Int. J. Oral Maxillofac. Surg. 2019; 48: 1289–1299
https://doi.org/10.1016/j.ijom.2019.04.014, available online at https://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Systematic Review
Clinical Pathology

Genetic predisposition for medication-related osteonecrosis of the jaws: a systematic review

J. Sandro Pereira da Silva, E. Pullano, N. S. Raje, M. J. Troulis, M. August
Department of Oral and Maxillofacial Surgery, Massachusetts General Hospital, Boston, Massachusetts, USA

J. Sandro Pereira da Silva, E. Pullano, N. S. Raje, M. J. Troulis, M. August: Genetic predisposition for medication-related osteonecrosis of the jaws: a systematic review. Int. J. Oral Maxillofac. Surg. 2019; 48: 1289–1299.

Abstract. The purpose of this study was to assess whether genetic variation is a predictor for the development of medication-related osteonecrosis of the jaws (MRONJ) in patients receiving bisphosphonate therapy for various conditions. A systematic review based on the PRISMA guidelines was performed. A search strategy was developed. Comprehensive searches of major databases were conducted for studies published January 2003 through July 2018. The PICOS strategy was used to develop the inclusion criteria. The analysis in each study was performed primarily using single nucleotide polymorphism (SNP) frequency mean values and odds ratios between cases and controls. A total of 3301 patients were enrolled in the 15 included studies (two genome-wide association studies, $n = 1877$; 10 candidate gene studies, $n = 1195$; three whole genome/whole exome studies, $n = 229$). Multiple myeloma was the most prevalent primary disease (54.8%). Zoledronate was prescribed in 68.8% of patients. No one SNP was definitively identified as a risk factor for the development of MRONJ. To date, studies have failed to show a single gene as a risk factor for MRONJ. Heterogeneity of case and control populations may be contributory. Next generation sequencing studies may help elucidate the role and interplay of genetic events in the development of MRONJ.

Key words: MRONJ; osteonecrosis; SNP; genetic; jaw; GWAS; candidate gene; bisphosphonate.

Accepted for publication 18 April 2019
Available online 13 May 2019

Medication-related osteonecrosis of the jaws (MRONJ) is a problem for patients who receive bisphosphonate therapy as part of treatment for various malignancies, including multiple myeloma, breast cancer, and prostate cancer, as well as for osteoporosis. Over the past two decades, since the first paper describing osteonecrosis of the jaw (ONJ) was published, researchers have tried to elucidate the underlying biological mechanism for this disease[1].

Genetic predisposition for MRONJ

Initially described as bisphosphonate-related osteonecrosis of the jaws (BRONJ), the disease was recently renamed MRONJ by the American Academy of Oral Surgeons (AAOMS;

2014 position paper) due to the discovery that the RANKL inhibitor denosumab and new anti-angiogenic therapies have also been found to trigger ONJ[2]. Thus far, the jaws are exclusively involved with this process of soft tissue breakdown, bony exposure, and secondary infection.

MRONJ is a clinical diagnosis based on signs and symptoms[3–6]. The incidence can vary widely, ranging from 0% to 16%[5,7–9]. Numerous studies have examined the role of systemic diseases, radiotherapy, corticosteroids, associated anti-angiogenic drugs, oral surgical procedures, and genetic variability as potential risk factors[4–6,8,10–12].

Many hypotheses have been raised concerning the pathogenesis of MRONJ. While the cause is currently unknown, pharmacogenetic studies have been developed to determine whether genetic differences influence the variability in patient responses to these drugs. Many genome-wide association studies (GWAS), candidate gene studies (CGS), and more recently whole genome/whole exome studies (WGS/WES) have been performed with the aim of better elucidating these genetic differences[13–15].

These types of genetic study utilize single nucleotide polymorphisms (SNPs). SNPs are single nucleotide changes in the DNA sequence that occur in the human genome. There are roughly 10 million present within the human genome[16]. They are typically located in the DNA between genes, causing minimal to no impact on the generalized phenotype of the organism. When they do occur within the gene or the regulatory regions nearby, they can affect transcription patterns and gene function. They have been theorized to serve as risk predictors for the responses of individuals to certain drugs and for the development of several diseases, including (most recently) nephrolithiasis, Alzheimer's disease, psoriasis, and tuberculosis[17–20]. The identification of reliable associated SNPs for MRONJ, while not directly indicative of causation, may lead to a better understanding of the pathophysiology of this disease, and also to better diagnostic tests that can predict the development of this debilitating disease.

The purpose of this systematic review was to investigate putative genetic markers and their association with the development of MRONJ in patients taking bisphosphonates, incorporating recent papers that have been published since the last systematic review on this subject matter.

Materials and methods

Literature search strategy and selection criteria

A systematic review based on the Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) guidelines was used[21]. A search strategy was developed and a comprehensive search of the major databases was conducted (PubMed, Web of Science, ScienceDirect, and Scopus) to find all pertinent articles published on this topic from January 2003 through July 2018. Only articles published in English were considered.

The PICOS strategy (population, intervention, comparison, outcomes, study type) was used to develop the inclusion criteria: the population (P) consisted of patients who had received a diagnosis of MRONJ according to the AAOMS criteria; the intervention (I) was the use of bisphosphonates administered intravenously (IV) or orally (PO) for the treatment of an underlying malignancy or condition; the comparison (C) was done with patients using bisphosphonates without any development of MRONJ and/or healthy patients without a history of either bisphosphonate use or a diagnosis of MRONJ; the outcomes (O) were a genetic association between bisphosphonate use and MRONJ measured through a statistically significant P-value ($P < 0.05$) and an odds ratio (OR) with a 95% confidence interval (CI); study types (S) were clinical human studies evaluating the genetic predisposition for MRONJ development using CGS, GWAS, or WGS/WES, including randomized controlled trials, controlled clinical trials, retrospective studies, and case–control studies.

The following exclusion criteria were applied: case reports, technical reports, animal or in vitro studies, review articles, studies not published in English, studies that did not report the data required to perform a systematic review (P-values/ORs), and studies that did not assess SNPs as the basis of comparison.

Data extraction and analysis

Data were extracted independently by the review authors. The following information was extracted from each study: first author and year of publication; genetic study design; primary disease diagnosis; demographic characteristics of case (ONJ) and control (bisphosphonate-using and/or healthy) patients; sample size; populations from which case and control patients were selected; type of bisphosphonate(s) used; SNPs analyzed; gene and chromosome location of SNPs; and relevant statistical data (P-values and ORs).

