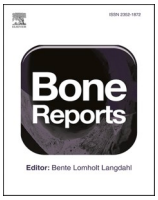
Bone Reports 18 (2023) 101648

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr



Genome-wide association study of osteonecrosis of the jaw in Danish patients receiving antiresorptive therapy for osteoporosis: A case-control study

Alma B. Pedersen [a,b,*], Sven E. Nørholt [c,d], Lars Rejnmark [e,f], Bente Langdal [e,f], Thomas Starch-Jensen [g], Henrik T. Sørensen [a,b]

[a] Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Allé 43-45, 8200 Aarhus N, Denmark
[b] Department of Clinical Medicine, Aarhus University, Olof Palmes Allé 43-45, 8200 Aarhus N, Denmark
[c] Department of Oral and Maxillofacial Surgery, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark
[d] Section of Oral and Maxillofacial Surgery, Department of Dentistry and Oral Health, Aarhus University, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark
[e] Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Palle Juul-Jensens Boulevard 165, 8200 Aarhus N, Denmark
[f] Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Boulevard 165, 8200 Aarhus N, Denmark
[g] Department of Oral and Maxillofacial Surgery, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark

ARTICLE INFO

Keywords:
Antiresorptive treatment
Bisphosphonates
Case control
Genes
Osteonecrosis of the jaw
Pharmacogenomics
Single nucleotide polymorphism

ABSTRACT

Background: Prior studies of the pharmacogenomics of osteonecrosis of the jaw (ONJ) have had various methodological limitations, including using candidate gene selection as their sole strategy, a small number of ONJ cases, or a study population based on an oncology setting.
Objectives: The aim of our case-control study was to evaluate previously reported associations between genetic factors and ONJ, which were based on either genome-wide association studies (GWAS) or candidate gene approaches. Furthermore, we aimed to identify genetic risk factors for ONJ by using GWAS to determine single-nucleotide polymorphisms (SNPs) with statistically significant differences in frequency between ONJ patients and osteoporosis controls.
Methods: Patients with medically confirmed ONJ and who were registered in the Scandinavian Cohort of ONJ patients were included. Controls from the general population were matched on age (±5 years), sex, and cumulative antiresorptive drug exposure. The ONJ diagnosis date for cases corresponded to the index date for matched controls. DNA isolation, genotyping, and data analyses were performed by Q2/EA Genomics using standard protocols and best practices. Blood or tissue samples for 55 ONJ cases and 125 controls were collected. Due to the low quality of the tissue samples, final analyses were based on blood samples of 40 ONJ cases and 124 controls.
Results: We detected no significant genome-wide associations. Of the 43 SNPs with ONJ association in prior studies, none were replicated in our study.
Conclusions: Even though our study sample is the largest to date, we had limited statistical power for GWAS but adequate power for replication analyses. Our study provides no evidence for any genetic predisposition to ONJ. Future studies could increase their statistical power by combining ONJ GWAS datasets and by performing a meta-analysis or pursuing a sequencing strategy in order to identify rare variants.

1. Introduction

Osteoporosis is a common and chronic disease that increases the risk for fracture in both men and women, especially as they age. Worldwide, osteoporosis is associated with >8.9 million fractures annually (Wright et al., 2014). Not only do fractures incur health-care costs and decrease patients' independence and mobility, they also increase mortality risk for up to 10 years after the incident event (Bliuc et al., 2009). Although

* Corresponding author at: Department of Clinical Medicine, Aarhus University, Olof Palmes Allé 43-45, 8200 Aarhus N, Denmark.
E-mail addresses: abp@clin.au.dk (A.B. Pedersen), sven.erik.noerholt@aarhus.rm.dk (S.E. Nørholt), rejnmark@clin.au.dk (L. Rejnmark), bente.langdahl@aarhus.rm.dk (B. Langdal), thomas.jensen@rn.dk (T. Starch-Jensen), hts@clin.au.dk (H.T. Sørensen).

https://doi.org/10.1016/j.bonr.2022.101648
Received 13 October 2022; Received in revised form 12 December 2022; Accepted 14 December 2022
Available online 15 December 2022

antiresorptive osteoporosis agents are proven effective for fracture prevention, there has been a decline in antiresorptive prescription in recent years due to safety issues such as elevated risks of atypical fractures of the femur (Balkhi et al., 2018) and osteonecrosis of the jaw (ONJ) (Ruggiero et al., 2014), even though the absolute risks of these events are actually very low.

ONJ is a rare but serious adverse event of antiresorptive medication (Ruggiero et al., 2014). ONJ causes considerable pain, exposes bone in the oral cavity, and diminishes oral-health-related quality of life due to sequestrectomy or surgical resection of necrotic bone. The reported incidence of ONJ in the osteoporosis population ranges from 0.001 % to <0.15 % and may be only slightly higher than the incidence observed in the general population (Khan et al., 2015). Although several acquired risk factors (e.g., recent oral surgery, tooth extraction, denture use, duration of exposure to antiresorptive medications, poor oral hygiene, and the presence of comorbid conditions such as diabetes, obesity, alcohol abuse, or steroid use) have been described in the literature (Ruggiero et al., 2014; Khan et al., 2015), the pathophysiology is not well understood. Potential hypotheses include inhibition of osteoclastic bone resorption and remodeling; oral risk factors associated with inflammation, infection and osteomyelitis; and inhibition of angiogenesis leading to avascular necrosis (Fung et al., 2015).

Since ONJ occurs only in some individuals despite the presence of the described acquired risk factors, several pharmacogenetic studies have been undertaken to examine ONJ genetic risk factors (Katz et al., 2011; Kim et al., 2015; Marini et al., 2011; Nicoletti et al., 2012; Sarasquete et al., 2008; Lee et al., 2019; La Ferla et al., 2012). These studies have various methodological limitations, including using candidate gene selection as the sole strategy and a small number of ONJ cases (the largest genome-wide association study [GWAS] to date included 38 cases) (Lee et al., 2019). Much of the research on the genetic risk factors for ONJ has been done on an oncology population; however, the incidence of ONJ is noted to be higher in oncology patients than in patients with osteoporosis (Fung et al., 2015; Goodwin et al., 2017). Few pharmacogenomics studies have focused on osteoporosis or metabolic bone disorders without cancer, and no conclusive results are available (Kim et al., 2015; Fung et al., 2015; Lee et al., 2019; Hu and E, 2008), due to poor study design, small population size, or confounding caused by population structure and recent genetic admixture.

