thangaraj G, Manakov V, Cucu A, Fournier C, Layer PG. Inflammatory effects of TNFα are counteracted by X-ray irradiation and AChE inhibition in mouse micromass cultures. Chem Biol Interact. 2016;259:313-318. doi:10.1016/j.cbi.2016.03.027 1.

39. Pierce H, Zhang D, Magnon C, et al. Cholinergic Signals from the CNS Regulate G-CSF-Mediated HSC Mobilization from Bone Marrow via a Glucocorticoid Signaling Relay. *Cell Stem Cell*. 2017;20(5):648-658.e4. doi:10.1016/j.stem.2017.01.002

40. Eimar H, Tamimi I, Murshed M, Tamimi F. Cholinergic regulation of bone. *J Musculoskelet Neuronal Interact*. 2013;13(2):124-132.

41. Bataille C, Mauprivez C, Haÿ E, et al. Different sympathetic pathways control the metabolism of distinct bone envelopes. *Bone*. 2012;50(5):1162-1172. doi:10.1016/j.bone.2012.01.023

42. Pohlers D, Brenmoehl J, Löffler I, et al. TGF-β and fibrosis in different organs - molecular pathway imprints. Biochim Biophys Acta - Mol Basis Dis. 2009;1792(8):746-756. doi:10.1016/j.bbadis.2009.06.004

43. Zhang S. The role of transforming growth factor β in T helper 17 differentiation. Immunology. 2018;155(1):24-35. doi:10.1111/imm.12938

44. Van Dijk L V., Abusaif AA, Rigert J, et al. Normal Tissue Complication Probability (NTCP) Prediction Model for Osteoradionecrosis of the Mandible in Patients With Head and Neck Cancer After Radiation Therapy: Large-Scale Observational Cohort. *Int J Radiat Oncol Biol Phys*. 2021;000(00). doi:10.1016/j.ijrobp.2021.04.042

45. Schache AG, Powell NG, Cuschieri KS, et al. HPV-related oropharynx cancer in the United Kingdom: An evolution in the understanding of disease etiology. *Cancer Res*. 2016;76(22):6598-6606. doi:10.1158/0008-5472.CAN-16-0633

**Figure 1 -** A) Forward selection trajectories indicate that individuals without ORN are easier to predict than ORN cases. B) Optimised 18 SNP model predicting ORN. While all controls are well predicted some ORN cases are still misclassified.

1. Abbreviations

HNC: Head and neck cancer, ORN: Osteoradionecrosis , SNP: Single nucleotide polymorphism, PCR: Polymerase chain reaction, ROC: Receiver operator characteristic, AUC: Area under curve, CRUK: Cancer research UK, HOPON: Hyperbaric Oxygen for the Prevention of OsteoradioNecrosis, GWAS: Genome wide association study, STAT: standardised average toxicity score, GALGO: genetic algorithm based predictive modelling, RF: random forests, UVA: univariate analysis, MVA: multivariate analysis, PCA: principle component analysis. [↑](#footnote-ref-1)