The analysis in each study was performed using SNP frequency mean values, linkage disequilibrium (for WGS/WES), and the OR between cases and controls for those SNPs being more or less prevalent in the presence of MRONJ.

Most included studies were at moderate risk of selection bias through selecting a subset of case patients from a treatment facility, group, or country and then matching the controls based on the case population. Thus, these are subsets of patients that may not be representative of the disease population as a whole[22]. It must be considered that any reported variations could be population-specific variations rather than an overarching finding. Additionally, only articles published in English were considered, leaving the possibility for selection bias on the part of the authors.

Results

Literature search strategy and selection criteria

The primary database searches yielded 376 studies, with three records supplied from outside sources. Of these, 281 remained after duplicates were removed. After the evaluation of the abstracts and titles, 248 articles were removed, leaving 33 articles. Of these 33 articles, 18 did not meet the inclusion criteria, leaving 15 articles for qualitative analysis (Fig. 1).

Data extraction and analysis

Studies varied in their focus in terms of SNPs analyzed. The GWAS analyzed numerous SNPs (Sarasquete et al., 500,568 SNPs; Nicoletti et al., 731,422 SNPs) and reported only those that achieved statistical significance[23,24]. Sarasquete et al. utilized the Affymetrix GeneChip Mapping system, whereas Nicoletti et al. utilized the Illumina OmniExpress BeadChip for SNP analysis[23,24]. These GWAS, particularly that by Sarasquete et al., led other investigators to evaluate SNP rs1934951 in gene *CYP2C8* in an attempt to reproduce its significant association with MRONJ, with mixed results[25–27].

The CGS as a whole analyzed from one gene to multiple genes and from one SNP to multiple SNPs within each target gene. Most CGS used the TaqMan SNP genotyping assay to amplify and quantify the SNPs. Analysis focused primarily on the comparison of SNP frequency in case patients relative to either bisphosphonate

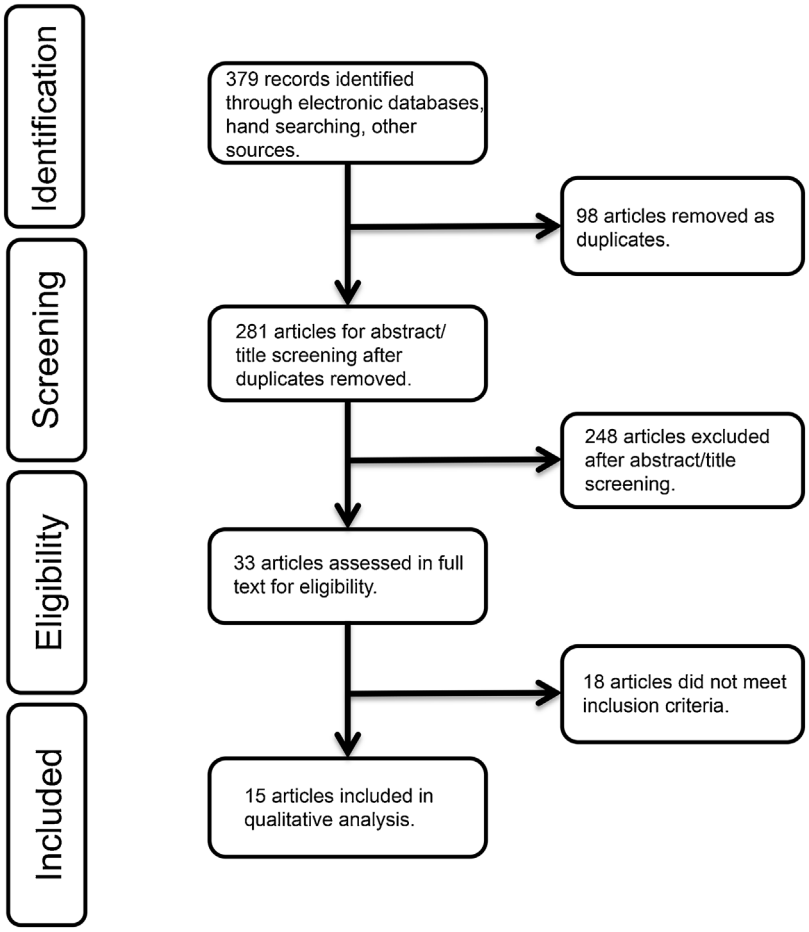

Fig. 1. Flowchart of the PRISMA literature search strategy.

controls, healthy controls, or both. There was no uniformity among the 10 CGS when it came to the control groups that were evaluated.

The WGS/WES utilized the Illumina OMNI SNP chip microarray (Kim et al.), a Gene Expression Omnibus profile (Sun et al.), or the Human Agilent exome kit (Yang et al.)[28–30]. Each focused on a different number of genes and took different approaches to the data analysis. Kim et al. and Sun et al. chose to construct protein function networks and enrichment analyses, whereas Yang et al. focused on allele frequency and linkage disequilibrium for their analysis[28–30]. Refer to Tables 1–3 for the description of the genotyping and amplification systems utilized.

A total of 3301 patients were enrolled in the 15 included studies (two GWAS, $n = 1877$; 10 CGS, $n = 1195$; three WGS/WES, $n = 229$). Several studies had incomplete demographic information, due to the following: not reporting demographic information, reporting the initial demographic data as a combination of case and controls, or reporting initial demographic information but then excluding patients, thus rendering reported values not indicative of the studied sample populations. The entire reported patient population was 56.7% female and 43.3% male. Multiple myeloma was the most prevalent underlying disease (54.8%), followed by breast cancer (17.9%) and prostate cancer (17.4%). Zoledronate was prescribed in 68.8% of patients and was the most commonly used medication, followed by pamidronate (14.0%) and alendronate (6.8%). See Table 4 for complete listing of demographic information for the case and control patients in all included studies.

No one SNP was definitively identified as a risk factor for the development of MRONJ. However, several SNPs were hypothesized to be partially contributory and achieved significant *P*-values within the individual papers. Within GWAS (Table 1), Sarasquete et al. found the SNP rs1934951 (gene *CYP2C8*) to be significant (corrected *P*-value = 0.02; OR 12.75, 95% CI 3.7–43.5)[23]. However, this was not definitively corroborated by subsequent candidate gene studies (Table 2). English et al. ($P = 0.47$; OR 0.63, 95% CI 0.165–2.42) and Such et al. ($P = 0.13$) both found insignificant evidence for rs1934951 being contributory[25,26]. Nicoletti et al. found SNP rs17024608 within gene *RBMS3* to be significantly correlated among MRONJ case patients ($P = 7.47 \times 10^{-8}$), with an increased OR of 5.8 (95% CI 3.0–11.0)[24]. This particular SNP was not mentioned in the other studies analyzed in this review.