We hypothesized that there are single-nucleotide polymorphisms (SNPs) associated with an increased risk of developing ONJ in patients exposed to antiresorptive drugs. Evaluation of previously reported SNPs and SNPs within candidate genes can establish additional associations that do not reach genome-wide significance but are nevertheless relevant to understanding the biology of bone metabolism and ONJ.

Using a case-control design, we examined the genetic association between ONJ and SNPs identified in previous studies. In addition, we aimed to discover genetic risk factors for ONJ by using GWAS to find SNPs with statistically significant differences in frequency between ONJ and non-ONJ samples in patients with similar cumulative antiresorptive drug exposure.

2. Methods

2.1. Data sources

We retrieved data from the following sources:

1) The Scandinavian Cohort for ONJ was established to support an ongoing regulator-mandated post-authorization safety study of denosumab in Denmark, Norway, and Sweden, with clinically confirmed ONJ as adverse event. Systematic registration of incident ONJ cases by the oral and maxillofacial surgeons treating the patients started in 2011. In Denmark, ONJ treatment is centralized in six departments of oral and maxillofacial surgery, with the majority of patients diagnosed at the departments in Aarhus and Copenhagen. The identification and diagnosis of ONJ as well as the enrollment and reporting of ONJ in the Scandinavian Cohort of ONJ patients have been described elsewere (Schiodt et al., 2015).
2) The Danish National Health Service Prescription Database (DNHSPD) has information on all reimbursed prescriptions from community and hospital-based outpatient pharmacies in Denmark since 2004; these prescriptions are registered using the Anatomical Therapeutic Chemical (ATC) codes (Johannesdottir et al., 2012).
3) The Danish National Patient Registry (DNPR) (Schmidt et al., 2015) holds data on all inpatient admissions to Danish hospitals since 1977 and all outpatient clinic visits since 1995. Information on dental history and radiation therapy was collected from the DNPR.
4) The Civil Registration System (CRS) holds data on the vital status and migration of all Danish residents since 1968 (Schmidt et al., 2014). Using the unique civil registration number assigned to every Danish resident and recorded in the CRS, it is possible to uniquely link data between multiple registries.
5) The Danish National Pathology Registry holds data on tissue samples (Erichsen et al., 2010).

2.2. Study subjects

This study included Danish men and women with medically confirmed ONJ who were registered in the Scandinavian Cohort of ONJ patients during 2011–2017 ($n = 501$).

Several patients were excluded based on the following criteria: 1) patients aged <55 at time of diagnosis with ONJ; 2) patients with history of hospitalization for radiation therapy most likely to the head and neck at index date; and 3) patients without history of antiresorptive therapy. After exclusion criteria were applied, 200 potentially eligible ONJ cases were identified, of which 136 (68 %) were alive at the time of data extraction (May 2019), while 65 (32 %) were deceased. Only subjects with medically treated osteoporosis were included.

For each ONJ case, five controls were selected from the general osteoporosis population. Controls were matched on age (±5 years), sex, cumulative antiresorptive drug exposure (i the form of a number of antiresorptive drug prescriptions, any kind), and being alive at the time of data extraction. The ONJ diagnosis dates for cases correspond to the index dates for matched controls.

Controls were selected from the Aarhus University Hospital, Department of Endocrinology and Internal Medicine, Osteoporosis Outpatient Clinic. The clinic is highly specialized and one of the largest clinics in Denmark for the diagnosis and treatment of adult men and women with osteoporosis. The clinic receives patient referrals from hospitals and general practitioners situated in the Central Denmark Region, but the majority of patients live in the Aarhus area.

A total of 136 ONJ cases and 596 matched controls were invited to participate. If cases and controls agreed to participate, they provided written informed consent regarding use of their blood samples for research purposes at the time of enrollment in the study. Blood samples were drawn at the local hospital laboratory.

Of the invited individuals, 55 ONJ cases and 125 controls were enrolled in the study. The Danish National Pathology Registry was reviewed for available pathology codes and archived tissue samples of 64 deceased ONJ cases. A fresh blood sample (40/55) or an archived tissue sample (15/55) was provided for each ONJ case. The 125 controls provided a fresh blood sample (Fig. 1).

2.3. Power calculation

The power to detect genetic effects in a case-control study is a function of the sample size, the inheritance model, and allele frequency. Using the Genetic Power Calculator (Purcell et al., 2003; Genetic Power Calculator, 2008), we estimated that by including 50 ONJ cases and 100 controls, we would have 80 % power to detect genome-wide associations with the most common SNPs through a candidate-variant approach if