Balla et al. looked at SNP rs1934951 within the *CYP2C8* gene and found significance ($P = 0.02$) and a 19.2-fold OR for developing mandibular osteonecrosis[27]. Thus, including Sarasquete et al., two studies have found significance and two have failed to do so. Three of the four studies looked at European populations of mixed sex, whereas English et al. looked only at 'American men'. They all assessed different primary diseases as well: Sarasquete et al. and Such et al. looked only at multiple myeloma patients, English et al. assessed only prostate cancer, and Balla et al. looked at six different primary diseases. These results indicate that perhaps rs1934951 may not be significant, but further testing with more demographically similar case–control groups will be beneficial going forward.

For the other candidate gene studies, Katz et al. looked at SNPs located within five genes that are suspected to have roles related to osteoclastogenesis, differentiation, bone resorption, and bone mineral density. They were unable to find any statistical significance individually. However, when all five SNPs were combined into one ''genotype score ≥ 5'', they found that the presence of all five SNPs conferred a significant risk for the development of MRONJ ($P = 0.0097$), with an 11.20-fold OR (95% CI 1.80–69.86)[31].

Arduino et al. assessed three SNPs within the vascular endothelial growth factor (*VEGF*) gene. They did not find any significant difference in genotype distribution of the three SNPs between the case and bisphosphonate control patients ($P = 0.40$, $P = 0.78$, $P = 0.86$)[32]. No effect from age, sex, primary disease, or duration of therapy was found. They proceeded with a haplotype analysis and again were unable to find data that were significant when the *P*-values were corrected[32].

Di Martino et al. found that peroxisome proliferator-activated receptor gamma (*PPARG*) gene SNP rs1152003 was more prevalent among multiple myeloma patients treated with zoledronate who developed MRONJ relative to multiple

Table 1. Summary of genome-wide association studies (GWAS).

Author/year	Population	Underlying disease	BP type	Cases (*n*)	Controls (*n*)	Genotyping	SNP	Gene	Chr	*P*-value	*Pc*	OR (95% CI)
Sarasquete et al., 2008[23]	Spanish	MM	PM or ZOL planned for 2 years	22	65 BP controls	Affymetrix GeneChip Mapping, 500 K set; 500,568 SNPs analyzed	rs1934951	CYP2C8	10	1.07×10^{-6}	0.02	12.75 (3.7–43.5)
							rs1934980	CYP2C8	10	4.23×10^{-6}	0.09	13.88 (4.0–46.7)
							rs1341162	CYP2C8	10	6.22×10^{-6}	0.13	13.27 (3.5–49.9)
							rs17110453	CYP2C8	10	2.15×10^{-5}	0.46	10.2 (3.2–32.1)
Nicoletti et al., 2012[24]	Northwestern, Southern, Eastern European descent	OP, BC	IV ZOL for majority	30	17 BP controls, 1743 population controls	Illumina Human OmniExpress 12v1.0 BeadChip; 731,422 SNPs analyzed	rs17024608	RBMS3	3	7.47×10^{-8}	Not listed	5.8 (3.0–11.0)
							rs5768434	FAM19A5	22	1.17×10^{-7}		12.6 (4.9–32.2)
							rs11064477	PHB2	12	5.16×10^{-7}		21.7 (6.5–71.9)
							12-7016684	C1S	12	5.85×10^{-7}		21.1 (6.4–69.8)
							8-58133986	IMPAD1	8	3.10×10^{-6}		7.3 (3.1–16.9)
							rs1886629	KCNT2	1	5.53×10^{-6}		3.6 (2.1–6.5)
							rs7588295	CSRNP3	2	6.24×10^{-6}		8.6 (3.3–22.17)
							rs4431170	MARCH1	4	7.28×10^{-6}		5.1 (2.5–10.6)
							rs7740004	C6orf170	6	7.87×10^{-6}		5.9 (2.7–13.0)
							rs11189381	SFRP5	10	8.17×10^{-6}		6.8 (2.9–15.8)
							rs12903202	ALDH1A2	15	9.15×10^{-6}		4.0 (2.1–7.4)
							rs17751934	MEX3C	18	9.16×10^{-6}		5.0 (2.4–10.1)
							11-23990403	LUZP2	11	$9.94 \times 10-6$		12.7 (4.0–36.8)
							rs1678387	ABCC4	13	2.0×10^{-5}		5.3 (2.4–11.4)
							rs11934877	IGFBP7	4	0.0002		2.9 (1.6–5)

BC, breast cancer; BP, bisphosphonate; Chr, chromosome; CI, confidence interval; IV, intravenous; MM, multiple myeloma; OP, osteoporosis; OR, odds ratio; *Pc*, Bonferroni corrected *P*-value; PM, pamidronate; SNP, single nucleotide polymorphism; ZOL, zoledronate.

myeloma patients treated with zoledronate who did not develop MRONJ, with a 31.5-fold OR ($P = 0.0055$; 95% CI 2.31–422.32) and hypothesized a contributory link[33].

Marini et al. analyzed one SNP, rs2297480 A/C polymorphism, within the farnesyl pyrophosphate synthase (*FDPS*) gene[34]. Gene variants within *FDPS* had been associated with bone morbidity and sensitivity in a previous study by the same group among a sample of Caucasian women with osteoporosis, leading to this follow-up study looking at Caucasian patients receiving bisphosphonate therapy for multiple myeloma, breast cancer, and prostate cancer. They found that there was a significant association between the rs2297480 A/C polymorphism and ONJ ($P = 0.03$)[34].

La Ferla et al. assessed the contribution of estrogens and their metabolism to osteoclastic inhibition, and found that the aromatase g.132810C > T polymorphism was significantly more prevalent in, but not exclusive to, the MRONJ case patients relative to healthy controls ($P = 0.0439$; OR −2.83)[35]. This allele leads to higher aromatase activity and higher bone mineral density, and was found in less than 40% of the ONJ group and 20% of the bisphosphonate control group. Thus, they hypothesized that the presence of the TT allele merely increased the risk of ONJ and was not causative.

Stockmann et al. assessed the association of major histocompatibility complex (MHC) class II polymorphisms with MRONJ among multiple myeloma, breast cancer and prostate cancer patients taking IV bisphosphonates[36]. They found that, in addition to individual MHC II genes conferring increased odds of developing MRONJ, the HLA-DRB1*15-DQB1*06:02 haplotype specifically was significantly associated with the development of ONJ, conferring a 2.5-fold OR (95% CI 1.3–5.0). They additionally found that the presence of at least one of the haplotypes was highly associated with ONJ (OR 3.0, 95% CI 1.7–5.5), and suggested that MCH class II polymorphisms could represent genetic risk factors for the development of MRONJ[36].