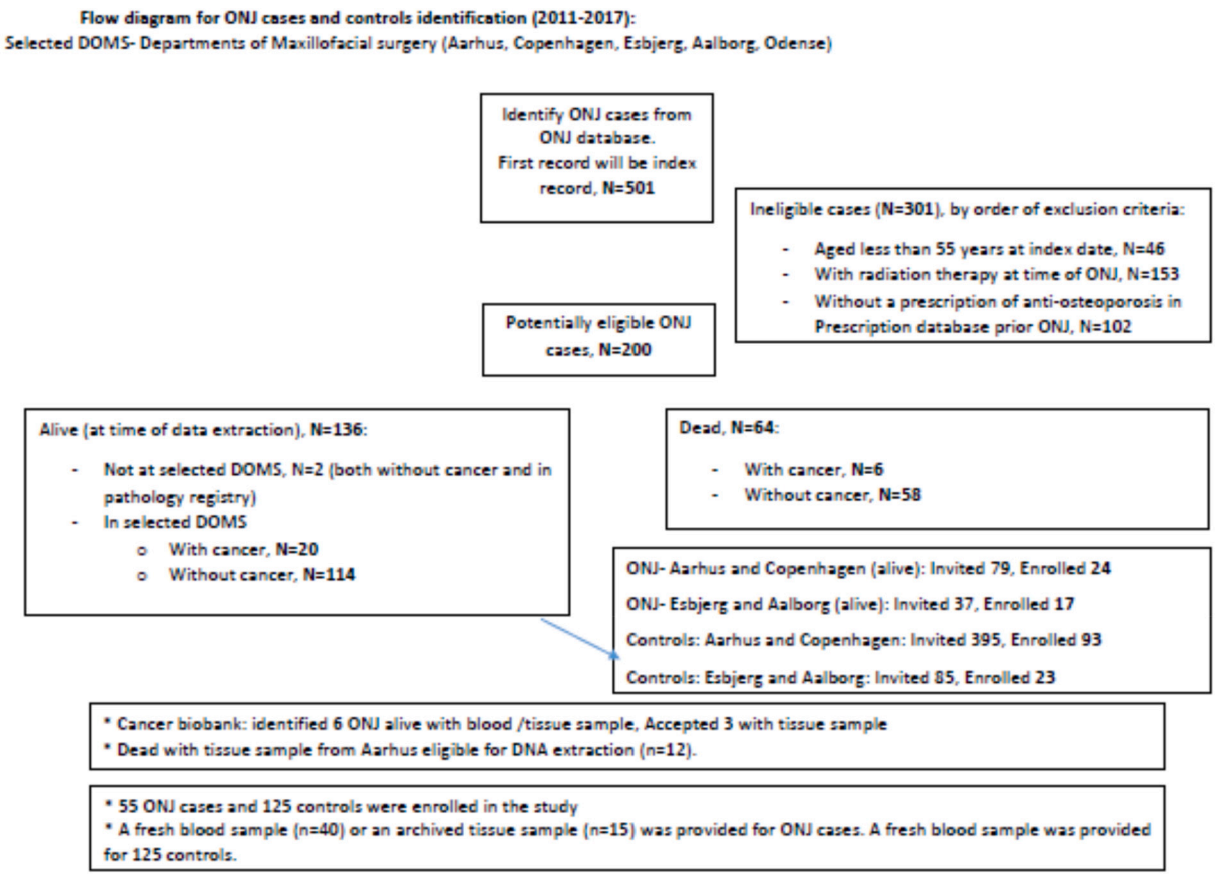


Fig. 1. Flow diagram.

they affect ONJ. The following parameters were used: model = "case-control for discrete traits"; $N = 150$ or 50 cases; control: case ratio = 2; model = additive; prevalence = 0.001; marker frequency = high-risk allele frequency; genotype relative risk Aa = risk in table below; genotype relative risk AA = risk in table below, squared; D-prime = 1; statistic = "allelic 1 df test".

2.4. Exposure

Information on the use of antiresorptive medication prior to the ONJ diagnosis date was collected from the DNHSPD. The ATC codes are presented in Appendix Table 1. The number of prescriptions from 2004 to the ONJ diagnosis or index date was calculated for each case and control. Controls and their case had to have had an equivalent number of prescriptions prior to the ONJ diagnosis date or index date (but not necessary the same type of antiresorptive medication). For intravenous bisphosphonates and subcutaneous denosumab prescribed in hospitals, treatment codes from the DNPR were used.

2.5. Analyses

Analyses were carried out in three steps: a quality-control check of genotype data, a genetic association study of focus SNPs from previous studies, and a GWAS analysis.

2.6. Quality control

Genotyping was performed using the Illumina Infinium HumanOmni2.5–8 BeadChip. Infinium processing was performed according to the standard Illumina workflow consisting of three steps: (1) whole-genome amplification of input DNA; (2) fragmentation; and (3) a two-step allele detection involving hybridization and single-base extension. Whole-genome amplification was carried out for 20–24 h at 37 °C, followed by enzymatic fragmentation for 1 h at 37 °C. DNA was purified by ethanol precipitation and resuspended prior to hybridization on Illumina Infinium HumanOmni2.5–8 at 48 °C for 16–24 h. Single-base extension and fluorescent labeling allowed allele specificity to be determined, and BeadChips were scanned and intensities determined using an Illumina iScan instrument. Genotypes were extracted from intensity data and called using a standard cluster file within the Illumina Genome Studio software.

Quality-control checks were performed on genotype data to potentially exclude low-quality samples and low-quality variants, by using standard protocols for GWAS quality control (Anderson et al., 2010). Using the PLINK software package v1.07 (Chang et al., 2015; Purcell et al., 2007; Renteria et al., 2013), variants were excluded if they were rare (minor allele frequency < 1 %), if they showed a low call rate (<95 %), or if they showed a deviation from Hardy-Weinberg equilibrium ($p < 0.001$), all of which suggest possible genotyping error. We also used PLINK to check individuals for genome-wide heterozygosity and excluded any who showed extreme values (>3 S.D.) and to check individuals pairwise for any cryptic relatedness (pairs with identity by descent >0.185 were excluded).

While Illumina measures ~2.5 M SNPs on their Omni SNP platform, not all are needed to make a population-group determination of participants. In the ONJ GWAS, we examined approximately 500,000 unambiguously measured (with respect to strand) SNPs (i.e., not A/T or G/C SNPs). In addition, we added subjects from transformed whole-genome sequence (WGS) data from those same SNP positions using Genome-in-a-bottle reference samples (GIAB) known as HG001-HG005. Ideally, we would have had Omni2.5 SNP chip data for these sample GIAB samples, but we could not easily find extensive SNP data on these

samples from the same platform. Thus, SNPs from WGS genotypes for the GIAB samples were recorded in a common format with the Illumina SNP data.

Genotypes were recorded into −1,0,1 (homozygous allele 1, heterozygous, homozygous allele 2), and principal components analysis (PCA) and correlation analysis were performed.

The characteristics of controls were checked to confirm that they remained in balance between cases and controls if samples were removed during the genotype quality-control analysis. The chi-squared test for count data and the *t*-test for continuous data were conducted using the R software package (R Core Team, 2020).

2.7. Candidate SNPs analysis

The candidate SNPs analysis used *p*-values and odds ratios and looked specifically at candidate variants previously reported in the literature (Katz et al., 2011; Kim et al., 2015; Marini et al., 2011; Nicoletti et al., 2012; Sarasquete et al., 2008) as associating with ONJ risk, to check whether they were replicated in this study by passing a nominal (non–genome-wide) significance level of $p < 0.05$.