The most recent CGS by Choi et al. looked at the same three SNPs within the *VEGF* gene that Arduino et al. had also analyzed and had found not significantly associated[32,37]. They also were unable to find a significant association between ONJ and either the rs699947 ($P = 0.126$; OR 0.5, 95% CI 0.23–1.38) or rs2010963 haplotypes between cases and controls ($P = 0.208$; OR 1.9, 95% CI 0.82–4.52).

Table 2. Summary of candidate gene studies (CGS).

Author/year	Population	Underlying disease	BP type	Cases (*n*)	Controls (*n*)	Genotyping	SNP	Gene	Chr	*P*-value	OR (95% CI)
English et al., 2010[25]	American men	PC	Most had IV ZOL (4 mg)	17	83 BP controls	Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1	rs1934951	CYP2C8	10	0.47	0.63 (0.165–2.42)
			IBA, ZOL + AL, ZOL + PM								
Such et al., 2011[26]	Spanish, Greek	MM	IV ZOL	42	37 BP controls, 45 population controls	TaqMan SNP genotyping assay	rs1934951	CYP2C8	10	0.13	Not reported
Katz et al., 2011[31]	American	MM	IV ZOL 4 mg, or IV PM 90 mg	12	66 BP controls	TaqMan SNP genotyping assay, pyrosequencing	rs1800012	COL1A1	17	0.55	1.69 (0.30–9.70)
							rs12458117	RANK	18	0.38	2.14 (0.39–11.71)
							rs243865	MMP2	16	0.11	3.49 (0.75–16.18)
							rs2073618	OPG	8	0.38	2.16 (0.38–12.23)
							rs11730582	OPN	4	0.21	2.97 (0.53–16.55)
Arduino et al., 2011[32]	Italian females	BC, MM	IV ZOL 4 mg over 15 min monthly	30	30 BP, 125 healthy controls	TaqMan	rs3025039	VEGF	6	0.40	0.57 (0.21–1.54)
							rs699947	VEGF	6	0.78	0.99 (0.31–3.18)
							rs2010963	VEGF	6	0.86	0.96 (0.37–2.53)
Di Martino et al., 2011[33]	Italian	MM	IV ZOL	9	10 BP controls	DMET Plus GeneChip, Affymetrix DMET Console	rs1152003	PPARG	3	0.0055	31.5 (2.31–422.32)
							rs10893	ABP1	7	0.023	Not reported
							rs4725373	ABP1	7	0.023	Not reported
							rs1049793	ABP1	7	0.023	Not reported
							rs2463437	CHST11	12	0.0198	Not reported
							rs903247	CHST11	12	0.0198	Not reported
							rs2468110	CHST11	12	0.0198	Not reported
							rs2097937	CROT	7	0.0198	Not reported
Marini et al., 2011[34]	Italian	MM, BC, PC	IV ZOL 4 mg	34	34 BP controls	GoTaq	rs2297480	FDPS	1	0.03	Not reported
Balla et al., 2012[27]	Hungarian	MM, OP, BC, PC, RC, CC	AL, PM, ZOL, IBA, RIS, CLO via IV, PO, or both	46	224 healthy controls	TaqMan SNP genotyping assay	rs1934951	CYP2C8	10	0.02 (AG genotype)	19.2× risk mandibular ONJ
La Ferla et al., 2012[35]	Italian	MM, BC, PC	IV ZOL	30	53 BP controls	TaqMan SNP genotyping assay, AmpliTaq Gold	rs10046	CYP19A1	15	0.0439	−2.83
							rs2234693	ESR1	6	>0.05	
							rs9340799	ESR1	6	>0.05	
Stockmann et al., 2013[36]	German	MM, BC, PC	IV ZOL, PM	94	110 BP controls	QIAamp DNA Mini Kit, single strand oligonucleotide kit	NA	HLA-DRB1*15	NA	0.014	2.3 (1.2–4.4)

Table 2 (*Continued*)

Author/year	Population	Underlying disease	BP type	Cases (*n*)	Controls (*n*)	Genotyping	SNP	Gene	Chr	*P*-value	OR (95% CI)
Choi et al., 2015[37]	South Korean	OP, RA	AL, IBA, RIS, ZOL	45	19 BP controls	DNA Link Inc.	NA	HLA-DRB1*01	NA	0.049	2.0 (0.99–4.1)
							rs699947	VEGF	5	0.126	0.5 (0.23–1.38)
							rs2010963	VEGF	5	0.208	1.9 (0.82–4.52)

AL, alendronate; BC, breast cancer; BP, bisphosphonate; CC, cervical cancer; Chr, chromosome; CI, confidence interval; CLO, clodronate; IBA, ibandronate; IV, intravenous; MM, multiple myeloma; NA, not applicable; ONJ, osteonecrosis of the jaws; OP, osteoporosis; OR, odds ratio; PC, prostate cancer; PM, pamidronate; PO, orally; RA, rheumatoid arthritis; RC, renal cancer; RIS, risedronate; SNP, single nucleotide polymorphism; ZOL, zoledronate.

Table 3. Summary of whole genome and whole exome studies (WGS/WES).

Author/year	Population	Underlying disease	BP type	Cases (*n*)	Controls (*n*)	Genotyping	SNP	Gene	Chr	*P*-value	OR (95% CI)
Kim et al. 2015[28]	South Korean	Not reported	AL, ZOL, RIS	16	126 healthy controls	FastQC v0.10.1 OMNI 2.5M SNP chip	NA	3819 genes	NA	NA	NA
Sun et al. 2015[29]	GEO database	MM	Not specified	11	10 BP controls, 5 healthy controls	Gene expression profile GSE7116 was downloaded from GEO database	NA	GSE7116 gene expression profile	NA	NA	NA
Yang et al. 2018[30]	American, Hungarian, Italian	MM, BC, PC, RC, CC	IV ZOL, PM	22 MM patients, 17 BC, CC, PC, RC patients with solid tumors	22 BP MM age/sex matched controls	Human Agilent v5,51 Mb exome kit, Illumina HiSeq 2500 sequencing	rs7896005	SIRT1	10	0.0052	0.07 (0.01–0.46)
							rs3758392	HERC4	10	0.0052	0.07 (0.01–0.46)
							rs10070440	SV2C	5	0.0143	11.68 (1.64–83.36)
							rs11938792	TBCK	4	0.0328	20.38 (1.28–324.20)

AL, alendronate; BC, breast cancer; BP, bisphosphonate; CC, cervical cancer; Chr, chromosome; CI, confidence interval; GEO, Gene Expression Omnibus; IV, intravenous; MM, multiple myeloma; NA, not applicable; OR, odds ratio; PC, prostate cancer; PM, pamidronate; RC, renal cancer; RIS, risedronate; SNP, single nucleotide polymorphism; ZOL, zoledronate.