2.8. GWAS analysis

The GWAS was based on well-established methods (Lewis and Knight, 2012; Sale et al., 2009). The GWAS analysis focused on the predictive power of SNPs as biomarkers for ONJ risk, as assessed through a difference in allele frequencies between cases and controls, from which an odds ratio and a p-value were calculated for each SNP. To minimize the number of tests performed, the simplest test for a case-control association, based on allele frequency and not accounting for individual diplotypes (proportion of heterozygotes/homozygotes), was implemented as the genome-wide test, using Fisher's exact test (as implemented in the PLINK software package (Chang et al., 2015; Purcell et al., 2007; Renteria et al., 2013) or equivalent). If any loci passed a genome-wide significance level of $p < 5 \times 10^{-8}$ (corresponding to a Bonferroni-corrected alpha of 5 %), the associations were explored through further models to see if the mode of inheritance was nonadditive (tests for dominant, recessive, and Cochran-Armitrage trend).

3. Results

3.1. Matching

Following genotype quality control, the case/control matching characteristics remained in balance (Table 1). However, dental procedures were more prevalent among ONJ cases than among matched controls.

Table 1
Balance of characteristics used to match ONJ cases with control subjects.

Characteristic	Cases ($n = 40$) (counts or quants)	Controls ($n = 124$) (counts or quants)	p-Value
Sex (female/male)	34/6	114/10	0.33
Age, years	67/63.8/73.2	68.5/64/74	0.90
Antiresorptive drug exposure[a]	1785/973/2723	1869/1008/2618	0.47
Any previous tooth extractions	47 %	22 %	0.05
Other oral surgeries	85 %	10 %	0.05
Regular visit to dentist	67 %	90 %	0.05
Paradentosis	31 %	27 %	0.35

The mean, 1st, and 3rd quartiles are reported.
ONJ: osteonecrosis of the jaw.
[a] In the form of a number of antiresorptive drug prescriptions, any kind.

3.2. Genotyping and quality control

Yields from fresh blood samples all met Q2/EA internal criteria and were considered adequate for genotyping. Yields from the archived tissue samples were lower than fresh-blood yields. Because the archived tissue samples were all ONJ cases and exclusion would adversely impact statistical power, they were submitted for genotyping despite the low DNA yield with the understanding that the results might not have satisfied the genotype quality-control metrics. A single control was missing a DNA sample.

Genotype call rates of the 164 blood samples were high (mean = 0.999, SD = 0.000365), and no single blood sample produced a call rate below the minimum call rate threshold of 0.95. In comparison, genotype call rates of the 15 archived tissue samples were all lower (mean = 0.479, SD = 0.218) than the minimum call rate threshold. Genotypes from the 15 archived tissue samples (all ONJ cases) were omitted from further analysis.

Two samples appeared closely related, with ~70 % in-common genotypes compared with ~45–50 % in-common genotypes in all other pairwise comparisons. Subject characteristics were missing for one control, and the genotype data for that sample were excluded from the study.

PCA (Fig. 1) confirmed that there was no underlying population structure in the study subjects with potential to confound the statistical analysis. Thus, the population was ethnically homogeneous. The data were consistent with study participants being of European ancestry, which indicates that the data were of good quality.

Genotyping data quality was also evaluated regarding variants. The genotyping platform assayed 2,380,364 SNPs. Tests for Hardy-Weinberg equilibrium, missingness, and low frequency reduced the total number of SNPs to 1,530,529. The total genotyping rate in the remaining individuals was 0.999151.

Following genotyping quality control, 40 ONJ cases, 124 controls, and 1,530,529 SNPs were used for the GWAS.

3.3. Candidate SNPs analysis

The candidate SNPs analysis investigated whether variants previously reported in the literature from GWAS or candidate gene studies could be replicated in this study by passing a nominal (non–genome-wide) significance level of $p < 0.05$. A total of 43 unique SNPs with rsIDs have been reported at 15 unique loci. In our study, 23 of the 43 SNPs were assayed directly by the Illumina Omni2.5 M microarray from the genotype data file. Among the 23 SNPs, 19 were matched on SNP ID, and 4 were matched on genomic position. One SNP was excluded afterwards from the analysis by genotype quality control, due to its low minor-allele frequency. Thus, 22 SNPs were selected for the genetic association study (Tables 2 and 3). The remaining 20 focus SNPs from the literature were not investigated directly by the Illumina Omni2.5 M microarray.

Of the 22 variants assayed directly, only rs1800012, a SNP which was identified in a candidate gene study, had a *p*-value <0.05 in our study. However, the odds ratio in our study was in the opposite direction of what was previously reported. Furthermore, adjusting for covariates of sex, age, smoking status, and cumulative exposure to antiresorptives increased the p-value of the signal observed from this variant above the replication threshold. To date, the most significant SNP identified by GWAS has been rs17024608 in *RBMS3* ($p = 7.47 \times 10^{-8}$, OR = 5.8). In our study with more cases, rs17024608 essentially produced no signal, with $p = 1$.

We also analyzed 20 target SNPs from the literature that were not investigated directly by the Illumina Omni2.5 M microarray but which could be in high linkage disequilibrium (LD) with a SNP on the array. Briefly, we found that 14 of the 20 target SNPs had a SNP on the array with very high LD ($r^2 > 0.95$) and one had a moderately high LD ($r^2 = 0.86$). However, for all these 15 SNPs, the smallest *p*-value observed in the study was 0.16 (target SNP = rs9340799, array

Table 2
Genetic association results of 22 selected SNPs (basic crude model).

SNP	Gene	CHR	BP	Tested allele	OR	P	MAF	NCHROBS
rs2297480	FDPS	1	155279482	C	1.389	0.2786	0.2239	326
kgp8956196	PPARG	3	12477055	G	1.273	0.3639	0.4177	328
rs17024608	RBMS3	3	29954690	G	1.036	1	0.07317	328
rs11730582	OPN	4	88896421	A	0.8051	0.4413	0.4909	328
rs11934877	IGFBP7	4	57941026	G	1.072	0.8598	0.1555	328
rs4431170	MARCH1	4	165284574	G	1.466	0.4214	0.05793	328
rs10070440	SV2C	5	75427935	G	0.8291	0.6377	0.2104	328
rs3025039	VEGF	6	43752536	A	1	1	0.125	328
rs10893	ABP1	7	150555915	G	0.7145	0.2744	0.3293	328
rs2097937	CROT	7	87030903	G	0.9457	1	0.1555	328
rs4725373	ABP1	7	150557622	A	0.7586	0.4075	0.319	326
rs11189381	SFRP5	10	99563198	G	2.094	0.5989	0.01524	328
rs17110453	CYP2C8	10	96829529	C	1.172	0.7248	0.1585	328
rs1934951	CYP2C8	10	96798548	A	0.9042	0.8779	0.2256	328
rs1934980	CYP2C8	10	96808973	G	0.9252	0.8779	0.2226	328
rs2463437	CHST11	12	105154087	G	0.8874	0.6851	0.3323	328
rs1678387	ABCC4	13	95717906	A	0.5043	0.5306	0.04268	328
rs10046	CYP19A1	15	51502986	A	1.048	0.8979	0.4787	328
rs12903202	ALDH1A2	15	58306793	G	0.5149	0.3482	0.08232	328
kgp747462	COL1A1	17	48277749	A	0.4292	0.04337	0.1799	328
kgp7931552	GRCh37	18	60027448	A	0.7241	0.5422	0.1098	328
rs17751934	MEX3C	18	49201814	A	0.3013	0.3066	0.03354	328

CHR: Chromosome.
SNP: SNP ID.
BP: Physical position (base-pair).
Tested allele: Minor allele Name (based on whole sample).
OR: Estimated odds ratio (for A1, i.e. A2 is reference).
P: Asymptotic p-value for this test.
MAF: Minor allele frequency.
NCHROBS: Non-missing allele count.

Table 3
Genetic association results of 22 selected SNPs (4 covariates adjusted model).

SNP	CHR	BP	Tested allele	BETA	SE	P	MAF	NCHROBS
rs2297480	1	155279482	C	0.3917	0.3347	0.2418	0.2239	326
kgp8956196	3	12477055	G	0.248	0.2487	0.3187	0.4177	328
rs17024608	3	29954690	G	0.06962	0.5258	0.8947	0.07317	328
rs11730582	4	88896421	A	−0.2399	0.2647	0.3647	0.4909	328
rs11934877	4	57941026	G	0.0822	0.3627	0.8207	0.1555	328
rs4431170	4	165284574	G	0.4078	0.5496	0.4581	0.05793	328
rs10070440	5	75427935	G	−0.1905	0.3196	0.5511	0.2104	328
rs3025039	6	43752536	A	−0.08997	0.4372	0.837	0.125	328
rs10893	7	150555915	G	−0.3368	0.3019	0.2645	0.3293	328
rs2097937	7	87030903	G	−0.07198	0.3757	0.848	0.1555	328
rs4725373	7	150557622	A	−0.2789	0.3027	0.3569	0.319	326
rs11189381	10	99563198	G	0.8971	0.9452	0.3426	0.01524	328
rs17110453	10	96829529	C	0.133	0.3509	0.7047	0.1585	328
rs1934951	10	96798548	A	−0.1413	0.3206	0.6594	0.2256	328
rs1934980	10	96808973	G	−0.1238	0.3202	0.699	0.2226	328
rs2463437	12	105154087	G	−0.1307	0.3017	0.6648	0.3323	328
rs1678387	13	95717906	A	−0.5585	0.7484	0.4555	0.04268	328
rs10046	15	51502986	A	0.000705	0.2679	0.9979	0.4787	328
rs12903202	15	58306793	G	−1.002	0.6123	0.1019	0.08232	328
kgp747462	17	48277749	A	−0.7726	0.416	0.06326	0.1799	328
kgp7931552	18	60027448	A	−0.3125	0.4511	0.4885	0.1098	328
rs17751934	18	49201814	A	−1.139	1.08	0.2917	0.03354	328

BETA: Regression coefficient.
SE: Standard error.
Values adjusted for sex, age, smoking status, and cumulative exposure to antiresorptives.
P: *p*-value.
MAF: minor allele frequency.
NCHROBS: Non-missing allele count.
BP: Physical position (base-pair).

SNP = rs9322331, LD $r^2 = 0.955$).

For the other five target SNPs, two had no published p-value, nor any evidence of variants in the CEU (Northern and Western European Ancestry) population (1000 Genomes or ALFA). Two SNPs showed only one haplotype (i.e., no variation) in the CEU population. There was only one SNP (rs5768453) in the NCBI database that had an LD r^2 value above 0.5 (0.59) and this SNP was not on the array. In addition, the observed haplotype associated with rs11064477 in the CEU population had a population frequency of <2 %. Thus, power would be very limited for this SNP in the current ONJ study.

3.4. GWAS analysis

The GWAS analysis was based on 1,530,529 SNPs. A quantile-quantile plot of logistic regression comparing observed to expected *p*-values was linear, suggesting there is no unexplained subpopulation bias in the dataset (Fig. 2). Furthermore, departures from linearity at the tail of the quantile-quantile plot are toward increased expected p-value, suggesting that even the most significant SNPs should not be considered suggestive or trending toward significance.

Plotting the test results versus genomic position in a Manhattan plot (Fig. 3) further emphasized the lack of genome-wide significant findings. No genome-wide significant SNPs were identified by our study.

4. Discussion

This study of 40 ONJ cases and 124 controls from the osteoporosis population matched on sex, age, antiresorptive drug exposure, and ONJ diagnosis date did not discover new associations or confirm previously reported associations between genetic factors and ONJ. Of 43 reported genetic associations from prior studies, 22 were directly assessed in this study, with none of them being replicated in our population. We did not detect any significantly associated SNP from the GWAS analysis.

4.1. Comparison with previous studies

The most significant SNP identified by GWAS has been rs17024608 in *RBMS3* by Nicoletti et al (Nicoletti et al., 2012), which our study did not confirm. Yang et al (Yang et al., 2021) identified three SNPs at suggestive level of significance, but none of these were confirmed in our study. Likewise, the SNP identified with a *p*-value <0.05 in our study, rs1800012, has previously been positively associated with ONJ, unlike the associations that we found (Katz et al., 2011). However, these previous studies differed methodologically from our study: they either examined patients with cancer (Katz et al., 2011), used only candidate gene studies (Katz et al., 2011), or defined use of antiresorptive agents differently than this study (Katz et al., 2011; Nicoletti et al., 2012). They (Kim et al., 2015; Marini et al., 2011; Nicoletti et al., 2012) primarily included patients who used zoledronate, while participants in this study had more varying prescriptions. However, the antiresorptive agents prescribed for patients in this study have varying potency; for example, denosumab and zoledronate have previously been associated with development of ONJ, while etidronate is less commonly associated with ONJ (Endo et al., 2017). Ideally, we would thus analyze our cohort based on which antiresorptive agents they used, although the ability to do so is limited by the number of patients included in the study and by the number of patients receiving some specific agents such as etidronate. Recruiting participants for ONJ studies is difficult, as seen by the low number of participants in the body of present literature, and although our study included a large number of patients compared to previous studies (Lee et al., 2019), this still resulted in problems regarding statistical power. Thus, candidate gene studies should be interpreted with caution, given that this study, despite its size, was not able to confirm any of the previously reported SNPs. In order to increase recruitment of patients and improve statistical power, future studies could benefit from the collection and storage of blood or tissue samples at the time of ONJ diagnosis. Considering the severity and complexity of ONJ disease, high age, and high level of multimorbidity which increases with age, this could be a way to improve genetic studies in osteoporosis patients with ONJ. A similar method is applied among cancer patients, since it is possible to retrieve tissues taken and stored during the diagnosis and treatment of cancer. However, the quality of tissue samples has to be improved or their usability will be low, as our study has shown. Further, to uncover genetic risk factors for ONJ, future studies could increase their statistical power by combining ONJ GWAS datasets and performing a meta-analysis or pursuing a sequencing strategy in order to identify rare variants.