They then looked at the genotype differences between cases and controls, and found that the rs2010963-GC co-dominant and -CC recessive genotypes were more significantly associated with MRONJ in the case patients as compared to the controls ($P = 0.03$ and $P = 0.04$, respectively)[37]. Thus, as these results differ from those found by Arduino et al., they went on to hypothesize that the differences they found were likely due to population-based genetic differences in *VEGF* expression.

Among WGS/WES (Table 3), Kim et al. found a significant difference in the expression of genes associated with post-translational modifications in ONJ patients and hypothesized that these expression modifications led to the impairment of osteoclast cell morphology and adhesion, interfering with function[28]. Sun et al. found that genes *TNF*, *IL1B*, and *DDX5* and a transcription factor of XBP1 appeared to be more down-regulated in MRONJ patients than in either the bisphosphonate controls or the healthy controls, highlighting potential molecular markers for bisphosphonate-related ONJ[29]. Most recently, Yang et al. found significant associations between two SNPs located within the same *SIRT1/HERC4* locus (rs7896005 and rs3758392, respectively) and decreased odds of developing ONJ among patients treated with bisphosphonates[30].

Please refer to Table 4 for a summary of all demographic parameters compiled, and to Table 5 for a comprehensive summary of all of the studies included in this systematic review.

Discussion

The aim of this systematic review was to find a definitive genetic marker, or markers, to predict the development of MRONJ. The development of accurate and reliable MRONJ risk assessment tests would be beneficial, as physicians could educate, treat, and even potentially prevent this medication complication in their patients. It has been suggested that genetic variation between individuals, as well as their underlying systemic disease, may increase or decrease the MRONJ risk[5,14,38].

GWAS allow the investigation of disease caused by genetic and drug response through the genotyping of over one million SNPs present in DNA, and has been shown to be a relatively unbiased method to study the incidence of MRONJ. They have a case–control design, more directly attributing a risk factor to a disease if the frequency of the minor allele in case subjects is significantly different to that in controls[39–41]. WGS/WES are similar to this, using sequencing of either the genome or exome and assessing variation between the case subjects and the controls. CGS investigate potentially relevant genes, screening fewer variants than GWAS, and approach their analyses with a specific hypothesis or hypotheses. The results do not carry much power without replication in subsequent studies[42]. Therefore, if results cannot be replicated for a particular SNP, such as *CYP2C8* rs1934951 in this review, it is challenging to draw definitive conclusions about the presence of an association.

The last meta-analysis on this topic was conducted by Zhong et al. in 2012 and focused on the *CYP2C8* SNP rs1934951. They commented on the differences in study population demographics and how they excluded the healthy controls from Balla et al. and Such et al. to create more matched case and control cohorts[43]. They were additionally unable to conclude that rs1934951 was significantly associated with the development of MRONJ. Since 2012, no further studies have been conducted analyzing *CYP2C8*. There are numerous potential genes that warrant deeper analysis to look at significance. However, as previously mentioned, Choi et al. concluded that there were likely genetic differences linked to different study populations, at least for the *VEGF* polymorphisms associated with MRONJ[37]. Therefore, associations linking *CYP2C8* to MRONJ may exist, but different popu-

Table 4. Demographics of case (MRONJ) and control patients in all included studies.

Definition	Case subjects (*n*)	Bisphosphonate control subjects (*n*)	Healthy control subjects (*n*)	Total (%)
Number of combined subjects	477	556	2268	3301
Sex[a]				
Male	77	164	109	350 (43.3)
Female	173	143	142	458 (56.7)
Total				808 (100)
Primary disease[a]				
Multiple myeloma	161	248		409 (54.8)
Prostate cancer	36	94		130 (17.4)
Breast cancer	74	60		134 (17.9)
Osteoporosis	36	19		55 (7.4)
Other	11	8		19 (2.5)
Total				747 (100)
Bisphosphonates used[a]				
Zoledronate	260	275		535 (68.8)
Pamidronate	38	71		109 (14.0)
Ibandronate	24	6		30 (3.8)
Alendronate	43	10		53 (6.8)
Risedronate	8	2		10 (1.3)
Clodronate	3	0		3 (0.4)
Zoledronate + alendronate	1	2		3 (0.4)
Zoledronate + pamidronate	1	1		2 (0.3)
Zoledronate → pamidronate	6	15		21 (2.7)
Pamidronate → zoledronate	2	10		12 (1.5)
Total				778 (100)

MRONJ, medication-related osteonecrosis of the jaws.
[a] Information was not uniformly specified or reported across all studies.

Table 5. Summary of all studies.

Author/year	Study type	Underlying disease	BP type	Cases (*n*)	Controls (*n*)	SNP	Gene	Chr	*P*-value	OR (95% CI)
Arduino et al. 2011[32]	Candidate gene	BC, MM	IV ZOL 4 mg over 15 min monthly	30	30 BP, 125 healthy controls	rs3025039	VEGF	6	0.40	0.57 (0.21–1.54)
						rs699947	VEGF	6	0.78	0.99 (0.31–3.18)
						rs2010963	VEGF	6	0.86	0.96 (0.37–2.53)
Balla et al. 2012[27]	Candidate gene	MM, OP, BC, PC, RC, CC	AL, PM, ZOL, IBA, RIS, CLO via IV, PO, or both	46	224 healthy controls	rs1934951	CYP2C8	10	0.02 (AG genotyp)	19.2× risk mandibular ONJ
Choi et al. 2015[37]	Candidate gene	OP, RA	AL, IBA, RIS, ZOL	45	19 BP controls	rs699947	VEGF	5	0.126	0.5 (0.23–1.38)
						rs2010963	VEGF	5	0.208	1.9 (0.82–4.52)
Di Martino et al. 2011[33]	Candidate gene	MM	IV ZOL	9	10 BP controls	rs1152003	PPARG	3	0.0055	31.5 (2.31–422.32)
						rs10893	ABP1	7	0.023	Not reported
						rs4725373	ABP1	7	0.023	Not reported
						rs1049793	ABP1	7	0.023	Not reported
						rs2463437	CHST11	12	0.0198	Not reported
						rs903247	CHST11	12	0.0198	Not reported
						rs2468110	CHST11	12	0.0198	Not reported
						rs2097937	CROT	7	0.0198	Not reported
English et al. 2010[25]	Candidate gene	PC	Most had IV ZOL (4 mg) IBA, ZOL + AL, ZOL + PM	17	83 BP controls	rs1934951	CYP2C8	10	0.47	0.63 (0.165–2.42)
Katz et al. 2011[31]	Candidate gene	MM	IV ZOL 4 mg, or IV PM 90 mg	12	66 BP controls	rs1800012	COL1A1	17	0.55	1.69 (0.30–9.70)
						rs12458117	RANK	18	0.38	2.14 (0.39–11.71)
						rs243865	MMP2	16	0.11	3.49 (0.75–16.18)
						rs2073618	OPG	8	0.38	2.16 (0.38–12.23)
						rs11730582	OPN	4	0.21	2.97 (0.53–16.55)
Kim et al. 2015[28]	Whole exome	Not reported	AL, ZOL, RIS	16	126 healthy controls	NA	3819 genes	NA	NA	NA
La Ferla et al. 2012[35]	Candidate gene	MM, BC, PC	IV ZOL	30	53 BP controls	rs10046	CYP19A1	15	0.0439	−2.83
						rs2234693	ESR1	6	>0.05	
						rs9340799	ESR1	6	>0.05	
Marini et al. 2011[34]	Candidate gene	MM, BC, PC	IV ZOL 4 mg	34	34 BP controls	rs2297480	FDPS	1	0.03	Not reported
Nicoletti et al. 2012[24]	GWAS	OP, BC	IV ZOL for majority	30	17 BP controls, 1743 population controls	rs17024608	RBMS3	3	$7.47 \times 10-8$	5.8 (3.0–11.0)
						rs5768434	FAM19A5	22	$1.17 \times 10-7$	12.6 (4.9–32.2)
						rs11064477	PHB2	12	$5.16 \times 10-7$	21.7 (6.5–71.9)
						12-7016684	C1S	12	$5.85 \times 10-7$	21.1 (6.4–69.8)
						8-58133986	IMPAD1	8	$3.10 \times 10-6$	7.3 (3.1–16.9)
						rs1886629	KCNT2	1	$5.53 \times 10-6$	3.6 (2.1–6.5)
						rs7588295	CSRNP3	2	$6.24 \times 10-6$	8.6 (3.3–22.17)
						rs4431170	MARCH1	4	$7.28 \times 10-6$	5.1 (2.5–10.6)
						rs7740004	C6orf170	6	$7.87 \times 10-6$	5.9 (2.7–13.0)
						rs11189381	SFRP5	10	$8.17 \times 10-6$	6.8 (2.9–15.8)
						rs12903202	ALDH1A2	15	$9.15 \times 10-6$	4.0 (2.1–7.4)
						rs17751934	MEX3C	18	$9.16 \times 10-6$	5.0 (2.4–10.1)
						11-23990403	LUZP2	11	$9.94 \times 10-6$	12.7 (4.0–36.8)
						rs1678387	ABCC4	13	$2.0 \times 10-5$	5.3(2.4–11.4)
						rs11934877	IGFBP7	4	0.0002	2.9 (1.6–5)

Sarasquete et al. 2008[23]	GWAS	MM	PM or ZOL planned for 2 years	22	65 BP controls	rs1934951	CYP2C8	10	$1.07 \times 10{-}6$	12.75 (3.7–43.5)
						rs1934980	CYP2C8	10	$4.23 \times 10{-}6$	13.88 (4.0–46.7)
						rs1341162	CYP2C8	10	$6.22 \times 10{-}6$	13.27 (3.5–49.9)
						rs17110453	CYP2C8	10	$2.15 \times 10{-}5$	10.2 (3.2–32.1)
Stockmann et al. 2013[36]	Candidate gene	MM, BC, PC	IV ZOL, PM	94	110 BP controls	NA	HLA-DRB1*15	NA	0.014	2.3 (1.2–4.4)
						NA	HLA-DRB1*01	NA	0.049	2.0 (0.99–4.1)
Such et al. 2011[26]	Candidate gene	MM	IV ZOL	42	37 BP controls, 45 population controls	rs1934951	CYP2C8	10	0.13	Not reported
Sun et al. 2015[29]	Whole genome	MM	Not specified	11	10 BP controls, 5 healthy controls	NA	GSE7116 Gene Expression Profile	NA	NA	NA
Yang et al. 2018[30]	Whole exome	MM, BC, PC, RC, CC	IV ZOL, PM	22 MM; 17 BC, PC, RC, CC	22 BP MM age/sex matched controls	rs7896005	SIRT1	10	0.0052	0.07 (0.01–0.46)
						rs3758392	HERC4	10	0.0052	0.07 (0.01–0.46)
						rs10070440	SV2C	5	0.0143	11.68 (1.64–83.36)
						rs11938792	TBCK	4	0.0328	20.38 (1.28–324.20)

AL, alendronate; BC, breast cancer; BP, bisphosphonate; CC, cervical cancer; Chr, chromosome; CI, confidence interval; CLO, clodronate; GWAS, genome-wide association studies; IBA, ibandronate; IV, intravenous; MM, multiple myeloma; NA, not applicable; ONJ, osteonecrosis of the jaws; OP, osteoporosis; OR, odds ratio; PC, prostate cancer; PM, pamidronate; PO, orally; RA, rheumatoid arthritis; RC, renal cancer; RIS, risedronate; SNP, single nucleotide polymorphism; ZOL, zoledronate.

lations need to be tested. This potentially holds true for all of the genes that have been tested in the previous studies. As genetic analyses become more feasible and accessible to researchers, these polymorphisms might be revisited and examined within several populations to be able to say with more certainty whether an association with MRONJ exists.

One limitation of this review was the inability to retrieve complete demographic records for several of the studies. Eight of the 15 studies did not have complete demographic information; demographics were either reported as combined between cases and controls or were reported before exclusion criteria were applied. This rendered the cumulative demographic statistics challenging to use for future analysis, and of questionable significance due to poorly matched case–control groups.

The implication of this study, for researchers and clinicians alike, is that MRONJ is a complex, likely multifactorial condition that still warrants further research in order to discover associated genetic biomarkers. More studies, especially GWAS and WGS/WES, would allow the discovery of novel associations and the corroboration of previously noted significant SNPs. If certain SNPs are shown to be consistently correlated, then there is potential for future targeted therapies, assuming their function is known. These targeted therapies could range from pharmacological treatments for MRONJ to more individualized patient care. Patients at risk could be more closely monitored throughout treatment, or given alternative therapeutic options.