One important difference between our ONJ cases and controls is a higher prevalence of recent dental surgeries among ONJ patients. Currently, clinicians must continue to rely on known risk factors, including dental surgeries, rather than genetic screening in order to make early predictions of ONJ disease.

4.2. Study limitations

This GWAS was limited to detecting common SNPs with relatively large effects on ONJ risk. Rare variants may make a significant genetic contribution that cannot be assessed by this study. Additionally, if ONJ risk is highly polygenic, the contribution to the risk will be spread across many common and rare SNPs, and no one variant may be genome-wide significant. The GWAS also inherently carries a modeling assumption of

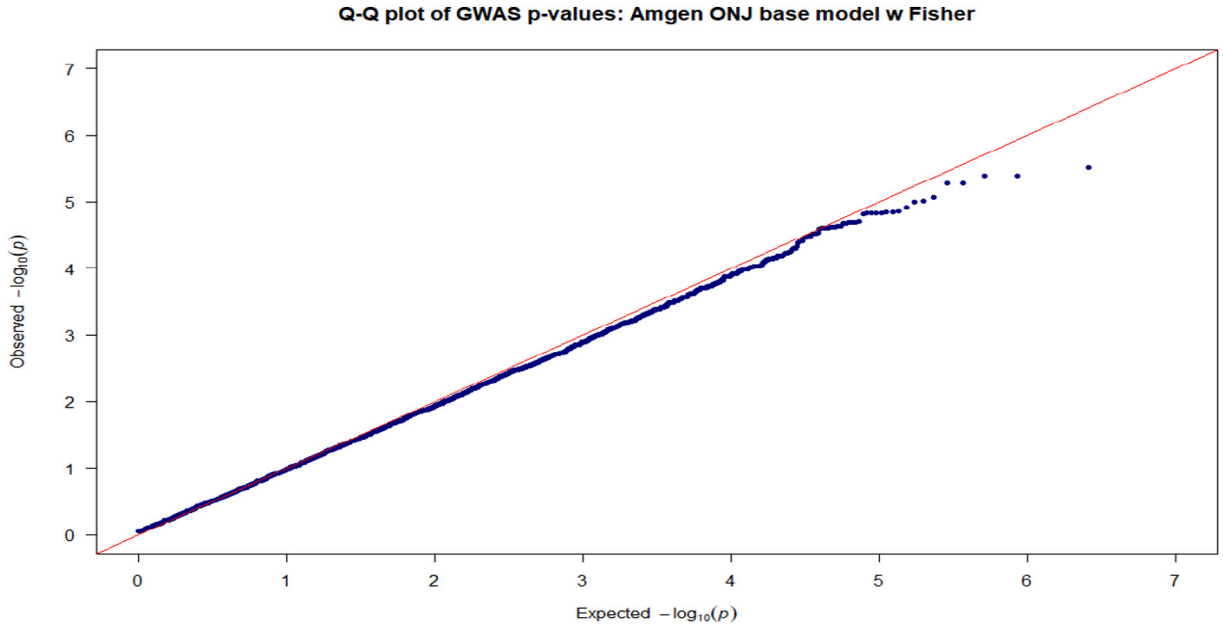


Fig. 2. Quantile-quantile Plot of GWAS *p*-values.