In conclusion, GWAS, CGS, and WGS/WES to date have all failed to show a single gene as a risk factor for the development of MRONJ, although several genes have shown potential increased or decreased association. The limitation in the number of base pairs analyzed in the genotyping and the use of preexisting reference SNPs may have contributed to this. The heterogeneity of the case and control populations across the 15 studies may additionally be contributory, as there may be separate genetic markers denoting MRONJ risk across different populations and with distinct diagnoses. Going forward, increasing the number of next generation sequencing studies may help to further elucidate the role and interplay of genetic events in the development of MRONJ. Repetition with different study populations would help to strengthen the association of specific SNPs by highlighting the possible pathophysiological links between the related genes and MRONJ

and targeting areas to identify for diagnosis, treatment, and/or prevention.

Competing interests

The authors do not profess any conflicts of interest.

Ethical approval

Not required.

Patient consent

Not required.

Funding

This work was funded in part by the Massachusetts General Hospital Department of Oral and Maxillofacial Surgery Education and Research Fund and supported by the Center for Applied Clinical Investigation (CACI). These parties had no say in any aspect of the study design or manuscript preparation.

References

1. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. *J Oral Maxillofac Surg* 2003;**61**:1115–7.
2. Ruggiero SL, Dodson TB, Fantasia J, Goodday R, Aghaloo T, Mehrotra B, O'Ryan F. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw—2014 update. *J Oral Maxillofac Surg* 2014;**72**:1938–56.
3. Khan AA, Morrison A, Hanley DA, Felsenberg D, McCauley LK, O'Ryan F, Reid IR, Ruggiero SL, Taguchi A, Tetradis S, Watts NB, Brandi ML, Peters E, Guise T, Eastell R, Cheung AM, Morin SN, Masri B, Cooper C, Morgan SL, Obermayer-Pietsch B, Langdahl BL, Al Dabagh R, Davison KS, Kendler DL, Sándor GK, Josse RG, Bhandari M, El Rabbany M, Pierroz DD, Sulimani R, Saunders DP, Brown JP, Compston J, International Task Force on Osteonecrosis of the Jaw. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. *J Bone Miner Res* 2015;**30**:3–23.
4. Ikebe T. Pathophysiology of BRONJ: drug-related osteoclastic disease of the jaw. *Oral Sci Int* 2013;**10**:1–8.
5. Dodson TB. The frequency of medication-related osteonecrosis of the jaw and its associated risk factors. *Oral Maxillofac Surg Clin North Am* 2015;**27**:509–16.
6. Campisi G, Fedele S, Fusco V, Pizzo G, Di Fede O, Bedogni A. Epidemiology, clinical manifestations, risk reduction and treatment strategies of jaw osteonecrosis in cancer patients exposed to antiresorptive agents. *Future Oncol* 2014;**10**:257–75.
7. Wang EP, Kaban LB, Strewler GJ, Raje N, Troulis MJ. Incidence of osteonecrosis of the jaw in patients with multiple myeloma and breast or prostate cancer on intravenous bisphosphonate therapy. *J Oral Maxillofac Surg* 2007;**65**:1328–31.
8. Assaf AT, Smeets R, Riecke B, Weise E, Gröbe A, Blessmann M, Steiner T, Wikner J, Friedrich RE, Heiland M, Hoelzle F, Gerhards F. Incidence of bisphosphonate-related osteonecrosis of the jaw in consideration of primary diseases and concomitant therapies. *Anticancer Res* 2013;**33**:3817–924.
9. Kajizono M, Sada H, Sugiura Y, Soga Y, Kitamura Y, Matsuoka J, Sendo T. Incidence and risk factors of osteonecrosis of the jaw in advanced cancer patients after treatment with zoledronic acid or denosumab: a retrospective cohort study. *Biol Pharm Bull* 2015;**38**:1850–5.
10. McLeod NMH, Brennan PA, Ruggiero SL. Bisphosphonate osteonecrosis of the jaw: a historical and contemporary review. *Surgeon* 2012;**10**:36–42.
11. Giovannacci I, Meleti M, Manfredi M, Mortellaro C, Greco Lucchina A, Bonanini M, Vescovi P. Medication-related osteonecrosis of the jaw around dental implants: implant surgery-triggered or implant presence-triggered osteonecrosis? *J Craniofac Surg* 2016;**27**:697–701.
12. Diz P, Limeres J, Fedele S, Seoane J, Diniz M, Feijoo JF. Is oral bisphosphonate-related osteonecrosis of the jaw an endemic condition? *Med Hypotheses* 2012;**78**:315–8.
13. Hamadeh IS, Ngwa BA, Gong Y. Drug induced osteonecrosis of the jaw. *Cancer Treat Rev* 2015;**41**:455–64.
14. Fung PL, Nicoletti P, Shen Y, Porter S, Fedele S. Pharmacogenetics of bisphosphonate-associated osteonecrosis of the jaw. *Oral Maxillofac Surg Clin North Am* 2015;**27**:537–46.
15. Lombard T, Neirinckx V, Rogister B, Gilon Y, Wislet S. Medication-related osteonecrosis of the jaw: new insights into molecular mechanisms and cellular therapeutic approaches. *Stem Cells Int* 2016;**2016**:8768162.
16. Genetics Home Reference: Genomic research. Lister Hill National Center for Biomedical Communications, US National Library of Medicine, National Institutes of Health, Department of Health and Human Services, July 17, 2018.
17. Mitra P, Maity B, Pal DK, Das M. Polymorphisms of PTH (parathyroid hormone) gene and risk of kidney stone disease: a case–control study from West Bengal, India. *Urology* 2018;**121**:79–85.
18. Chen XX, Guo RR, Cao XP, Tan L, Tan L. Alzheimer's disease neuroimaging initiative: the impact of GAB2 genetic variations on cerebrospinal fluid markers in Alzheimer's disease. *Ann Transl Med* 2018;**6**:171.
19. Stawczyk-Macieja M, Szczerkowska-Dobosz A, Re?baa K, Gabig-Cimińska M, Nowicki RJ, Haraś A, Cybulska L, Kapińska E. ERAP1 and HLA-C*06 are strongly associated with the risk of psoriasis in the population of northern Poland. *Postepy Dermatol Alergol* 2018;**35**:286–92.
20. Wu S, Huang W, Wang D, Wang Y, Wang M, Zhang M, He JQ. Evaluation of TLR2, TLR4, and TOLLIP polymorphisms for their role in tuberculosis susceptibility. *APMIS* 2018;**126**:501–8.
21. Moher D, Liberati A, Tetzlaff J. Altman DG; the PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;**6**:e1000097.
22. Kopec JA, Esdaile JM. Bias in case–control studies: a review. *J Epidemiol Community Health* 1990;**44**:179–86.
23. Sarasquete ME, Garcia-Sanz R, Marin L, Alcoceba M, Chillón MC, Balanzategui A, Santamaria C, Rosiñol L, de la Rubia J, Hernandez MT, Garcia-Navarro I, Lahuerta JJ, González M, San Miguel JF. Bisphosphonate-related osteonecrosis of the jaw is associated with polymorphisms of the cytochrome P450 CYP2C8 in multiple myeloma: a genome-wide single nucleotide polymorphism analysis. *Blood* 2008;**112**:2709–12.
24. Nicoletti P, Cartsos VM, Palaska PK, Shen Y, Floratos A, Zavras AI. Genomewide pharmacogenetics of bisphosphonate-induced osteonecrosis of the jaw: the role of RBMS3. *Oncologist* 2012;**17**:279–87.
25. English BC, Baum CE, Adelberg DE, Sissung TM, Kluetz PG, Dahut WL, Price DK, Figg WD. A SNP in CYP2C8 is not associated with the development of bisphosphonate-related osteonecrosis of the jaw in men with castrate-resistant prostate cancer. *Ther Clin Risk Manag* 2010;**6**:579–83.
26. Such E, Cervera J, Terpos E, Bagán JV, Avaria A, Gómez I, Margaix M, Ibiñez M, et al. CYP2C8 gene polymorphism and bisphosphonate-related osteonecrosis of the jaw in patients with multiple myeloma. *Haematologica* 2011;**96**:1557–9.
27. Balla B, Vaszilko M, Kósa JP, Podani J, Takács I, Tóbiás B, Nagy Z, Lazáry Á., Lakatos P. New approach to analyze genetic and clinical data in bisphosphonate-induced osteonecrosis of the jaw. *Oral Dis* 2012;**18**:580–5.
28. Kim JH, Ko YJ, Kim JY, Oh Y, Hwang J, Han S, Kim S, Lee JH, Han DH. Genetic investigation of bisphosphonate-related osteonecrosis of the jaw (BRONJ) via whole exome sequencing and bioinformatics. *PLoS One* 2015;**10**:e 0118084.
29. Sun J, Wen X, Jin F, Li Y, Hu J, Sun Y. Bioinformatics analyses of differentially expressed genes associated with bisphosphonate-related osteonecrosis of the jaw in