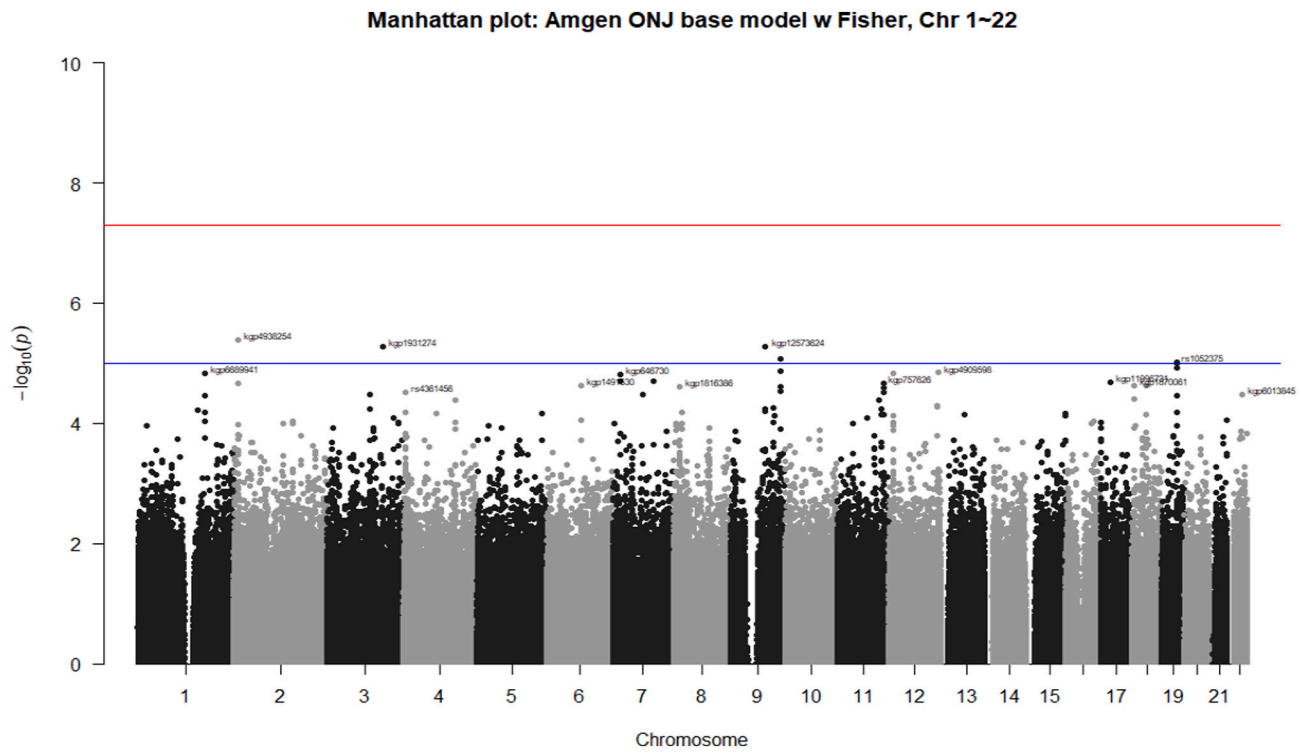


Fig. 3. Manhattan plot of GWAS p-values for chromosomes 1 to 22.

additivity; nonadditivity between genetic effects at different loci may mask genetic effects. For example, if an individual has an increased ONJ risk only if they have a particular combination of two alleles at two SNPs in different genes, rather than each SNP contributing independently to risk, then the risk calculated for each of these SNPs by GWAS will be an underestimate and may not pass the threshold for detection.

Although no significant SNP from the GWAS analysis was identified, we do not doubt the quality of our genotype data. The very high SNP call rates per sample, the expected results from principal component analysis, the detection of the person of non-Danish ancestry, the detection of a near relative in the SNP data who was excluded from the study, the expected level of SNPs not meeting Hardy-Weinberg equilibrium assumptions, the SNPs in LD with target SNPs having concordant minor-allele frequency values with the CEU population, etc., all indicated that there were no issues with the genotype data itself (Hu and E, 2008). We were not able to do matching on the specific antiresorptive drug prescription, rather on the number of specific antiresorptive drugs of any kind. However, the majority of the cases and controls had received bisphosphonates.

4.3. Clinical implications

Developing ONJ may be multifactorial and thus a result of predisposing factors and genetic factors. Given the possibility of nonadditivity between genetic effects, it is possible that coupling the risk of developing ONJ to genetic deviations is unrealistic. Although ONJ is a severe disease, it is also rare among patients without cancer. Thus, screening for genetic variants possibly linked to ONJ does not seem to be a plausible approach, based on the present results.

5. Conclusions

Despite including more ONJ cases than any prior GWAS, our study did not detect any genome-wide significant associations. Of the 43 SNPs with ONJ association in prior studies, none were replicated in our study. Thus, screening for genetic variants possibly linked to ONJ does not seem to be a plausible approach, based on these results.

Ethical approval

This research involving human subjects and their genomic data was approved by the Institutional Review Board of the Danish National Committee on Health Ethics (No. 1-10-72-190-17). All clinical investigation was performed in accordance with the Declaration of Helsinki.

Patient/research participant consent

Written informed consent was obtained from all living subjects prior to their participation.

Funding

This work was supported by institutional research funding from Amgen Inc. USA, to and administered by Aarhus University.

Consent for publication

All authors have reviewed the final version of the manuscript and approved it for publication.

CRediT authorship contribution statement

Conceptualization: Henrik T. Sørensen; Data curation, formal analysis, and methodology: Alma B. Pedersen, Sven E. Nørholt, Bente Langdahl, Lars Rejnmark, Thomas Starch-Jensen, Henrik T. Sørensen; Funding acquisition: Henrik T. Sørensen; Project administration and supervision: B. Pedersen, Henrik T. Sørensen; Roles/writing - original draft: Alma B. Pedersen; writing -review & editing: Alma B. Pedersen, Sven E. Nørholt, Bente Langdahl, Lars Rejnmark, Thomas Starch-Jensen, Henrik T. Sørensen.

Role of the funding source

The funder had participated in the design of the study, analysis and interpretation of the data.

The funder had no role in the collection and management of the data.

The funder had no role in preparing or approval of the manuscript, or in the decision to submit the manuscript for publication.

Submission declaration and verification

This manuscript has not been published in whole or in part, nor is it being considered for publication elsewhere.

Declaration of competing interest

Alma B. Pedersen and Henrik T. Sørensen are employed at the Department of Clinical Epidemiology at Aarhus University Hospital, which is involved in studies with funding from various pharmaceutical companies as institutional research grants. One such study, specifically an ongoing regulator-mandated postauthorization safety study of denosumab among women with postmenopausal osteoporosis, funded by Amgen Inc. through institutional funding to Aarhus University, is related to and has overlapping populations with the present study.

Alma B. Pedersen: None.

Henrik T. Sørensen: None.

Sven E. Nørholt: None.

Lars Rejnmark: None.

Bente Langdahl: Institutional research grants from Amgen; advisory board and speakers bureau: UCB, Amgen, Gedeon-Richter, Astellas, Astra-Zeneca.

Thomas Starch-Jensen: None.

Data availability

The authors do not have permission to share data.

Acknowledgements

We thank all participants for taking the time to participate in this study. Also, we thank Q2 Solutions –a Quintiles Quest Joint Venture- EA Genomics, USA, for performing genetic analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2022.101648.

References

Anderson, C.A., Pettersson, F.H., Clarke, G.M., Cardon, L.R., Morris, A.P., Zondervan, K. T., 2010. Data quality control in genetic case-control association studies. Nat Protoc 5 (9), 1564–1573.

Balkhi, B., Seoane-Vazquez, E., Rodriguez-Monguio, R., 2018. Changes in the utilization of osteoporosis drugs after the 2010 FDA bisphosphonate drug safety communication. Saudi Pharm. J. 26 (2), 238–243. https://doi.org/10.1016/j.jsps.2017.12.005 [published Online First: 2018/09/01].

Bliuc, D., Nguyen, N.D., Milch, V.E., et al., 2009. Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. JAMA 301 (5), 513–521. https://doi.org/10.1001/jama.2009.50.

Chang, C.C., Chow, C.C., Tellier, L.C., et al., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4, 7. https://doi.org/10.1186/s13742-015-0047-8.