patients with multiple myeloma. *Onco Targets Ther* 2015;**8**:2681–8.
30. Yang G, Hamadeh IS, Katz J, Riva A, Lakatos P, Balla B, Kosa J, Vaszilko M, Pelliccioni GA, Davis N, Langaee TY, Moreb JS, Gong Y. SIRT1/HERC4 locus associated with bisphosphonate-induced osteonecrosis of the jaw: an exome-wide association analysis. *J Bone Miner Res* 2018;**33**:91–8.
31. Katz J, Gong Y, Salmasinia D, Hou W, Burkley B, Ferreira P, Casanova O, Langaee TY, Moreb JS. Genetic polymorphisms and other risk factors associated with bisphosphonate induced osteonecrosis of the jaw. *Int J Oral Maxillofac Surg* 2011;**40**:605–11.
32. Arduino PG, Menegatti E, Scoletta M, Battaglio C, Mozzati M, Chiecchio A, Berardi D, Vandone AM, Donadio M, Gandolfo S, Scully C, Broccoletti R. Vascular endothelial growth factor genetic polymorphisms and haplotypes in female patients with bisphosphonate-related osteonecrosis of the jaws. *J Oral Pathol Med* 2011;**40**:510–5.
33. Di Martino MT, Arbitrio M, Guzzi PH, Leone E, Baudi F, Piro E, Prantera T, Cucinotto I, Calimeri T, Rossi M, Veltri P, Cannataro M, Tagliaferri P, Tassone P. A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with zoledronic acid-related osteonecrosis of the jaw in multiple myeloma patients: analysis by DMET microarray profiling. *Br J Haematol* 2011;**154**:525–39.
34. Marini F, Tonelli P, Cavalli L, Cavalli T, Masi. Falchetti A, Brandi ML. Pharmacogenetics of bisphosphonate-associated osteonecrosis of the jaw. *Front Biosci* 2011;**3**:364–70.
35. La Ferla F, Paolicchi E, Crea F, Cei S, Graziani F, Gabriele M, Danesi R. An aromatase polymorphism (g.132810C>T) predicts risk of bisphosphonate-related osteonecrosis of the jaw. *Biomark Med* 2012;**6**:201–9.
36. Stockmann P, Nkenke E, Englbrecht M, Schlittenbauer T, Wehrhan F, Rauh C, Beckmann MW, Fasching PA, Kreusch T, Mackensen A, Wullich B, Schett G, Spriewald BM. Major histocompatibility complex II polymorphisms are associated with the development of anti-resorptive agent-induced osteonecrosis of the jaw. *J Craniomaxillofac Surg* 2013;**41**:71–5.
37. Choi H, Lee JH, Lee JH, Kim JH. Genetic association between VEGF polymorphisms and BRONJ in the Korean population. *Oral Dis* 2015;**21**:866–71.
38. Peer A, Khamaisi M. Diabetes as a risk factor for medication-related osteonecrosis of the jaw. *J Dent Res* 2015;**94**:252–60.
39. Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 2010;**363**:166–76.
40. Easton DF, Eeles RA. Genome-wide association studies in cancer. *Hum Mol Genet* 2008;**17**(R2):109–15.
41. Bush WS, Moore JH. Chapter 11: genome-wide association studies. *PLoS Comput Biol* 2012;**8**:e1002822.
42. Daly AK, Day CP. Candidate gene case–control association studies: advantages and potential pitfalls. *Br J Clin Pharmacol* 2001;**52**:480–99.
43. Zhong DN, Wu JZ, Li GJ. Association between CYP2C8 (rs1934951) polymorphism and bisphosphonate-related osteonecrosis of the jaws in patients on bisphosphonate therapy: a meta-analysis. *Acta Haematol* 2013;**129**:90–5.

Address:
Meredith August
Massachusetts General Hospital
Department of Oral and Maxillofacial Surgery
55 Fruit Street
Warren 1201
Boston
MA 02114
USA
Tel.: +1 617 726 8222; Fax: +1 617 726 6195
E-mail: maugust@partners.org