Endo, Y., Kumamoto, H., Nakamura, M., et al., 2017. Underlying mechanisms and therapeutic strategies for bisphosphonate-related osteonecrosis of the jaw (BRONJ). Biol. Pharm. Bull. 40 (6), 739–750. https://doi.org/10.1248/bpb.b16-01020 [published Online First: 2017/06/02].

Erichsen, R., Lash, T.L., Hamilton-Dutoit, S.J., et al., 2010. Existing data sources for clinical epidemiology: the Danish National Pathology Registry and Data Bank. Clin. Epidemiol. 2, 51–56. https://doi.org/10.2147/clep.s9908 [published Online First: 2010/09/25].

Fung, P.L., Nicoletti, P., Shen, Y., et al., 2015. Pharmacogenetics of bisphosphonate-associated osteonecrosis of the jaw. Oral Maxillofac. Surg. Clin. North Am. 27 (4), 537–546. https://doi.org/10.1016/j.coms.2015.06.005.

Genetic Power Calculator, 2008. 2008 [program].

Goodwin, J.S., Zhou, J., Kuo, Y.F., et al., 2017. Risk of jaw osteonecrosis after intravenous bisphosphonates in cancer patients and patients without cancer. Mayo Clin. Proc. 92 (1), 106–113. https://doi.org/10.1016/j.mayocp.2016.09.015 [published Online First: 2016/11/24].

Hu, D.Z., E, 2008. Confounding in Genetic Association Studies and Its Solutions. Pharmacogenomics in Drug Discovery and Development. Humana Press.

Johannesdottir, S.A., Horvath-Puho, E., Ehrenstein, V., et al., 2012. Existing data sources for clinical epidemiology: the danish National Database of reimbursed prescriptions. Clin. Epidemiol. 4, 303–313. https://doi.org/10.2147/CLEP.S37587.

Katz, J., Gong, Y., Salmasinia, D., et al., 2011. Genetic polymorphisms and other risk factors associated with bisphosphonate induced osteonecrosis of the jaw. Int. J. Oral Maxillofac. Surg. 40 (6), 605–611. https://doi.org/10.1016/j.ijom.2011.02.002.

Khan, A.A., Morrison, A., Hanley, D.A., et al., 2015. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J. Bone Miner. Res. 30 (1), 3–23. https://doi.org/10.1002/jbmr.2405.

Kim, J.H., Ko, Y.J., Kim, J.Y., et al., 2015. Genetic investigation of bisphosphonate-related osteonecrosis of jaw (BRONJ) via whole exome sequencing and bioinformatics. PLoS One 10 (2), e0118084. https://doi.org/10.1371/journal.pone.0118084.

La Ferla, F., Paolicchi, E., Crea, F., et al., 2012. An aromatase polymorphism (g.132810C>T) predicts risk of bisphosphonate-related osteonecrosis of the jaw. Biomark. Med 6 (2), 201–209. https://doi.org/10.2217/bmm.12.14 [published Online First: 2012/03/28].

Lee, K.H., Kim, S.H., Kim, C.H., et al., 2019. Identifying genetic variants underlying medication-induced osteonecrosis of the jaw in cancer and osteoporosis: a case control study. J. Transl. Med. 17 (1), 381. https://doi.org/10.1186/s12967-019-2129-3 [published Online First: 2019/11/22].

Lewis, C.M., Knight, J., 2012. Introduction to genetic association studies. Cold Spring Harb. Protoc. 2012 (3), 297–306. https://doi.org/10.1101/pdb.top068163.

Marini, F., Tonelli, P., Cavalli, L., et al., 2011. Pharmacogenetics of bisphosphonate-associated osteonecrosis of the jaw. Front Biosci (Elite Ed) 3, 364–370.

Nicoletti, P., Cartsos, V.M., Palaska, P.K., et al., 2012. Genomewide pharmacogenetics of bisphosphonate-induced osteonecrosis of the jaw: the role of RBMS3. Oncologist 17 (2), 279–287. https://doi.org/10.1634/theoncologist.2011-0202.

Purcell, S., Cherny, S.S., Sham, P.C., 2003. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19 (1), 149–150.

Purcell, S., Neale, B., Todd-Brown, K., et al., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.

Renteria, M.E., Cortes, A., Medland, S.E., 2013. Using PLINK for genome-wide association studies (GWAS) and data analysis. Methods Mol. Biol. 1019, 193–213. https://doi.org/10.1007/978-1-62703-447-0_8.

Ruggiero, S.L., Dodson, T.B., Fantasia, J., et al., 2014. American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw–2014 update. J. Oral Maxillofac. Surg. 72 (10), 1938–1956. https://doi.org/10.1016/j.joms.2014.04.031.

Sale, M.M., Mychaleckyj, J.C., Chen, W.M., 2009. Planning and executing a genome wide association study (GWAS). Methods Mol. Biol. 590, 403–418. https://doi.org/10.1007/978-1-60327-378-7_25.

Sarasquete, M.E., Garcia-Sanz, R., Marin, L., et al., 2008. 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 112 (7), 2709–2712. https://doi.org/10.1182/blood-2008-04-147884.

Schiodt, M., Larsson Wexell, C., Herlofson, B.B., et al., 2015. Existing data sources for clinical epidemiology: Scandinavian cohort for osteonecrosis of the jaw - work in progress and challenges. Clin Epidemiol 7, 107–116. https://doi.org/10.2147/CLEP.S71796.

Schmidt, M., Pedersen, L., Sorensen, H.T., 2014. The danish civil registration system as a tool in epidemiology. Eur. J. Epidemiol. 29 (8), 541–549. https://doi.org/10.1007/s10654-014-9930-3 [published Online First: 2014/06/27].

Schmidt, M., Schmidt, S.A., Sandegaard, J.L., et al., 2015. The danish National Patient Registry: a review of content, data quality, and research potential. Clin. Epidemiol. 7, 449–490. https://doi.org/10.2147/CLEP.S91125.

Wright, N.C., Looker, A.C., Saag, K.G., et al., 2014. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J. Bone Miner. Res. 29 (11), 2520–2526. https://doi.org/10.1002/jbmr.2269.

Yang, G., Singh, S., McDonough, C.W., et al., 2021. Genome-wide association study identified chromosome 8 locus associated with medication-related osteonecrosis of the jaw. Clin. Pharmacol. Ther. 110 (6), 1558–1569. https://doi.org/10.1002/cpt.2397 [published Online First: 20210831].